

*Recent Advances in*  
**MEDICINAL PLANTS RESEARCH**  
of North East India



*Editor*  
**Dr. Mousmi Saikia**

**Department of Herbal Science and Technology**  
Anandaram Dhekial Phookan College (A.D.P. College)  
Nagaon, Assam, India



# Recent Advances in MEDICINAL PLANTS RESEARCH of North East India

**Editor**

Dr. Mousmi Saikia

*For,*  
ADP COLLEGE LIBRARY



**Department of Herbal Science and Technology**  
Anandaram Dhekiyal phookan College (A.D.P. College), Nagaon, Assam, India

**Recent Advances in Medicinal Plants Research of North East India** : Publication of proceedings of research papers and review articles presented during UGC sponsored National Seminar organized by Dept. of Herbal Science & Technology, ADP College, Nagaon on 11<sup>th</sup> & 12<sup>th</sup> November, 2016

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**Dr. S. U. Ahmed, M.Sc., Ph.D.**

Principal

Anandaram Dhekial Phookan College  
Nagaon, Pin-782001 (Assam)



## Message

It is a matter of happiness and pride to note that the Department of Herbal Science and Technology, Anandaram Dhekial Phookan College (A.D.P. College), Nagaon had successfully organized the 2nd National Seminar entitled "Recent Advances in Medicinal Plants Research of North East India" (RAMPR-NE 2016) on 11 and 12 November 2016. It was my privilege to welcome a few well known experts in the field of Plant sciences, who have come forward to strengthen our efforts in one of the most important themes of the present times.

From very ancient time's man have been relied on the healing properties of medicinal plants. As herbs are natural products they are free from side effects, they are comparatively safe, eco-friendly and locally available. Traditionally there are lots of herbs used for the ailments related to different seasons. There is a need to promote them to save the human lives. These herbal products are today are the symbol of safety in contrast to the synthetic drugs, that are regarded as unsafe to human being and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security.

As North east India is the biodiversity hotspot with five biosphere reserve and two belonging to Assam. It's our duty to discover, document, conserve and sustainably use the ethnic plants having multipurpose properties along with its medicinal values. In this context, I encourage our students and academia's to take up research related to Plant Science and promote them globally.

The organizers are going to publish the proceedings of the above symposium in a book form entitled "Recent Advances in Medicinal Plants Research of North East India". I appreciate the efforts of the organizing committee of the National Seminar, and the entire team of workers for this important task. I sincerely hope that the issues addressing in this proceedings will be useful for the future prospects.

10th Feb, 2017

(Dr. S.U. Ahmed)

Patron

National Seminar (RAMPR-NE 2016)

## From the Desk of the Organizing Secretary

Dear Seminar Participants and Distinguished Guests,

As the Organizing Secretary of the National Seminar, I wish to extend a warm welcome to all the distinguished delegates and all beloved participants present here at the Anandaram Dhekial Phookan College (A.D.P. College), Nagaon, Assam to participate in the National Seminar on "Recent Advances in Medicinal Plants Research of North East India" (RAMPR-NE 2016) organized by the Department of Herbal Science and Technology on 11<sup>th</sup> and 12<sup>th</sup> November 2016. The objective of the Seminar was to create awareness of documentation, conservation and utilization of plant resources for sustainable economic development and promotion of eco-friendly traditional and herbal products.

Therefore we the members of ADP College have come together to hold this Seminar and created a common platform to academicians, scientists, research scholars and students to share and disseminate information and knowledge of scientific research works related to Plant Science and to inform the scientific community that ADP College has started Herbal Science and Technology teaching and research programmes.

The Seminar is broadly encompassing the following sub-themes: Ethnomedicine, Traditional knowledge, Pharmacognosy, Pharmacology, Phytochemistry, Herbal Drug Formulations, Biotechnology, Nanotechnology, Biodiversity & Conservation, Natural Product Research and IPR related to Plant Science.

The seminar was inaugurated by Prof. R.C. Borah, Registrar of Mahapurusha Srimanta Sankardeva Viswavidyalaya (MSSV), Chief Guest for the occasion and the inaugural function was presided over by Dr. Sariff Uddin Ahmed, Principal of ADP College. Prof. PJ Handique, Department of Biotechnology, Gauhati University, delivered the Keynote Address followed by a plenary lecture highlighting the conspectus on Biodiversity, Bio-prospecting and Bio-piracy of Medicinal Plants of North East India.

The technical sessions were preceded by invited talks by eminent persons in the concerned area. The persons who delivered invited talks include Prof. Mohan Chandra Kalita, Department of Biotechnology, Gauhati University, Prof. Bishnu Prasad Sarma, Govt. Ayurvedic College and Dr. Ananad Ramteke, Associate Professor, Department of Molecular Biology and Biotechnology, Tezpur University. Prof. Sarma chaired a session and discussed the Potential of Herbs in the management of Type 2 Diabetes. Dr. Ramteke delivered a thematic presentation on Chemopreventive and Anticancer Potential of Medicinal Plants from North East India. Prof. Kalita opined on the various issues of Biodiversity and Sustainable management of Ethnomedicinal plants and Traditional Knowledge. He also left his view regarding preservation of germplasm of our endangered and threatened species of flora and fauna of our country. A total number of 41 papers were presented in the technical session including a few poster presentations.

This seminar could not have been successful without the generous support from UGC, Govt. of India. The Organizing Committee expresses its gratitude to the authors and reviewers for their valuable contribution.

My sincere thanks to every member of the committee for making this seminar successful and a memorable event.

The views expressed and the materials presented in this publication are those of the author(s). Steps are being taken to avoid mistakes in the proceedings; however, some mistakes still may remain for which I tender my apology to all concerned.

With best wishes,

**(Dr. Mousmi Saikia)**  
Organizing Secretary  
National Seminar, RAMPR-NE 2016 &  
Assist. Prof. & Head  
Deptt. of Herbal Science & Technology  
ADP College, Nagaon-782002  
e-mail : mousmi3@rediffmail.com  
hstadp@gmail.com

## **ACKNOWLEDGEMENT**

Our special thanks and appreciation goes to the contributors who have enriched this proceeding book. We wish to express our sincere gratitude to Dr. S.U. Ahmed, Principal, ADP College, for his continuous monitoring from the day of submitting proposal to UGC till the day of publication of the proceeding.

We duly acknowledge the peerless services from the concerned personalities and offer its best regards to Hon'ble Secretary, UGC, NERO, Guwahati, honorable distinguished guest, respected participants and respected colleagues for their help during the seminar.

We must convey our special thanks to the PG students of 1st & 3rd semester M.Sc. Herbal Science & Technology and Mr. Upakul Bora (office Bearer) who took the responsibility in every respect to make the seminar a successful one.

We are also thankful to all who helped us in any way to make the seminar a grand success. Our thanks also goes to the proprietor and workers of Krantikaal Prakashan who took the responsibility of printing the book within a very short time.

**Editorial Board**

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# Antioxidant, cytotoxic and protective activities of *Nyctanthes arbor-tristis* Linn

Anowar Hussain<sup>1</sup>, Pitambar Baishya<sup>1</sup>, †, Monoj Kumar Das<sup>1</sup>,  
†, AshuBhan Tikku<sup>2</sup>, \*and Anand Ramteke<sup>1</sup>, \*

<sup>1</sup>Cancer Genetics and Chemoprevention Research Group, Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur-28, Assam, India

<sup>2</sup>Radiation and Cancer Therapeutics Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi-67, India

Email: anand@tezu.ernet.in

Email: abtiku@mail.jnu.ac.in

† Contributed equally

## Abstract

In the present study, we investigated antioxidant, cytotoxic and protective activities of hydroalcoholic flower extract of *Nyctanthes arbor-tristis* Linn. (FENA), a traditionally used medicinal plant of India. The antioxidant activity of FENA were determined using in vitro chemical based reactions. Cytotoxic activity of FENA was investigated against human peripheral blood mononuclear cells (PBMCs) and prostate cancer cell line PC3. Protective activity of FENA was investigated against triton-x induced membrane damage and H<sub>2</sub>O<sub>2</sub> induced oxidative stress using human erythrocyte and PBMCs respectively. The results indicated that FENA scavenged 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), superoxide, hydroxyl radicals and inhibited Fe<sup>2+</sup> chelation with IC<sub>50</sub> value of 15.46 ± 0.28, 82.92 ± 4.40, 17.48 ± 0.48, 271.84 ± 23.65 and 168.96 ± 4.28 µg/ml respectively. FENA was also found to increase the viability of PBMCs and exhibited protective activity against triton-X induced membrane damage and H<sub>2</sub>O<sub>2</sub> induced oxidative stress. Phytochemical study revealed the presence of 71.20 ± 0.60µg gallic acid equivalent (GAE) phenolics and 520.88 ± 16.19µg epicatechin equivalent (ECE) flavonoids per mg FENA. These findings highlight that FENA could be considered as potential source of antioxidant and hence, might find application in food and pharmaceutical industries.

**Keywords:** *Nyctanthes arbor-tristis*, antioxidant, cytotoxicity, membrane stability, oxidative stress, phenolics and flavonoids.

## Introduction

Oxidative stress, generated as a result of imbalance between free radicals and cellular antioxidants has been recognized as the root cause of several degenerative diseases such as cardiovascular disease, cancer, neurodegenerative diseases, alzheimer, inflammation etc. Boosting of endogenous antioxidant defenses and/or administration of exogenous antioxidant has become salutary to combat the harmful consequences of free radicals (Lobo et al., 2010; Krishnaiah et al., 2011; Kasote et al., 2015). The synthetic antioxidants currently being used such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT) have been reported to cause liver damage and induce carcinogenesis (Krishnaiah et al., 2011). Therefore, antioxidants of natural origin have gained importance as an alternative to synthetic one. Also the inverse relationship between dietary intake of fruits and vegetables rich in antioxidants and incidence of human disease have attracted considerable attention for their use (Lobo et al., 2010; Wang et al., 2014). Recently, several herbs and spices have been identified as sources of antioxidants, but, none of the antioxidants were found to be effective (Pandey and Rizvi, 2009; Abbasi et al., 2015). Therefore, identification of medicinal plant and isolation of phytochemicals with antioxidant gained considerable attention in recent past.

*Nyctanthes arbor-tristis* Linn.,(Oleaceae), commonly known as night-flowering Jasmine or parijatha (in Hindi) is a well-known medicinal plant distributed in tropical and sub-tropical regions of south and south-east Asia. Different parts of this plant are extensively used in Ayurveda, Siddha-Ayurveda and Unani systems of medicine as alternative therapeutics to treat indigestion, as laxative, antidote, bitter tonic etc. (Agarwal and Puri, 2013; Bansal et al., 2015). The decoction prepared from leaves have been used to treat obstinate sciatica, malaria, intestinal worms etc. (Mishra et al., 2016); while the flowers are used as stomachic, carminative, astringent to bowel, antibilious, expectorant, hair tonic and in the treatment of piles and various skin diseases (Wang et al., 2013).

Modern pharmacological studies have found that the leaves possess anti-inflammatory (Saxena et al., 1984), analgesic, antipyretic (Saxena et al., 1987), immunostimulant (Puri et al., 1994), antispasmodic (Kumari et al., 2012), antimalarial activity (Kumari et al., 2012). Ghosh et al., (2015) isolated a carbohydrate polymer from the leaves and demonstrated their antitussive activity. The flowers have been proven to exhibit antibacterial and cytotoxic (Khatune et al., 2001), hypoglycemic and hypolipidemic (Rangika et al., 2015) and sedative activity (Ratnasooriya et al., 2005). Kakoti et al., (2013) demonstrated potent analgesic and anti-inflammatory activities of the stem bark. Despite tremendous medicinal properties, very few studies have reported antioxidant activity of this important plant. A study by Michael et al., (2013) indicated potent antioxidant activity of leaf extract (Michael et al., 2013). Ghose et al., (2015) isolated a water soluble fraction from the leaves that have significant radical scavenging

activities. Besides these, there are no reports available on the antioxidant activity of this valuable plant. Therefore, the present study was undertaken to evaluate the antioxidant and protective activity of the flower extract and to correlate with cytotoxicity and phytochemicals present. As the flowers are used as ingredients in various food preparations, the outcome of this study would be of great value for use in pharmaceutical industries.

## **Materials and methods**

### **Analytical chemicals**

2,2-diphenyl-1-picrylhydrazyl (DPPH), butylatedhydroxyanisole (BHA), nitrobluetetrazolium chloride (NBT), 2- deoxyribose, ferrozine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), trichloroacetic acid (TCA) 2-thiobarbituric acid (TBA), ascorbic acid, gallic acid, ethylenediaminetetraacetic acid (EDTA), HiSep LSM 1077, RPMI-1640, fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) etc. were purchased from HiMedia Laboratories (Mumbai, India). The rest of the chemicals like dimethylsulfoxide (DMSO), bovine serum albumin (BSA),folin-ciocalteu reagent (FCR), triton-X, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) etc. were of analytical grade, obtained from local firms of India.

### **Sample collection and preparation of extracts**

*N. arbor-tristis* flowers were collected from Baihata Chariali, Assam (India) and identified at Botanical Survey of India (BSI), Eastern Regional Centre, Shillong, Meghalaya (India) (BSI/ERC/Tech./Plant Iden./2015/306). The collected flower samples were rinsed in distilled water, dried in shade and grinded into powder form. The extract was prepared by cold maceration of powdered form of flower samples in hydroalcohol (80% ethanol v/v) for 7 days. The solvent was allowed to evaporate and the extracts thus obtained (FENA) were stored at -20°C until used.

### **Determination of antioxidant activities**

#### **ABTS radical scavenging assay**

Total antioxidant activity of FENA was determined by ABTS radical scavenging assay following the method of Arnao et al. (2001) with some modifications. The working solution was prepared by reacting the stock ABTS (7.4 mM) with potassium persulfate (2.6 mM) for 12 hours at room temperature in dark. The working solution was then diluted with methanol to an OD equivalent to  $1.100 \pm 0.02$  at 734 nm and mixed with various concentrations of FENA or BHA. After 2 hours of incubation in dark, the absorbance was measured at 734 nm using the spectrophotometer. The total antioxidant activity was calculated in terms of % scavenging of ABTS\*+ radical using the following equation:

$$\% \text{ABTS radical scavenging activity} = (\text{Abs control} - \text{Abs test sample}) / (\text{Abs control}) \times 100$$

### **DPPH radical scavenging activity**

DPPH radical scavenging activity of the FENA was determined according to the method as described by Kitts et al. (2000) and Shahidi et al. (2007) with slight modification. Briefly, DPPH solution of 0.135 mM in methanol was mixed with various concentrations of FENA or BHA in methanol in a total volume of 1 ml and vortexed thoroughly. After 30 minutes of incubation in dark, the absorbance of the reaction mixtures were measured at 517 nm. The scavenging activity was calculated using the following equation:

$$\% \text{ DPPH radical scavenging activity} = \frac{\text{Abs control} - \text{Abs test sample}}{\text{Abs control}} \times 100$$

### **Superoxide radical scavenging activity**

The superoxide radical scavenging activity was determined by alkaline DMSO method as described by Srinivasan et al. (2007) and Kumara et al. (2012). Briefly, NBT solution of 0.1 ml (1 mg/ml) was added to the reaction mixture which contains 1 ml of alkaline DMSO (1 ml DMSO containing 5 mM NaOH in 0.1 mL water) and 0.3 mL of the different concentrations of FENA or ascorbic acid (AA) in DMSO. The absorbance was measured at 560 nm against reagent blank consisting of DMSO. The scavenging activity was calculated using the following equation:

$$\% \text{ Superoxide radical scavenging activity} = \frac{\text{Abs control} - \text{Abs test sample}}{\text{Abs control}} \times 100$$

### **Hydroxyl radical scavenging activity**

The hydroxyl radical scavenging activity was determined based on quantification of the degradation product of 2-deoxyribose by condensation with TBA as originally described by Halliwell and Gutteridge (1981). In the reaction system, hydroxyl radical was generated by the Fenton reaction ( $\text{Fe}^{3+}$ -ascorbate-EDTA- $\text{H}_2\text{O}_2$  system). The reaction mixture (1 ml) contained 20 mM  $\text{FeCl}_3$ , 0.1 mM EDTA, 0.28 mM 2-deoxyribose, 0.2 mM  $\text{H}_2\text{O}_2$ , 0.3 mM ascorbic acid and various concentration of FENA or gallic acid. After 1 hour incubation at 37°C, 1 ml TBA (1 %, prepared in 50 mM NaOH) and 1 ml TCA (2.8 %, prepared in Milli Q water) was added and heated for 15 minutes on boiling water bath at 80°C. After cooling, absorbance was taken at 532 nm against reagent blank and the percentage inhibition was calculated using the following equation:

$$\% \text{ Hydroxyl radical scavenging activity} = \frac{\text{Abs control} - \text{Abs test sample}}{\text{Abs control}} \times 100$$

### **Fe<sup>2+</sup> chelation inhibitory activity**

The Fe<sup>2+</sup> chelation inhibitory activity of the extract was determined according to the method described by Dinis et al. (1994) with slight modifications. Different concentrations of the FENA or EDTA were added to a solution of 1 mM FeCl<sub>2</sub> (0.05 ml) and the reaction was started by adding 1 mM ferrozine (0.1 ml). The volume of the reactions was adjusted to 1 ml with methanol, shaken vigorously and left standing at room temperature for 10 minutes. After the mixture reached equilibrium, the absorbance of the solution was measured at 562 nm using spectrophotometer against blank consisting of methanol and MilliQ water. The inhibition of chromogen formation was calculated from the following equation:

$$\% \text{ Inhibition of Fe}^{2+} = \frac{\text{Abs control} - \text{Abs test sample}}{\text{Abs control}} \times 100$$

### **Reducing power assay**

The reducing power of FENA was determined according to the method of Zhu et al. (2011) with slight modification. To the reaction mixture containing equal volume of potassium buffer (0.2 M, pH 6.6) and various concentrations of FENA or ascorbic acid, 0.5 ml of potassium ferricyanide (1%) was added and incubated for 20 minutes at 50°C. After incubation, 0.1 ml TCA (10%) was added and centrifuged at 3000 rpm for 10 minutes. The supernatant was mixed with 0.5 ml MilliQ water and 0.5 ml Ferric Chloride (0.1%) and mixed thoroughly. The absorbance was measured spectrophotometrically at 700 nm against blanks consisting of all reagents except extract or standard.

### **Evaluation of cytotoxic activity**

The cytotoxic activity of FENA was evaluated by MTT assay using human peripheral blood mononuclear cells (PBMCs) and prostate cancer cell line PC3 (National Centre for Cell Sciences, Pune, India).

### **Isolation of PBMCs**

The PBMCs were isolated from human blood, collected voluntarily using HiSep LSM-1077 according to manufacturer protocol. Briefly, anticoagulated blood was diluted (1:1) with phosphate buffered saline (PBS) (pH 7.4) and layered on the top of HiSep, centrifuged at 700g for 30 minutes at room temperature and middle buffy layers containing the PBMCs were collected. The isolated PBMCs were washed with PBS and RPMI and viability was checked by trypan blue exclusion method using haemocytometer. Only the batches of isolation that have viable cells more than 95 % were used for subsequent studies.

### **Cell culture and treatment**

Isolated PBMCs were seeded at a density of  $\sim 5 \times 10^3$  cells/well in 96-well plate and grown for 6 hours in RPMI supplemented with 10% fetal bovine serum and 1% Pen Strep. Whereas the PC3 cells were seeded at a density of  $\sim 1 \times 10^3$  cells/well in 96-well plate and grown overnight in RPMI supplemented with 10% heat inactivated fetal bovine serum and 1% PenStrep. The cells were subsequently treated with FENA (12.5-200 $\mu$ g/ml) dissolved in DMSO and maintained at 37°C and 5% CO<sub>2</sub> in an incubator for 24 hours.

### **MTT assay**

At the end of treatment period, the cells were treated with 10% MTT dissolved in complete media for 3 hours. The formazan crystals formed in PBMCs were dissolved in acidified isopropanol, whereas in PC3, they were dissolved in DMSO after discarding the treatment media and the optical density was measured at 570 nm and 690 nm. The % cell viability was calculated considering the untreated as 100 % viable.

### **Evaluation of protective activity**

#### **Membrane stability assay**

The membrane stability assay was performed to evaluate the protective activity of FENA against triton-X induced membrane lysis of human erythrocytes as described by Powel et al. (2000) with minor modifications. Briefly, the erythrocytes, collected during isolation of PBMCs were suspended in PBS, treated with various concentrations of FENA and/or triton-X (0.01%) and incubated at 37°C for 35 minutes. After incubation, the reaction tubes were agitated slowly (10 minutes), cooled for 5 minutes and centrifuged at 6500 rpm. The absorbance of the clear supernatant was measured at 576 nm using spectrophotometer and the % erythrolysis was calculated by considering the triton-X treatment as 100% lyse.

#### **Oxidative stress protective assay**

Protective activity of FENA against oxidative stress induced by H<sub>2</sub>O<sub>2</sub> was evaluated by measuring the viability of human PBMC cultured in vitro, as described by Ramteke et al (2012). Briefly, the isolated PBMCs were pre-treated with various concentrations of FENA for 6 hours followed by 0.5% H<sub>2</sub>O<sub>2</sub> treatment for 24 hours. The viability of PBMCs were evaluated by MTT based assay as described earlier.

#### **Phytochemical analysis**

##### **Qualitative screening**

The FENA was subjected to preliminary phytochemical screening with the battery of chemical tests for the detection of organic constituents present. Small amounts of

extract were dissolved in appropriate solvent and following tests were performed: ferric chloride and alkaline test for phenolics, Shinoda test for flavonoid, Wagner test for alkaloids, Borntrager's test for glycosides and Salkowski and Liebermann-Burchard's reaction for steroids (Kokate, 1994; Khandelwal, 2005).

### **Spectral analysis**

The UV-VIS and FTIR spectral analysis were performed to detect the characteristic peaks of phytochemicals present in the FENA. Briefly, the FENA dissolved in ethanol (1 mg/ml) was scanned using spectrophotometer in the wavelength ranging from 200 to 800 nm. For FTIR analysis lyophilized powder of plant material (NAF) and FENA were used. The analysis was performed on a potassium bromide disc using FTIR spectrophotometer (Spectrum-100, Perkin Elmer, Singapore) in the spectral range 4000-400  $\text{cm}^{-1}$ .

### **Estimation of phenolic and flavonoid content**

The content of total phenolics was determined using Folin-ciocalteu reagent (FCR) as described by Singlet and Rosi (1965) with minor modification. Briefly 400  $\mu\text{l}$  of  $\text{Na}_2\text{CO}_3$  solution was added to a mixture of FENA and 500  $\mu\text{l}$  of FCR (10 fold diluted) and incubated at 22°C for 2 hours. The absorbance was measured at 725 nm. The phenolic content was calculated from gallic acid standard curve and finally expressed as  $\mu\text{g}$  gallic acid equivalent (GAE) per mg FENA.

The content of total flavonoids was estimated according to the method as described by Zhishen et al. (1999) with slight modification. Briefly, the extract was mixed with 1.5% of  $\text{NaNO}_2$  after 5 minutes of which aluminium chloride ( $\text{AlCl}_3$ ) was added. Thereafter, 200 mM NaOH was added and the absorbance was measured at 510 nm. The phenolic content was calculated from epicatechin standard curve and finally expressed as  $\mu\text{g}$  epicatechin equivalent (ECE) per mg extract.

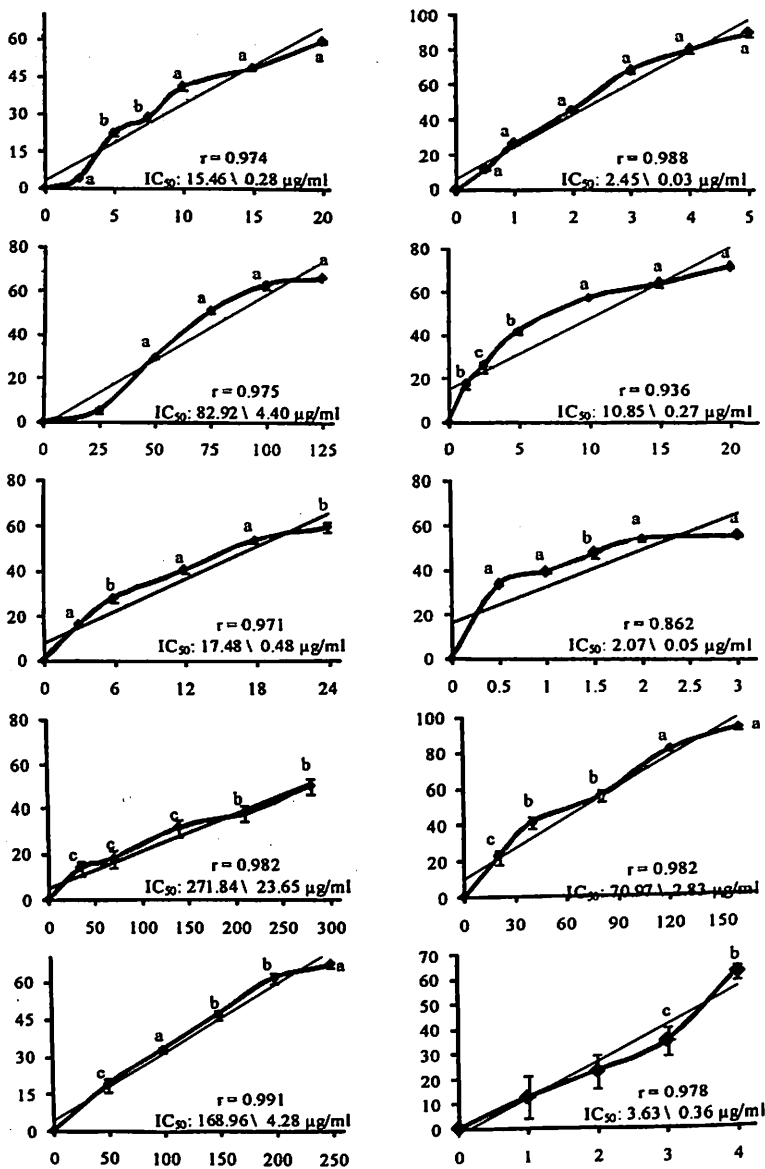
### **Statistical analysis**

All the results were expressed as Mean  $\pm$  sem (n=3). Results were statistically analyzed using ANOVA, and a value of  $p \leq 0.001$ ,  $p \leq 0.01$  and  $p \leq 0.05$  were considered significant. The analysis was carried out using GraphPad Prism 7.0 (GraphPad Software, Inc., CA, USA).

### **Results and discussion**

In this work, antioxidant, cytotoxic and protective activities of flower extract of *N. arbor-tristis* (FENA) were studied. The antioxidant activity of FENA was evaluated employing chemical based reactions, whereas cytotoxic activity of FENA was assessed by measuring the viability of human PBMCs and prostate cancer cell line PC<sub>3</sub>. Protective

activity of FENA was evaluated against triton-X induced membrane damage and H<sub>2</sub>O<sub>2</sub> induced oxidative stress using human erythrocytes and PBMCs respectively. Qualitative, quantitative and spectral analysis were performed to detect the phytochemicals present in FENA.



**Fig. 1.** Antioxidant activity of FENA and standard. A) ABTS radical scavenging activity, B) DPPH radical scavenging activity, C) Superoxide radical scavenging activity, D) Hydroxyl radical scavenging activity and E) Fe<sup>2+</sup> chelation inhibitory activities were evaluated by *in vitro* cell free chemical based reactions as described in 'Material and methods'. Values are mean \ sem; n=3; <sup>a</sup>p=0.001, <sup>b</sup>p=0.01 and <sup>c</sup>p=0.05 compared to control.

### **FENA exerts promising antioxidant activity**

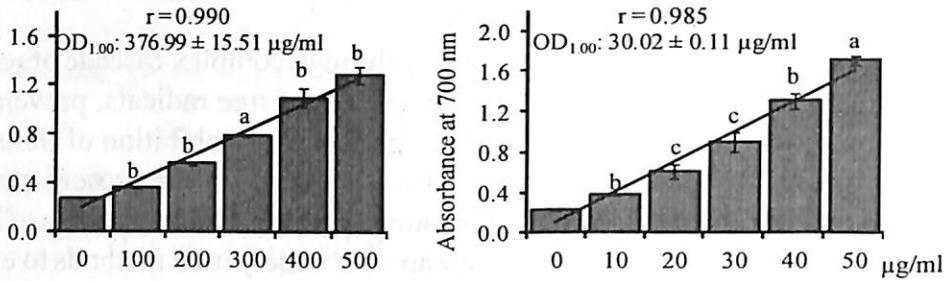
Antioxidants are known to exert their activity through complex cascade of reactions such as prevention of hydrogen abstraction, scavenging of free radicals, prevention of chain initiation, peroxide decomposition, reducing capability, inhibition of transitional metal ion chelation etc (Ud-Daulaa et al., 2016). Therefore, in the present study, we have used several chemical based assays to measure the antioxidant functions of FENA. The ABTS and DPPH radical scavenging assays are two widely used methods to evaluate the antioxidant activity in vitro. Both the synthetic free radicals ABTS<sup>•+</sup> and DPPH accept electron or hydrogen from antioxidants and become stable radicals (Hazra et al., 2008; Aksoy et al., 2013). In the present study, we have observed dose dependent increase in the ABTS and DPPH radical scavenging activity of FENA with IC<sub>50</sub> value 15.46 ± 0.28 and 82.92 ± 4.40 µg/ml respectively; whereas, the standard BHA showed similar activity with IC<sub>50</sub> value 2.45 ± 0.03 and 10.85 ± 0.27 µg/ml respectively [Fig. 1(A,B)].

The superoxide anionic radicals, generated as a by-product of mitochondrial respiration and fatty acid oxidation, are known to be very harmful to the cellular components as it can result into hydrogen peroxide, hydroxyl radical (OH<sup>•</sup>), singlet oxygen (O<sup>-</sup>), peroxyxynitrite (ONOO<sup>•</sup>) etc. (Hazra et al., 2008; Srinivasan et al., 2007; Kumara et al., 2012). The present study revealed that FENA scavenge superoxide radicals in a dose dependent manner with IC<sub>50</sub> value 17.48 ± 0.48 µg/ml [Fig. 1(C)]. The hydroxyl radical (OH<sup>•</sup>) is one of the major active oxygen species that cause lipid peroxidation and exerts various biological damages such as DNA damage. As such prevention of OH<sup>•</sup> generation and/or inhibition of chain reaction initiated by OH<sup>•</sup> is one of the approaches in antioxidant mechanism (Lipinski, 2011). Results of the present study indicated dose dependent scavenging of OH<sup>•</sup> radical with IC<sub>50</sub> value 271.84 ± 23.65 µg/ml [Fig. 1(D)].

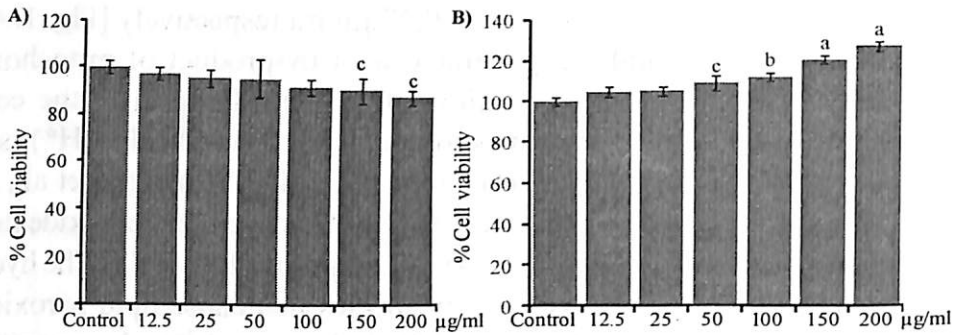
Transitional metal such as iron stimulates lipid peroxidation through Fenton reaction and accelerates lipid peroxidation by converting lipid hydroperoxides into peroxy and alkoxy radical which perpetuate chain reaction (Halliwell, 1991; Duh et al., 1999). Here, we observed dose dependent inhibition of Fe<sup>2+</sup> chelation by FENA with IC<sub>50</sub> 168.96 ± 4.28 µg/ml [Fig. 1(E)]. The reducing potential of substances from Fe<sup>3+</sup> to Fe<sup>2+</sup> is a significant indicator of antioxidant activity, as it exerts antioxidant activity by preventing free radical chain initiation by donating hydrogen atom, decomposing peroxides and scavenging free radical (Yildirim et al., 2000). In the present study, FENA exhibited dose dependent reducing potentials with OD 1.00 at 376.99 ± 15.51 µg/ml of FENA [Fig. 2].

### **FENA exhibits differential cytotoxic activity**

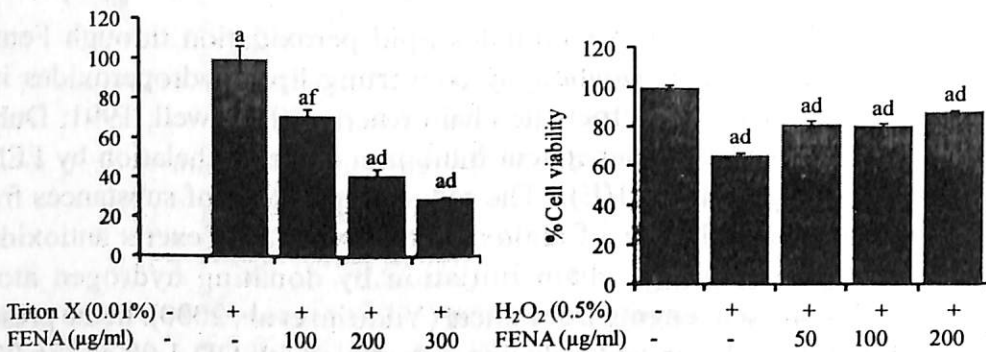
The promising in vitro antioxidant activity of FENA encouraged us to study their effects in biological system. Therefore, we evaluated the cytotoxic effects of FENA on



**Fig. 2. Reducing power activity of FENA and standard.** The reducing power activity of FENA and standard were evaluated by *in vitro* cell free chemical based reactions as described in 'Material and methods'. Values are mean ± sem; n=3; <sup>a</sup>p≤0.001, <sup>b</sup>p≤0.01 and <sup>c</sup>p≤0.05 compared to control.



**Fig. 3. Cytotoxic effect of FENA.** The cytotoxic effect of FENA was evaluated against human prostate cancer cell line (PC3) and peripheral blood mononuclear cell (PBMC) by MTT assay as described in 'Material and methods'. Values are mean ± sem; n=3; <sup>a</sup>p≤0.001, <sup>b</sup>p≤0.01 and <sup>c</sup>p≤0.05 compared to control.



**Fig. 4. Protective effects of FENA.** The protective effects of FENA was evaluated against A) triton-X and B) H<sub>2</sub>O<sub>2</sub> induced cellular damages using human erythrocytes and peripheral blood mononuclear cell (PBMC) respectively, as described in 'Material and methods'. Values are mean ± sem; n=3; <sup>a</sup>p≤0.001, <sup>b</sup>p≤0.01 and <sup>c</sup>p≤0.05 compared to control. sem; n=3; <sup>d</sup>p≤0.001, <sup>e</sup>p≤0.01 and <sup>f</sup>p≤0.05 compared to triton-X or H<sub>2</sub>O<sub>2</sub> treatment.

human PBMCs and PC3 cells by MTT based assay. Finding of our study demonstrated that FENA decreases the viability of PC3 cells in a dose dependent manner and at 200 µg/ml, the viability is reduced by 13% ( $p \leq 0.05$ ). Interestingly, the viability of PBMCs were increased upon treatment with FENA by 5-27% and maximum increase was observed at 200 µg/ml of FENA (by 27%,  $p \leq 0.001$ ) [Fig. 3]. The selective cytotoxic effect of FENA might be due to differential physiology of normal and cancer cells and hence might find application in antioxidant therapy without causing side effects to the normal cells (Pittella et al., 2009).

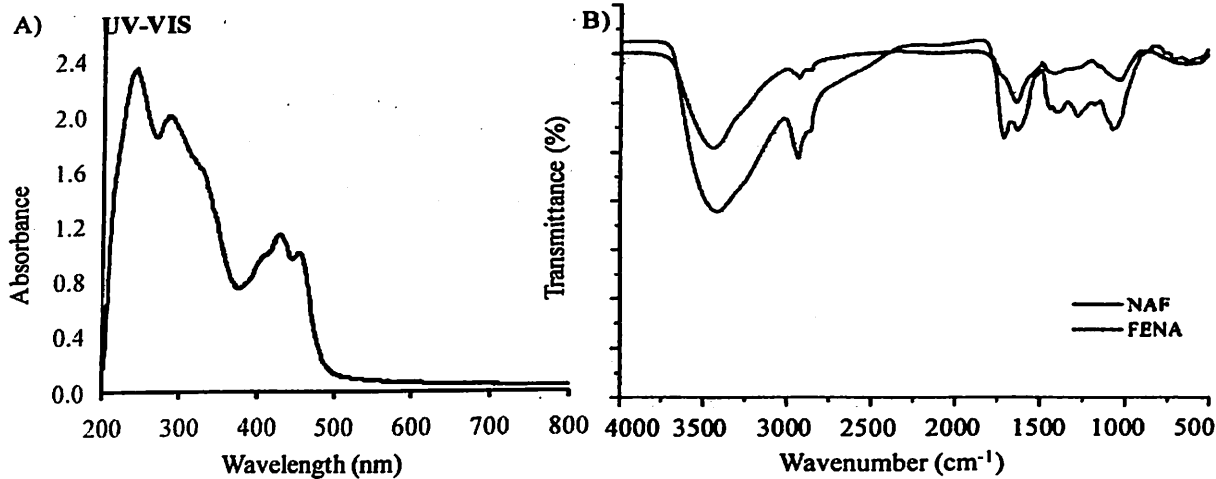
### **FENA confers protective activity**

Healthy cellular environment is primarily dependent on the integrity of the cell membrane. Exposure of cells to injurious substances such as triton-X leads to cytolysis through membrane rupture, rendering the cell more prone to secondary damages induced by free radicals (Gadgoli and Shelke, 2010). Therefore, in the present study, we evaluated the protective activity of FENA against triton-x induced membrane damage. The results obtained indicate that FENA can significantly inhibit the triton-X induced erythrolysis upto 31% ( $p \leq 0.001$ ) [Fig. 4(A)]. Similar magnitude of membrane protective activity of methanolic extract of *N. arbor-tristis* and crocetin, a carotenoid, against hypotonic solution was observed by Gadgoli and Shelke (2010). As the membrane of erythrocytes are similar to the lysosomal membrane, the potent protective activity of FENA can also be used to prevent the release of inflammatory mediators in the early phase of inflammation (Amrite et al., 2006; Gadgoli and Shelke, 2010).

Hydrogen peroxide ( $H_2O_2$ ), an oxidizing agent is known to cause oxidative damage to the cells. There is evidence of decrease in the viability of cells and release of lactate dehydrogenase to the exterior of cell, treated with  $H_2O_2$  accompanied by decrease in cellular antioxidants (Ramteke et al., 2012; Hussain and Ramteke, 2012). Here, in the present study, we observed that PBMCs pretreated with FENA for 6 hours followed by  $H_2O_2$  treatment (24 hours) increases the viability upto 87% ( $p \leq 0.001$ ) as compared to only  $H_2O_2$  treated PBMCs where viability was only 66% ( $p \leq 0.001$ ) [Fig. 4(B)]. This increase in the viability of cells might be due to increase in cellular antioxidant level and/or membrane stabilizing activity.

### **FENA is rich in phenolic and flavonoid content**

Qualitative chemical based reactions were performed to detect the group of phytochemicals present in FENA. As shown in Table 1, FENA contains several group of secondary metabolites such as phenolics, flavonoids, glycosides, phytosterols etc. To confirm the qualitative test, we performed spectral analysis of FENA in UV-VIS and FTIR range. As shown in Fig. 5(A,B), we observed the presence of sharp peaks of several groups of phytochemicals. The peaks of FTIR in the range of 3640-3610, 3500-3200, 1600-1585, 1500-1400, 1335-1250 and 900-675  $cm^{-1}$  confirmed the presence of



UV-VIS spectra		FTIR spectra		
Wavelength (nm)	Absorption	Frequency (cm <sup>-1</sup> )	Bond	Functional group
241	2.339	3640-3610	O-H stretch, free hydroxyl	Alcohols, phenols
285	2.000	3500-3200	O-H stretch, H-bonded	
327	1.605	2830-2695	H-C=O: C-H stretch	Aldehydes
408	0.968	1600-1585	C-C stretch (in-ring)	Aromatics
429	1.128	1500-1400		
455	0.994	1360-1290	N-O symmetric stretch	Nitro compounds
		1335-1250	C-N stretch	Aromatic amines
		900-675	C-H "oop"	Aromatics

**Fig. 5. Phytochemical analysis of FENA.** The phytochemicals present in FENA was evaluated qualitatively by A) UV-VIS B) FTIR spectral analysis. The distinctive peaks of UV-VIS and FTIR spectra and functional groups of the phytochemicals present in FENA are shown in tabular form (C).

phenolic compounds and flavonoids in the FENA [Fig. 5(C)] (Stuart, 1997; Dumas and Miller, 2003; D'souza et al., 2008). Quantitative estimation revealed that  $71.20 \pm 0.60 \mu\text{g}$  gallic acid equivalent (GAE) phenolics and  $520.88 \pm 16.19 \mu\text{g}$  epicatechin equivalent (ECE) flavonoids were present per mg FENA.

## Conclusion

In conclusion, the results suggest that the high amount of phenolic and flavonoid compounds might be responsible for the observed antioxidant and protective activity. As the flower extract is non-toxic to humans it can be exploited as a good source of antioxidant in the food and pharmaceutical industries. However, extensive investigation is needed to corroborate our findings along with toxicological profiling and isolation and characterization of active principles responsible thereof.

## Acknowledgment

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**Conflict of interest:** The authors declare that they have no competing interests.

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# Medicinal Plants of North East India: A conspectus on Biodiversity, Bioprospecting and Biopiracy

**Pratap Jyoti Handique**

Department of Biotechnology, Gauhati University, Guwahati-781014

Email: [pjhandique@rediffmail.com](mailto:pjhandique@rediffmail.com)

Phone: 9435012920

## **Abstract**

North Eastern region of India embracing the states of Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Tripura and Sikkim is very much rich in medicinal plant populations. This region is one of the richest repositories of medicinal and aromatic plants in the world due to its unique topography, high rainfall and various vegetation types. This region houses around 1800 species of medicinal plants including several rare, endangered and endemic species, which are being used in ethno-medicinal and modern medicinal practices. Around 50 plant species grown wild in NE India are exploited in large quantities for preparation of ayurvedic and modern medicine including healthcare products. About 26 species of medicinal plants out of 32 species prioritized for cultivation and trade in India are found wild and semi-cultivated condition in these states. However, the biodiversity of the medicinal plants species are not fully explored and evaluated till today. On the other hand, bioprospecting of medicinal plants for novel and important phytochemical is now a global issue. Screening for elite clones and identification of diverse plant genetic resources using biotechnological tools are receiving momentum along with phytochemical investigations. Attempts are being made at various levels in various places for screening and assessment of valuable wild medicinal plants. However, such attempts are very much limited in North Eastern region of India. Another problem in the area is biopiracy. Biopiracy is a situation where indigenous knowledge of nature is exploited for commercial gain with no compensation to the indigenous people themselves. Traditional knowledge (TK) has always been an easily accessible treasure and thus has been susceptible to misuse. The TK, particularly, related to the treatment of various diseases of human has provided leads for development of biologically active molecules by the technology rich countries. Moreover, TK is often misappropriated,

because it is conveniently assumed that since it is in public domain, communities have given up all claims over it. The instances of biopiracy related to medicinal plants are available NE India. The present paper gives a conspectus of scientific work on these three aspects viz, biodiversity, bioprospecting and biopiracy of medicinal plants of NE India. The paper is based on field investigation and analysis of available published and unpublished information.

**Key words:** Biodiversity, Bioprospecting, Biopiracy, Medicinal plants, North-East, Ethnomedicinal.

## **Introduction**

North East India is a biodiversity rich region falls under Indo-Burma and Himalaya biodiversity hot-spots. All the eight political states of this region namely Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Tripura and Sikkim are equally rich in plant diversity. The inhabitants of this region have long tradition of herbal medicinal practices which contributed to identify a large number of plant species as medicinal plants as well as medicinal sources. It is well known that the plants are one of the most significant sources of medicine. The application of various plants as medicine dates back to prehistoric period. Over six thousand years ago, the ancient Chinese were the first to use the natural vegetation as medicine. In India, the Ayurvedic system of medicine has been for over three thousand years. A rich heritage of knowledge in curative and preventive plant based medicine was available in ancient Indian scholastic works like the Atharva Veda, Charaka and Sushruta.

An estimate suggests that 13,000 plant species are known to have worldwide use as drugs. Twenty five thousand plant species are known for their medicinal uses, phytochemical tests have been performed in about 5000, and nearly 1100 species have been extensively exploited in Ayurvedic, Unani and Allopathic medicines. It is reported that almost forty one percent of the prescriptions in the USA and fifty percent in Europe contains constituents from natural products. This trend of using natural products is rising visibly.

In recent years scientific investigation on medicinal plants is gaining momentum. Systematic efforts are seen to take place not only for botanical identification of important medicinal plants but to extract and characterize active principles, selection of elites for better production of important biomolecules, in vitro culture of plant and plant parts for production of phytochemical, and clinical trials of the medicinal plant products. These investigations are collectively termed as 'bioprospecting'. However, such investigations are still in infancy in North East India though this region houses a large number of important medicinal plants and a time tested tradition of herbal practices. This write up emphasizes the importance of bioprospecting of rich medicinal plant diversity of NE India.

Another problem in the area is biopiracy. Biopiracy is a situation where indigenous

knowledge of nature is exploited for commercial gain without any compensation to the indigenous people who the owner of the knowledge system. Traditional knowledge (TK) has always been an easily accessible treasure and thus has been susceptible to misuse. The TK, particularly, related to the treatment of various diseases has provided leads for development of biologically active molecules by the technology rich countries. Moreover, TK is often misappropriated, because it is conveniently assumed that since it is in public domain, communities have given up all claims over it. The instances of biopiracy related to medicinal plants are available NE India. The present paper gives a overview of scientific work on these three aspects viz, biodiversity, bioprospecting and biopiracy of medicinal plants of NE India. The paper is mainly based on field investigation and analysis of available published and unpublished information

### **Medicinal plant diversity of NE India**

North Eastern Region of India is a treasure house of medicinal plants. It is to be noted that this region is inhabited by the largest number of tribes (220 nos.) in India. Most of the tribes lead an intricate life totally dependent on the forest plants. This region houses around 1800 species of medicinal plants including several rare, endangered and endemic species, which are being used in ethno-medicinal (i.e. traditional or folk) and modern medicinal practices (Table 1). Around 50 plant species grown wild in NE India are exploited in large quantities for preparation of ayurvedic and modern medicine including health care products. On the other hand about 26 species of medicinal plants out of 32 species prioritized for cultivation and trade by the National Medicinal Plant Board (NMPB) are found wild and semi-cultivated condition in these states. One of the important characters of the medicinal plant population in NE India is the existence of several ecotypes of individual species. Laboratory investigations revealed variations among the ecotypes in the production of secondary metabolites.

Till the recent past all the states of NE India were fairly wooded with thick vegetation all round. But with the acceleration in urbanization and as a result of bringing greater hilly forest areas under shifting cultivation or jhuming, the forests are confined mainly to reserve forests and patches of inaccessible terrain. Moreover, unregulated exploitation of forest plant wealth by a section of motivated people and many other socio-economic factors are responsible for depletion of forest cover in this region. However, it is to be mentioned that even after facing massive deforestation, this region is still rich in species diversity of medicinal plants. Even many forest areas are remaining uninvestigated for medicinal plant wealth till date.

### **Some highly valuable and important medicinal plants of NE India**

Most of the plant species available in NE India are not only with high medicinal value but are 'endemic' (found only in NE India), rare and highly priced. Some of the

very prominent example of such plants are - (1) *Aquilaria malaccensis* (Agar wood, Sachi, Agar, Agaru) - Older agar wood is the source of Agar. The wood is stimulant, antiasthmatic, carminative, tonic, aphrodisiac and astringent. It is used in diarrhoea, dysentery, gout, rheumatism and paralysis. The wood on distillation yields an essential oil. The oil has astringent, acrid, bitter, depurative, alexeteric and antiloprotic properties. It is used to cure various skin diseases, ulcers, eczema, rheumatoid arthritis, cough, asthma, cardiac problems, thyroid cancer and tumour. (2) *Coptis teeta* (Mishmi Tita) - the rhizome is used for stomachache, nervous diseases and cough, (3) *Gloriosa superba* (Glory lily, Surjya-sikha, Ulotkondal)- the rhizome is used in the treatment of gout, rheumatism and also to induce polyploidy, (4) *Rouvolfia serpentina* (Sarpagandha, Arekson tita)- used in the treatment of hypertension and as a sedative and tranquilizing agent, (5) *Hydnocarpus kurzii* (Chalmugra) - used in the treatment of leprosy, (6) *Homalomena aromatica* (Sugandh-mantri, Gandh-kasu) - used as active ingredient in cosmetics and herbal beauty products, (7) *Taxus baccata* (Taxus, Yew) - used for extraction of Taxol, a high cost medicine for breast cancer, (8) *Panax pseudoginseng* (Indian ginseng, Rejuvenating plant) - used as rejuvenating medicine (e.g 30 plus capsules), (9) *Swertia chirayita* (Chiretta, Chirota) - the whole plant of which at flowering stage constitutes the drug 'Chirata' or 'Brown -Chiretta' and used in the treatment of asthma, liver disorders, fever and general debility.

All these plants have great demand in drug industries. Large quantities of these plants are being exploited from various places of NE India. Such collection and trade is both opportunistic and secretive. *Taxus* is an example. This plant was once available in Arunachal Pradesh. But it was exploited in large scale and now the natural population is almost vanished. It is to be noted that one kilogram of "Taxol" (the chemical extracted from leaf and bark of *Taxus* plant) costs about 140 crore rupees which is really a huge amount for a plant product.

### **Bioprospecting of Medicinal plants**

Bioprospecting is basically the search for commercially valuable biomolecules and genetic resources in plants, animals and microorganisms. These resources may be used in food production, pest control, and the development of new drug and for other related biotechnological applications. Mere exploration or taxonomic cataloguing of plant and animal species is not bioprospecting. Of late bioprospecting for new chemicals in plants that have some medical or commercial use becomes a high priority area worldwide. While it is a high risk area for investors, it can have massive returns. It is worth to be mentioned that of the world's 25 top-selling pharmaceuticals, 10 were originally sourced from plants (Table 2). In 1995, these accounted for nearly 14 billion US dollar in global sales.

Before 1992, biological resources were considered a common heritage of

humankind. Scientists could take samples from anywhere in the world without any specific permission. The "Convention on Biological Diversity" (CBD, 1992) re-affirms the sovereign rights of countries over the biological resources within their borders. Though not granted property upon natural resources, biodiversity-rich countries are committed to [1] conserve their biodiversity, [2] develop it for sustainable use and [3] share fairly the benefits resulting from this use. In short, bioprospecting has to be allowed by the biodiversity-rich country and must benefit the country including the communities that traditionally use these resources, as well as the corporations (usually from developed countries) or universities collecting the bioresource.

Bioprospecting must follow the new rules of international treaties and national laws. More specifically, it must respect the "informed consent" (the source country must know what will be done with the resource, and how benefits will be shared; and must give permission for collecting), and the "fair agreement on benefit sharing" (benefits may include support for conservation, research, equipment, technologies, knowledge transfer, development and royalties). Bioprospecting may be considered as biopiracy when these principles are not respected. Some even argue bilateral agreements of bioprospecting between a country or a community and a corporation are a sort of juridical validation of biopiracy toward traditional communities whose values and rights are not considered and respected.

Bioprospecting of medicinal plants for novel and important phytochemical is now a global issue. Moreover, screening for elite clones and identification of diverse plant genetic resources using biotechnological tools are receiving momentum along with phytochemical investigations. Attempts are being made at various levels in various places for screening and assessment of valuable wild medicinal plants. However, such attempts are very much limited in North Eastern region of India. So, it is considered very much important and urgent to make a consortium effort to evaluate the valuable medicinal plant resources of NE India.

### **Biopiracy of Medicinal plants of NE India**

Biopiracy is the commercial development of naturally occurring biological materials, such as plant substances or genetic cell lines, by a technologically advanced country or organization without fair compensation or payment to the peoples or nations in whose territory the materials were originally discovered. There are quite a lot of instances available on the use of wild plants by international companies to develop medicines, without recompensing the countries from which they are taken. Moreover, there are cases available where indigenous knowledge of nature is exploited for commercial gain with no costs paid to the indigenous people themselves. It is to be noted that biopiracy is not only inter-country event but also is intra-country. Exploitation of traditional knowledge (TK) of under developed /developing regions of India by

"developed researcher" and "companies" is an agonizing fact.

There is possibility for continuation of biopiracy of codified Indian traditional knowledge, because, these information exist in regional languages, and due to language barrier the patent offices are unable to search these information as prior art, before granting patents. Formulations used for the treatment of human ailments from traditional knowledge are time-tested since they have been in practice for centuries. The reliability of the traditional medicine systems coupled with the absence of such information with patent offices, provides an easy opportunity for intruders for getting patents on these therapeutic formulations derived from traditional or folklore medicine systems.

The grant of patents on non-patentable knowledge (related to traditional medicines), which is either based on the existing traditional knowledge of the developing world, or a minor variation thereof, has been causing a great concern to the developing world. Patent granted on neem, turmeric, basmati rice etc., are the serious examples of biopiracy of traditional knowledge of India. In many of these cases our country had to fight for revocation of the granted patents. However, revocation may not be a feasible option possible for all the patents taken on the traditional knowledge since it involves huge costs and time. Another painful truth is that we do not know about all the patents that contain the TK of various Indian communities. In addition, we are not giving proper importance to preserve and protect all forms of traditional knowledge. In many instances, our people share information to gain for popularity and ordinary personal benefit. Publication of information without giving proper recognition to the owner-ethnic group and inattention to the existing legal provisions that protects and safeguards the wild resources also encourage biopiracy.

Table 1: State wise estimated numbers of Medicinal plant in NE India

Sl. No	State	No. of Plant Species
1.	Arunachal Pradesh	547
2.	Assam	458
3.	Manipur	310
4.	Meghalaya	315
5.	Mizoram	285
6.	Nagaland	325
7.	Sikkim	378
8.	Tripura	253

Table 2: Most prescribed Medicinal Agents from Plants

Medicinal agent	Activity	Plant source
Steroid from Diosgenin	Anti-fertility agents	<i>Dioscorea deltoidea</i>
Codeine	Analgesic	<i>Papaver somniferum</i>
Atropine	Anti-cholinergic	<i>Atropa belladonna</i>
Hyoscyamine	Anti-cholinergic	<i>Hyoscyamus niger</i>
Digoxin	Cardio-tonic	<i>Digitalis lanata</i>
Scopolamine	Anti-cholinergic	<i>Datura metel</i>
Digitoxin	Cardiovascular	<i>Digitalis purpurea</i>
Pilocarpine	Cholinergic	<i>Pilocarpus jaborandi</i>
Quinidine	Anti-malarial	<i>Cinchina ledgerans</i>
Reserpine	Antihypertensive	<i>Rauwolfia serpentina</i>

## Conclusion

North East India is one of the richest repositories of medicinal and aromatic plants in the world due to its unique topography, high rainfall and various vegetation types. But this rich phytodiversity is not fully evaluated and explored in search of novel bioactive compounds except the information available from folklore or from local medicine men. In these circumstances, an over all reorganization and motivation is required among all the stake holders to ensure the effective management and effective conservation with sustainable utilization of the rich medicinal plant diversity of NE India. In this context, institutional and human capacity building is considered as one of the most important factor for successful conservation of plant resources. Besides, extensive exploration programme for enumeration of individual species giving accurate and adequate information on habit, habitat, taxonomic identification keys, traditional medicinal uses and available trade information have to be undertaken. It is further required to design proper and coordinated program for bioprospecting, molecular taxonomic study, clinical trials and development of individual species using adequate scientific tools and techniques. At the same time, we need a concerted effort to protect our TK. To discourage biopiracy, it is considered as important to create more easily accessible non-patent literature databases on TK of India so that the patent examiners can more easily search and retrieve non-patent literature as prior art before granting a patent.

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# Potential of Herbs in the Management of Type II Diabetes Melitus

**Bishnu Prasad Sarma**

Dept. of Kayachikitsa (medicine), Govt. Ayurvedic College & Hospital,  
Guwahati, Assam

Email: dr.bpsarma@gmail.com

## **Introduction**

Diabetes is now considered as the world's most dreaded slow killing disease. "76% of people with diabetes will be living in the developing countries in 2030."...WHO. More Than 235 million people worldwide have Diabetes mellitus and 80% of the deaths due to Diabetes mellitus occur in low and middle income countries. Further Diabetes mellitus and its complications have a significant economic impact on individuals, families, health systems and countries. From the above facts we can easily comprehend the global significance of the disease Diabetes mellitus which have become an epidemic world over. But what exactly is Diabetes mellitus?

Diabetes mellitus is a chronic disease that occurs either when the pancreas doesn't produce enough insulin or the body cannot effectively use the insulin it produces.

Type 1: Diabetes is characterized by deficient insulin production and requires daily administration of insulin. Symptoms include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, weight loss, vision changes and fatigue.

Type 2: Diabetes results from the body's inactive use of insulin. Type 2 Diabetes mellitus comprises 90% of people around the world, and is largely the result of excess body weight and physical inactivity.

Symptoms may be similar to those of Type 1 Diabetes, but are often less marked. As a result, the disease may be diagnosed several years after onset. Gestational Diabetes is hyperglycaemia with onset or first requisition during pregnancy. Symptoms of gestational Diabetes are similar to Type 2 Diabetes. Gestational Diabetes is most often diagnosed through prenatal screening.

The complications of Diabetes are serious enough to cause death and most of the deaths are due to complications. The important consequences of Diabetes are increased risk of heart disease and stroke 50 % of people with Diabetes die of cardiovascular d

diseases. Further neuropathy in the feet increases the chance foot ulcers and eventual limb amputation. Diabetic retinopathy is an important cause of blindness, and occurs as a result of long-term accumulated damage to the small blood vessels in the retina. Diabetes is also among the leading causes of kidney failure and 10-20% of people with diabetes die kidney failure.

**Management of Diabetes mellitus:** Other than prevention of Diabetes through healthy diet, regular physical activity and maintaining a normal body weight, the diabetics can take the help of drugs and lead a perfectly healthy life. In Ayurveda the ancient system of medicine, a triangular approach is used to manage the diabetics. The triangular approach consists of diet, drugs and yogic exercises to maintain a proper and balanced treatment for Diabetes.

**Diet:** In Ayurveda, Charak and Susrut has realized the importance of dietary restrictions in Diabetes and many principles given by them hold true even today.

A carefully planned diet is one of the dietary treatment is one of the major tools in the management of Diabetes. Even though the immediate objective of the dietary treatment is to control blood sugar, the ultimate goal must be to enable the person to live a normal span of life in health and comfort. A well planned diet plays a very important role in making the blood sugar and the lipids within the normal range. If the Diabetic person is lean he was prescribed a nourishing diet and in case of an obese person fasting and physical exercise is prescribed.

### **The Do's and Don'ts in food for a Diabetic patient :**

Total restriction on food articles like sugar, jams, biscuits, cakes, puddings, chocolates, icecream, pastries, syrups etc. The food items to be taken in moderation are bread, potato, thick soups, fried foods, dryfruits, foods with excessive flour like macaroni etc. Such patients can eat some foods without much restrictions like meat, fish, eggs, tomato, turnip, lettuce, cucumber, cabbage, radish, thin butter milk etc.

General dietary guidelines include no radical change in type of food normally eaten, to take small frequent meals instead of 2-3 large meals, salad eating to be encouraged. The American Diabetic Association recommends usual calorie intake of 1200-1400 calories per day. The carbohydrate should be less than or equal to 60% of the total calories, protein .8 gms/kg body wt, fats 30% of the total calories.

**Drugs:** There are large numbers of drugs of herbal or herbo mineral origin mentioned in Ayurveda which claim to be effective for the treatment of Diabetes. Ayurveda, the treasure box of herbal drug has the potential to offer oral hypoglycemic agents without the toxic effects. Some are mentioned below -

**Amlaki:** Natural insulin out-put has increased when diabetics have taken supplementary vitamin C. Amlaki is the richest source of Vitamin C. 100 gms of dried fruit contains 650 mg of vitamin C no other plants has such a high content of vitamin

C. 100 gms of oranges contains only 60 mg. Therefore one fruits of this plant is equivalent to ten oranges. Have fresh Amlaki is season time and dry powder 3-4 gm daily in of season.

***Pterocarpus marsupium* (Vijayasaar):** *P. marsupium* extract has been shown to reduce the glucose absorption from the gastrointestinal tract in mice and produce a fall in blood sugar in dogs and alloxan-induced diabetic rats. It has also been shown to reduce the acute hyperglycaemic response induced by anterior pituitary extract. It has been claimed that this extract causes pancreatic beta-cell regeneration by the flavanoid fraction (epicatechin). In a study on alloxan-induced diabetic rats, it was shown to decrease blood sugar levels, improve insulin levels and increase synthesis of proinsulin. In another study, epicatechin isolated from the bark of *P. marsupium* has been shown to protect animals against the diabetogenic effect of alloxan. It has also been shown to have a hypocholesterolaemic effect.

**Gudmar (*Gymnema Sylvestre*):** It has been named "Gurmar", meaning "sugar-destroyer", because of its property of abolishing the taste of sugar. The drug causes significant reduction in blood sugar in anterior pituitary treated, Hyperglycaemic subjects, suggesting its use in maturity onset Diabetes mellitus. The components have also been observed inhibiting the taste buds for sweet and tends to reduce the glucose absorption from the mucosae.

***Momordica Charantia* (Karela):** The fruits and seeds yield a polypeptide considered to be similar to bovine insulin, which has been shown to have a hypoglycaemic effect in all types of diabetes. It also promotes peripheral utilisation of glucose and potentiates the action of tolbutamide.

**Fenugreek Seeds (*Trigonella foenum-graceum*):** Hypoglycaemic activity has been evaluated and confirmed by Mencil E et. al & Mishinsky et al; Published in Lancet. These are very commonly used for controlling diabetes in India. Effectiveness of these seeds mentioned in all Ayurvedic literatures and Greek & Latin Pharmacopia. A teaspoonful of these seeds can be gulped daily with a glass of water. The director, National Institute of Nutrition, Hydrabad recomends inclusion in daily diet of methi in amount 50 to 100 gm as an effective supportive therapy in the management of diabetes.

***Tinospora cordifolia* (Giloe):** The watery extract of this plant is known as "Indian quinine". It acts as a tonic in debilitating disease. The aqueous extract has been shown to exert significant hypoglycaemic effects. It inhibits hepatic glucose release caused by adrenalin.

**Jamun Patra (*Syzygium jambos*):** This herb has been reported of having a stimulating effect on the B-cells. of islets of Langerhan's to secrete more insulin, may also increase the utilisation of sugar either by direct stimulation of sugar uptake or via the mediation of enganced insulin secretion.

**Banana:** Recent study shows that banana is good for heart. Journal of American

Medical Association says "Banana and skimmed milk furnish a simple and effective method for weight reduction in treating patients for diabetes. Unripe Banana cooked as vegetable is good for diabetics.

**Karipatta (*Murrayakoengii*):** Clinical and experimental study showed hypoglycemic effect. Ethanol extracts (80%) of *M.koenigii* shows significant hypoglycemia effect in normal rats and it has significant anti- hyperglycemic effect in both types of diabetic model rats.

- A clinical trial was done with *dillenia indica* on 40 cases of type 2 diabetes mellitus (NIDDM) as hypoglycemic agent.

Dose - 30 gm daily in two divided doses half an hour before lunch and dinner with warm water.

**Duration:** 24 weeks

## Results

Among 40 cases of type 2 diabetes mean FBS level before treatment was 158+16.1 and after treatment with the trial drug the mean difference in each follow up had increased gradually from 139+8.1 at 8 weeks to 119+4.1 at 16 weeks and 98.7+1.1 at 24th week of treatment (Table 1). The mean difference in case of PPBS had increased gradually from 180+5.6 to 168.45+12.1 and 155.9+16.7 at 8th, 16th and 24th weeks of treatment respectively (Table 2). The initial mean HbA1c was 8.7 and this was reduced to 6.8 after treatment (Table 3). Clinically no adverse effect was reported during this 24 weeks study.

**Table :1 Effect of *Dillenia indica* on FBS**

N= 40	BT	FU1	BT-FU1	FU2	BT-FU2	FU3	BT-FU3
Mean±SD	158±16.1	139.2±8.1	18.3±11.4	119.3±4.1	38.2±15.4	98.7±1.1	58.8±16
SE			1.8		2.4		2.5
t value			10		16		23.7
P value			< 0.001		<0.001		<0.001

**Table :2 Effect of *Dillenia indica* on PPBS**

N= 40	BT	FU1	BT-FU1	FU2	BT-FU2	FU3	BT-FU3
Mean±SD	212.7±11	180.5±10.8	32.1±17.6	168.4±12	44.2±15.9	155.9±16.7	56.6±18.3
SE			2.79		2.52		2.89
t value			11.51		17.55		19.62
P value			<0.001		<0.001		<0.001

**Table :3 Effect of *Dillenia indica* on HbA1c**

N= 40	MEAN ±SD			SE	t value	P value
	BT	AT	BT-AT			
	8.7±1.0	6.8±0.8	1.9±0.7	0.11	17.2	<0.001

### Conclusion

The result of the therapeutic trial showed that the trial drug *Dillenia indica* was very effective in controlling the blood glucose level. It needs to be mentioned that the trial drug needs further evaluation on large number of patients using different study designs. Because of its significant hypoglycaemic effect it can prove to be very valuable as oral hypoglycaemic drug to manage Diabetes mellitus and its complications without the adverse effects of synthetic oral hypoglycaemic Agents (OHA).

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Fig1: Amalaki



Fig2: Gudmar



Fig3: Karela



Fig 4: *Tinospora cordifolia*



Fig 5: Jamunpatra



Fig 6: Karipatta



Fig 7: *Dillenia indica*

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# Ethnobotanical study of the Mishing tribe living In Fringe villages of Kaziranga National Park of Assam, India

**Anima Kutum and Apurba Saikia**  
Department of Botany, D.R. College  
Golaghat-785621, Assam, India

## **Abstract:**

The people of North-East India are rich in traditional knowledge of plants and plants products from ancient time. The Mishing tribe is traditionally dependent on forest resources to complete their livelihood. A total 75 plant species belong to 45 families has been recorded in the present study that used in various aspects of ethenobotanical importance by Mishing tribe of forest fringe villages of Kaziranga national park. Among the families recorded poaceae was the largest with altogether 6 species followed by Euphorbiaceae 2 species and Malvaceae, Caesalpinaceae, Rautaceae, Lamiaceae, Zinziberaceae with 3 species.

**Key words:** Traditional knowledge, Ethnobotany, Fringe village, Livelihood, Mishing tribe, Kaziranga National Park

## **Introduction:**

Assam is a region of many culture and traditions, races and ethnic. The folk culture is still vital in this region. Most tribal communities still largely depend on non timber for their traditional system of medicine.

The Mishing or Mishing also called Miri. The missings are East Asian, or more specifically a Southeast Asian sub-race of the Mongoloid race. It is not known exactly where they migrated from, but it is popularly believed that they were dwellers of the hills of present day Arunachal Pradesh. According to available knowledge of history and folklores the mishing were the Adis who migrated to Assam. Legends say that they came into contact with more civilized communities living in plains of Assam as early as in 16th century. There is no any written history of missing about their migration from hills to the plains of assam. Though they belong to Tani group of tribes and they used to be hill dwellers, they started living on the banks of rivers in plains of assam.

Plants provide humankind with our most basic resources- food, medicine, fiber and a whole array of other useful products. The use of natural drugs whether traditional or modern, has originated directly or indirectly from folklore and rituals known as ethnomedico-botany. The use of herbal medicine reflects the long history of human interaction with the environment. Different tribes inhabiting different parts of Assam with an intricate life totally dependent on the forest resources for their livelihood. Local communities of North-East India are extremely knowledgeable about the local plant resources and their utilization(Sharmah, 2006, 2010,). Mishing tribal have been inhabiting in and around different parts of the Kaziranga national park. Mishing is a tribal community belonged to Mongoloid group- a multitude of people that followed Austr-Asiatic races to India(Singh et al. 1996) Livelihood system of Mishing people is traditionally dependent on forest resources. They are agriculturist, hardworking and very much peace loving. But, in-spite of their ceaseless tail and their peaceful co-existence with their Assamese non-tribal neighbors, they have remained literally and economically poor and backward. In Assam they are distributed in most parts of the northern bank of the river Brahmaputra. Mishing have distinct entities from the rest of the tribes of Assm with their special culture and tradition (Baruah and Kalita,2007). The fringe tribal villages of Kaziranga national park are depended on forest for preparation of medicine, food, country-drinks, fiber, detergent, construction materials, fishing, fire wood etc. Considering the traditional knowledge for the welfare of human being, a number of researches have been undertaken in this field in India from time to time. Some of the important publications include Arora (1990), on native food plants of the tribal in North-eastern India.

#### **Materials and Methods:**

The study was carried out in and around Kaziranga national park( $26^{\circ}30' N$  to  $26^{\circ}45' N$  to  $93^{\circ}08 E$  to  $93^{\circ}36'E$ ) located on the banks of the Brahmaputra River in the far North East of India. Detailed field studied was conducted during September 2015 to April 2016 and ethnobotanically important plants were collected with interviewing the villagers of different age and sex. Local traditional healers were also engaged during collection of plant samples. All relevant information such as parts used, mode of preparation, used pattern along with morphological features an ecological information were recorded during field work and herbarium were prepared. Plant samples were identified by comparing their morphology as well as laboratory studies, with local floras and morph graphs and with the help of plant taxonomist. The voucher herbarium were submitted to the departmental herbarium of Debraj Roy College, golaghat.

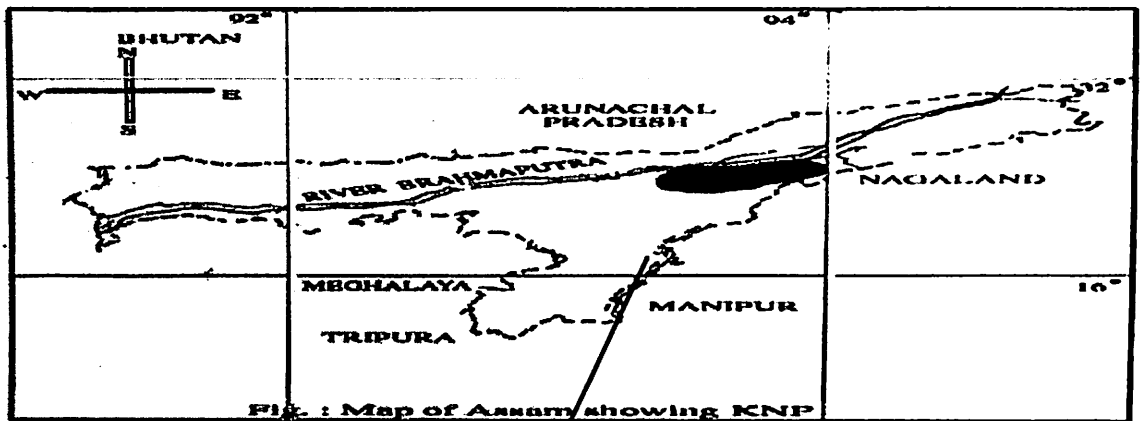


Fig. : Map of Assam showing KNP

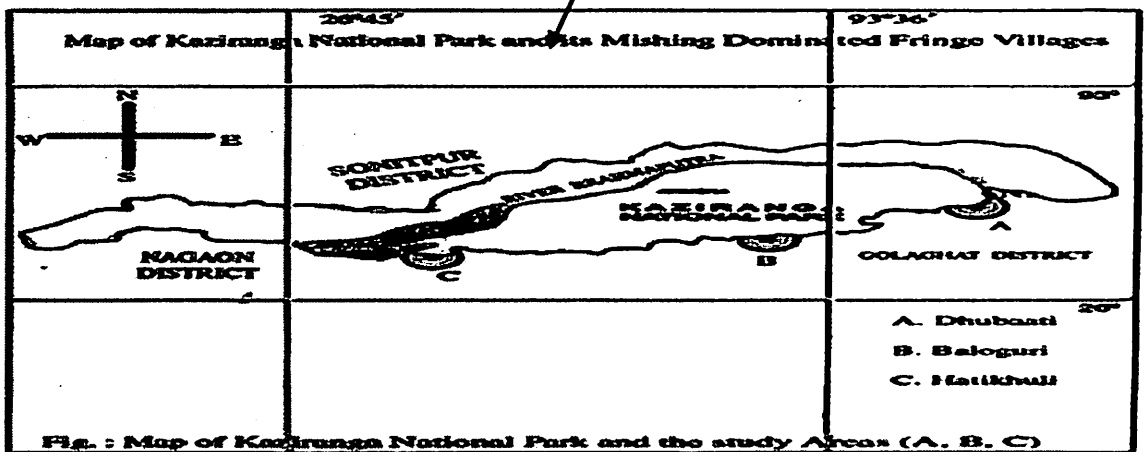


Fig. : Map of Kaziranga National Park and the study Areas (A, B, C)

Fig:1 Map of study area showing the study site in triangle shape.

### Result and discussion:

Ethnobotanical knowledge on forest resources is the local knowledge that is unique to a given culture or a human society. Traditional knowledge basically related with the socio-economic activities of a particular community that included health care, food security and natural resources management in rural communities etc. A total 75 species belonging to 45 families of ethnobotanically important plants were recorded in the present study. Among the families recorded Poaceae was the largest with altogether 6 species followed by Euphorbiaceae 2 species and Malvaceae, Caesalpinaceae, Rautaceae, Lamiaceae, Zinziberaceae with 3 species. The detailed ethnobotanical importance, utilization pattern of every plant part are given in table 1.

Public environmental awareness is very important for the study area. Protection of land, water, fauna, flora and atmosphere must become the joint responsibility of the people and the Government. Involvement of fringe tribal people in the management of

natural resources is essential for promoting all these natural resources. Present study has enabled in understanding the mishing people of this region socio-economic and their relation with plants especially the uses of medicinal plant.

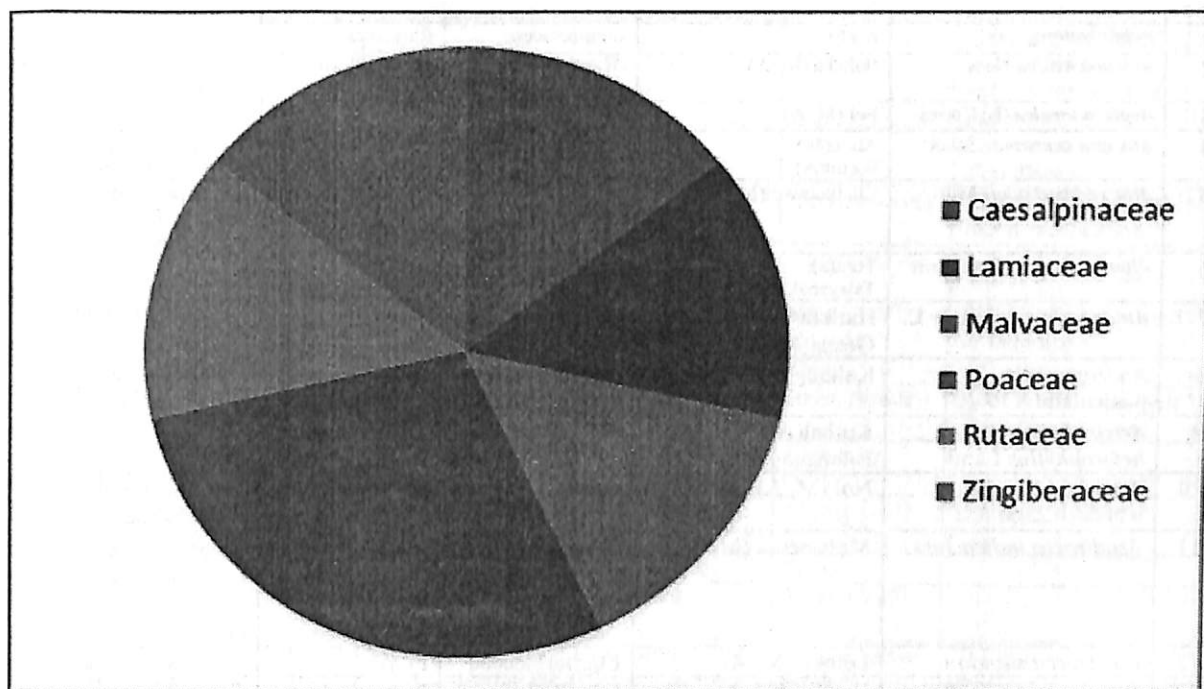


Fig:2 Pie diagram of Maximum used plant families by Mishing tribe of study area

**Table. 1: Medicinal plants used by Mishing People of Study Area.**  
N: B (M= Mishing, A= Assamese)

Sl. No	Scientific Name	Local Name	Family	Part used	Medicinal Use
1	<i>Achas malorogloss</i>	Karful	Zingiberaceae	Rhizomes	Used as food
2	<i>Adhatodavasica</i> Nees	Bahaka (M, A)	Acanthaceae	Leaves Flowers	Leaves juice used for relieve cough.
3	<i>Aegle marmelos</i> (L) Correa	Bel (M, A)	Rutaceae	Fruit, leaves	---
4	<i>Alocasia acuminata</i> Schott.	Ange(M), kochu(A)	Araceae	Shoots, leaves, tubers	Treatment of malaria, blood purification
5	<i>Aloe verabarbarnis</i> Mill	Chalkouwori(M, A)	Liliaceae	Leaves	Leaves are used in treatment of cuts, burns and eczema
6	<i>Alpinia alughas</i> (Retz) Rose	Tora(a), Talayanakhan(M)	Zingiberaceae	Leaves	
7	<i>Amaranthus spinosus</i> L.	Hatikhutora(A), Geang(M)	Amaranthaceae	Leaves	Dysentery treatment
8	<i>Andrographis paniculata</i>	Kalmegh (M, A)	Acanthaceae	Leaves	Leaves are used febrifuge, tonic
9	<i>Arteocarpous heterophyllus</i> Lamk	Kothal(A), Bilangaai(M)	Moraceae	Stem, leaves fruit	---
10	<i>Arundo donax</i> L.	Nol (M, A)	Poaceae	Spikes	Treatment of bagly skin disease
11	<i>Azadiracta indica</i> Juss.	Mohaneem (M, A)	Meliaceae	Twig, leaves	Twigs are used for brushing. Leaves are used cure of small pox.
12	<i>Baccaurea sapida</i> L.	Leteku (M, A)	Euphorbiaceae	Pulp	Used as antioxidants
13	<i>Bacopa monnieri</i> (L.) Pennell	Brahmi (M, A)	Scrophulariaceae	Leaves	Tonic for nerves, mental diseases ,brachitis.
14	<i>Bambusa balcoon</i> Roxb	Bhalooka bah (M, A)	Poaceae	Pulp	---
15	<i>Bombax ceiba</i> L.	Simalu(A), Singeem(M)	Malvaceae	Roof, flower, Fruit	Roots used as medicine
16	<i>Butea monosperma</i> (Lamk) Taub	Polash (M, A)	Fabaceae	Gum	Tannis are treatment for diarrhea.
17	<i>Calamus rotang</i> L.	Bet, Jeying (M, A)	Palmaceae	Apical portion	Deworming
18	<i>Cassia fistula</i> L.	Sonaru (M, A)	Caesalpiniaceae	Leaves, bark, fruit	---
19	<i>Celosia argentia</i> L.		Amaranthaceae	Young leaves, shoots	Seed are used in diarrhea
20	<i>Centella asiatica</i> Urb	Bormanimuni(A), Bortanmanimuni((M)	Apiaceae	Leaves, flower	Stomatic disorder, carminative
21	<i>Ceratopteris thalictroides</i> (L.)	Panidhekia(A), Okangoing(M)	Parkeriaceae	Fronds	Blood purification

22	<i>Cinnamomum veram</i> Presl	Dakeni (M, A)	Lauraceae	Bark	The bark is used in treatment of diarrhea and vomiting
23	<i>Cinnamomum, tamala</i> Nees.	Tejpat (M, A)	Lauraceae	Leaves	---
24	<i>Cissus quadrangula</i> L	Herhurualata (M, A)	Vitaceae	Tendrilleaves	Tendril leaf is used in join broken fracture
25	<i>Clerodendrum cloebrookianum</i> L.	Ne fāfu(A), Pakcoom(M)	Verbenaceae	Leaves	Leaves are used in skin diseases, blood pressure
26	<i>Clerodendrum. Indicum</i> (L) Kuntz	Akalbih (M, A)	„	Leaves	Leaves are used in skin diseases
27	<i>Colocasia esculenta</i> (L.) Schott.	Kachu(A), Angee(M)	Araceae	Tender leaves	Treatment of malaria, blood purification
28	<i>Corchorus capsularis</i> L.	Morapat(A), Mura(M)	Tiliaceae	Stem, Leaves	.....
29	<i>Costus speciosus</i> (Koen-ex Retz.) Smith	Jam-lakhuti (M, A)	Costaceae	Shoot, rhizome	Rhizome juice is used in Jaundice
30	<i>Cynodon dactylon</i> Pers	Duboribon (M, A)	Poaceae	Grass	
31	<i>Datura stramonium</i> L	Dhatura (M, A)	Solanaceae	Root, flower	Root is used treatment of bite by mad dog
32	<i>Dendrocalamus hamiltoni</i> , Neesat Arn	Banh(A), Wha(M)	Poaceae	Soft stem	.....
33	<i>Dillenia indica</i> L	Outenga(A), Champa(M)	Dilleniaceae	---	Diarrhea, dysentery
34	<i>Dioscorea alata</i> L	Kathalu(A), Ali(M)	Dioscoreaceae	Tuber	---
35	<i>Diplazium esculentum</i> (Retz.) SW	Dhekia(A), okang(M)	Athyriaceae	Tender leaf	---
36	<i>Drymaria cordata</i> (L.) wild ex Roemer	Lajabori (M, A)	Caryophyllaceae	Tender leaves, shoots	It is used in sinus problem, cuts, wounds
37	<i>Ficus glomerata</i> Roxb	Dimboru(A), Takpiyang(M)	Moraceae	Leaves, stem	---
38	<i>Flacourita cataphracta</i> L.	Ponniol (M, A)	Flacortiaceae	Fruit	---
39	<i>Garcinia cowa</i> L	Kujithekera (M, A)	Guttigerea	Fruit	Dried fruit is used in dysentery
40	<i>Garcinia xanthohymus</i> Hook. F & Th	Teportenga (M, A)	Guttiferaceae	Fruit	---
41	<i>Gossipium herbaceum</i> L.	Kopah(A), Sipag(M)	Malvaceae	Fruit	---
42	<i>Hibiscus Suddarifa</i> L.	Tengamora (M, A)	Malvaceae	Leaves, fruits	Leaves used in dysentery of man and domestic animals
43	<i>Houttuynia cordata</i> Thunb	Mosundori (M, A)	Saururaceae	Leaves	Leaves used in dysentery

44	<i>Hydrocotyle sibthopoides</i> L.	Harumanimuni(A), Bormamanimuni(M)	Apiaceae	Leaves, flowers	„
45	<i>Imperatocyl indrica</i> L.	Ulukher(A), Taseseleng(M)	Poaceae	Leaves	
46	<i>Lagerstroemia flos-reginae</i> Retz.	Azar (M, A)	Lythraceae	Wood	---
47	<i>Lawsoni ainermis</i> L.	Jetuka (M, A)	Lythraceae	Leaves	Leaves used for healthy hair, skin diseases.
48	<i>Leucas indica</i> (L.) R.Br.exVatke	Doron(A), Durun(M)	Lamiaceae	Root, leaves	Relieve burning sensation, noston.
49	<i>Lygodium flexuosum</i> L.	Kopoujongi(M)	Schizaceae	---	---
50	<i>Magifera indica</i> L.	Aam(A), Keddi(M)	Anacardiaceae	Fruit, leave,	---
51	<i>Meliosma pinnata</i> (Roxb.) Maxim.	Borpichola(A), Dermiesing(M)	Sabiaceae	Young leaves	
52	<i>Meliosma simplicifoila</i> (Roxb.) Walp.	Dhopattitta(A), Nitak(M)	Sabiaceae	Tender leaves, young shoot	
53	<i>Mentha spicata</i> L.	Pudina (M, A)	Lamiaceae	Leaves	Leaves fever & bronchitis
54	<i>Michelia champaka</i> L.	Champa (M, A)	Magnoliacea	Stem, bark, root	Bark is used in treatment of rheumatism root juice is used women period
55	<i>Mimuso pselengi</i> L.	Bokul (M, A)	Saptaceae	Fruit, Bark	Bark is medicinal
56	<i>Moringa pterygosperma</i> Gaertn	Sajina (M,A)	Moringaceae	Leaves flower, seed	Leaves used in nerves, debility, asthma, epilepsy
57	<i>Murraya koenigii</i> Spreng	Narasingha(A), Nor-hing(M)	Rutaceae	Leaves	Leaf juice is used treatment of dysentery
58	<i>Musa balbisiana</i> (Rety) Rose	Bhinkol (M, A)	Zingiberaceae	Leaves	Tuberculosis, worm
59	<i>Myrica esculenta</i> Ham		Myricaceae	Bark	Used to relief rheumatism
60	<i>Nycthenes arbor-tristis</i> L.	Sewali (M, A)	Oleaceae	Leaves, flower	Leaf juice efficacious internal worm
61	<i>Nymphaea nouchali</i> Burms.f.	Vateful(A), Aluick(M)	Nymphacaceae	Tubers, rhizomes, fruit	---
62	<i>Ocimum sanctum</i> L.	Tuloshi(A), Tulohi(M)	Lamiaceae	Leaves, seed	Leaves are used cough, seed-eye diseases
63	<i>Oryza sativa</i> L.	Dhan(A), Amm(M)	Poaceae	Caryopsis, straw	---

64	<i>Phyllanthus embellica</i> L.	Amlokhi (M, A)	Euphorbiaceae	Fruit	Fruits are used in diarrhea, dysentery
65	<i>Piper nigrum</i> L.	Bonorajaluk (M, A)	Piperaceae	Leaves, seed	---
66	<i>Rubus moluccanus</i> L.	Jetulipoka (M, A)	Rosaceae	Fruit	---
67	<i>Sapindus mukorossi</i> Gaertn.	Ritha(A), Haital-bang(M)	Sapindaceae	Fruit, Stem	---
68	<i>Saraca asoca</i> (Roxb.) De Wilde	Asok (M, A)	Caesalpinaceae	Flower, seed	Flowers are treatment of hemorrhagic dysentery
69	<i>Scoparia dulcis</i> L.	Bondhonia(A), Jaluk(M)	Scrophulariaceae	Stem, leaves, flower	---
70	<i>Solanum indicum</i> L.	Titabhekuri(A), Bangko(M)	Solanaceae	Fruit	Killing worm
71	<i>Spilanthes paniculata</i> L.	Aohoni(A), Malsa(M)	Asteraceae	Leaves	---
72	<i>Syzygium cumine</i> (L.) Skeels	Jamu (M, A)	Myrtaceae	Fruit	---
73	<i>Tamarindus indica</i> L.	Teteli (M, A)	Caesalpinaceae	Stem, Seed	---
74	<i>Vitex negundo</i> L.	Pochotia (M, A)	Verbenaceae	Root, twigs, leaves	Leaves roots are febrifuges tonic
75	<i>Zanthoxyl amoxyphyllum</i> Edgn.	Mesenga(A), Onger(M)	Rutaceae	Tender shoots Tender shoots	

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# Ethnomedicinal Plants used by Bodo Community of Nagaon District, Assam

**Bijumoni Borah<sup>1</sup> & Suranjan Bhowal<sup>2</sup>**

<sup>1</sup>Department of Botany, ADP College, Nagaon, Assam

<sup>2</sup>Department of Zoology, ADP College, Nagaon, Assam

Email of correspondence: [bijumoni70@gmail.com](mailto:bijumoni70@gmail.com)

## **Abstract:**

Assam is known as "Hot spot" for ethnomedicinal plants. From time immemorial plants had been used by human being for his very existence. From proper utilization of raw materials in a developing country like India the need of natural sources is emphasized. Ethnobotanical lore is interlinked with some knowledge of general flora of medicinal plants of the area of work. Thus we can hope to utilize the plant resources of an area by collection of information on flora and proper evaluation of earlier experiences. The present work is an attempt towards floristic exploration of medicinal plants used by Bodo community of the area. The present communication deals with 44 important medicinal plant species and their parts used by Bodo community in the treatment of their different ailments.

**Key words:** Ethnomedicinal plants, Bodo community, Treatment, Ailments.

**Introduction:** Ethnobotany is the study of useful plants prior to commercialization and eventual domestication. It includes the use of plants by both tribal and non-tribal communities without any implication of primitive and developed societies (Wickens 1990), Jain (1987) described ethnobotany as the total natural and traditional relationship between man and his surrounding plants resources. Medicinal plants are those plants which have some chemical ingredients stored in their tissues produce definite physiological action on both man and animal and act accordingly on proper application for curing diseases.

## **Materials and methods**

### **Study Area**

The study was conducted on ethnomedicinal plants used by Bodo community of Nagaon District Assam. The District is geographically located at the latitude of 25° 30'

- 26°45' North and the longitude 92°15' - 93°20' East. The average altitude is about 60 m. The Bodo community, one of the oldest inhabitants of Assam mostly inhabited in the villages of Dhing (Saharia), Kathiatoli, Kampur, Kaliabor and Samaguri area of Nagaon district. The present study was conducted during 2015 to 2016 covering various parts of Nagaon district inhabited by Bodo people.

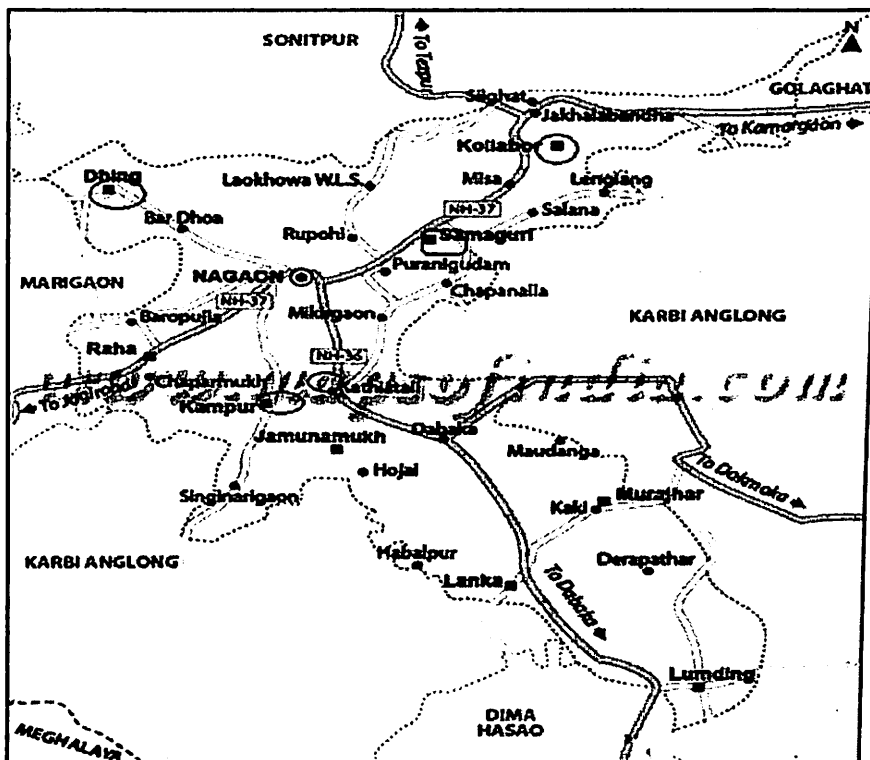


Fig 1: Map of Nagaon District (Study area are marked in circles)

### Methods of information and data collection

The present study was done by field survey in various parts of Nagaon district inhabited by Bodo people. Interviews was carried out (Vaidyas and local people). Plants were collected and prepared following the herbarium technique (Jain and Rao 1977) and specimens were indentified with the help of flora of Assam (Kanjilal et al 1934-1940). The voucher specimens were deposited in the Herbarium of Botany Department, ADP college, Nagaon. The species are arranged according to alphabetical order by botanical name, family, local names, parts used, diseases and does regimes.

### Result

The survey work carried out throughout the study area revealed certain plant species which are medicinally very important. The plants were enlisted in table 1.

Table 1: Ethnobotanical aspects of the plants used by the people of Study area

Sl no.	Species Name	Family	Assamese name	Bodo name	Part used
1	<i>Aegle marmelos</i> L.	Rutaceae	Bel	Beldongphung	Root and leaf
2	<i>Allium sativum</i> L.	Liliaceae	Noharu	Shambramgufur	Leaf and bulb
3	<i>Alocasia macrorrhiza</i> (L) G Don.	Araceae	Kolakachu	Thasogwswm	Rhizome ,tender leaf
4	<i>Aloe vera</i> (L) Burma.	Liliaceae	Chalkonwori	Salkhungri	Leaf
5	<i>Amaranthus spinosus</i> L.	Amaranthaceae	KhuturaSak	Khuthrasugwnang	Whole plant
6	<i>Amorphopallus paeoniifolius</i> (Dennst) Nicolson.	Araceae	Olkoshu	Thasogwsu	Leaf and corm
7	<i>Ananas comosus</i> (L) Meer.	Bromeliaceae	Anaros	Anarwsh	Leaf and fruit
8	<i>Anthocephalus chinensis</i> (L) Rich.	Rubiaceae	Kadom	Khodom	Bark and leaf
9	<i>Areca catechu</i> L.	Arecaceae	Tamul	Goi	Root
10	<i>Azadirachta indica</i> Juss.	Meliaceae	Neem	Nimdongfang	Root, leaf, stem ,bark
11	<i>Bacopa monierie</i> (L) Penn.	Scrophulariaceae	BrahmiSak	Brahmimengong	Whole plant
12	<i>Calotropis gigantea</i> (L)R.Br.	Asclepiadaceae	Akon	Aungkhon	Leaf,bark and latex
13	<i>Carica papaya</i> L.	Caricaceae	Amita	Mwdwmpful	Fruit and latex
14	<i>Cassia fistula</i> L.	Caesalpinaceae	Sonaru	Sonaridongfang	Root ,leaf and fruit
15	<i>Catharanthus roseus</i> (L) D. Don.	Apocynaceae	Naqyantora	Dawdaibibar	Stem and leaf
16	<i>Centella asiatica</i> (L)	Apiaceae	Bormanimuni	Gedetjatmanimuni	Whole plant
17	<i>Cissus quadrangula</i> L.	Vitaceae	Harjoralota	Hara joraina ilewa	Whole plant
18	<i>Clitoria ternatea</i> L.	Fabaceae	Aparajita	Ophorajita	Root ,bark and seed
19	<i>Curcuma aromatic</i> Salisb.	Zingiberaceae	Bon-halodhi	Khathri	Rhizome
20	<i>Curcuma longa</i> (L)	Zingiberaceae	Halodhi	Hal dai	Rhizome ,

21	<i>Cuscuta reflexa</i> Roxb.	Cuscutaceae	Akashilota	Gwmwlewa	Stem and seed
22	<i>Datura stramonium</i> L.	Solanaceae	Datura	Thutura	Root ,leaf and fruit
23	<i>Dillenia indica</i> L.	Dilleniaceae	Owtenga	Thaigir	Fruit and shoot
24	<i>Euphorbia nerifolia</i> (L) Sehund.	Euphorbiaceae	Sizu	Sijou	Root and latex
25	<i>Ficus religiosa</i> L.	Moraceae	Anhot	Phakridongfang	Bark and leaf
26	<i>Gossypium herbaceum</i> L.	Malvaceae	Kopah	Khun	Root
27	<i>Hibiscus rosa-sinensis</i> L.	Malvaceae	Joba	Jobabibar	Leaf and flower

28	<i>Lawsonia inermis</i> (L).	Lythraceae	Jetuka	Jenthoka	Leaf, bark and seed
29	<i>Leucas aspera</i> (Roth) Spreng.	Lamiaceae	Doron	Boromphutra	Root and leaf
30	<i>Litsea salicifolia</i> (Roxb. Ex. Nees.) Hook. F.	Lauraceae	Digh-lati	Golaudongfang	Leaf and bark
31	<i>Mangifera indica</i> L.	Anacardiaceae	Am	Thajjou	Leaf, bark, fruit, seed
32	<i>Mentha viridis</i> L.	Lamiaceae	Pudina	Phudina	Whole plant
33	<i>Mimosa pudica</i> L.	Mimosaceae	Nilajibon	Nilajihagra	Leaf, stem and root
34	<i>Momordica charantia</i> L.	Cucurbitaceae	Titakerela	Kerelagwkha	Leaf and fruit
35	<i>Moringa oleifera</i> Lamk.	Moringaceae	Sajina	Mwdai	Leaf, root, bark and fruit
36	<i>Murrya koenigii</i> (L) Spreng.	Rutaceae	Narahsingha	Nursingh	Leaf
37	<i>Nyctanthes orbor-tristis</i> L.	Oleaceae	Sewaliphul	Shewalibibar	Root, leaf and flower
38	<i>Ocimum canum</i> Sims	Lamiaceae	Kola tuloshi	Thulungshi	Root and leaf
39	<i>Oxalis corniculata</i> L.	Oxalidaceae	Tangeshitenga	Singrimukhai	Whole plant
40	<i>Paederia scandens</i> Lour	Rubiaceae	Bhedailata	Khifilewa	Root and leaf
41	<i>Phyllanthus emblica</i> L.	Euphorbiaceae	amlokhi	Amlai	Root, leaf, fruit and seed
42	<i>Piper betel</i> L.	Piperaceae	Pan	Phathai	Leaf
43	<i>Psidium guajava</i> L.	Myrtaceae	Madhurium	So fari	Leaf and bark
44	<i>Punica granatum</i> L.	Punicaceae	Dalim	Dalimdonfang	Root, bark, fruit and seed

## Enumeration of Species

### 1. *Aegle marmelos* L.

**Local name:** Bel(As), Beldongphung (Bodo)

**Family:** Rutaceae

**Parts used:** Root and leaf.

**Medicinal uses:** Root chewed in vomiting

Warmed extract of bel with extract of the root of *Ricinus communis* is given in chest pain (20 ml once daily for 10 days). Decoction with bark of *Bambaxcieba* is given in paralysis (About 5 ml trice daily till for a month). Leaf juice is given in constipation (About 5 ml trice daily till cure).

Infusion with leaves of *Nyctanthes* and *Ocimum sanctum* is prescribed in influenza (About 5 ml trice daily till cure).

### 2. *Anana scomosus* (L) Meer.

**Local name:** Anaros (As), Anarwsh (Bodo).

**Family:** Bromeliaceae.

**Parts used:** Leaf and fruit.

**Medicinal uses:** Leaf crush and soaked in water for 2-3 hours and liquid is given to hasten childbirth (about 30 ml once or twice within a gap of one hour)

Juice is given as vermifuge (about 20 ml on empty stomach for three days).

### **3. *Areca catechu* L**

**Family:** Arecaceae

**Local name:** Tamul (As);Goi (Bodo).

**Parts used :** Root.

**Medicinal uses :** Decoction with roots of *Citrus medica*, *Piper longum* and rhizome of *Zingiber officinale* is prescribed in asthma and in whooping cough(about 20 ml thrice daily for a month).

### **4. *Aloe vera* (L) Burma. *Aloe barbadensis* Mill.**

**Local name:** Chalkonwori (as), Indian Aloe (Eng.), Salkhungri (Bodo)

**Family:** Liliaceae.

**Pats used :** Leaf.

**Medicinal uses:** Leaf juice is applied to burn injuries (twice daily till the healing of wound). It is also used for curing piles and inflammation. It is also applied to wounds of abscesses and naphtha. Paste applied on forehead is said to reduce typhoid fever, Juice is given in peptic ulcers (about 20 ml twice daily for a week). Juice is also given as vermicide (about 10ml once daily in the morning on empty stomach for three days). Juice heated to get a stocky mass and then mixed with boiling water. This is given as laxative (about 5gm in 100 ml water is given before bed time for adults and for children half of the above dose is given.

### **5. *Amaranthus spinosus* Linn.**

**Local Name:** KhuturaSak (As); Loves lies bleeding (Eng.), Khuthrasugwang (Bodo)

**Family:** Amaranthaceae

**Parts used:** Whole plant.

**Medicinal uses:** Root poultice is applied on boil to hasten suppuration. Juice is given in diarrhoea (about 25ml twice daily till cure). It is also useful in feeling of burning sensation during urination, Decoction with leaves of *Cannabis sativa* is given in Jaundice (about 20ml twice daily for a month). Whole plant is used as vegetable, Which is said to be useful as laxative, resumptive, stomachic and as lactagogue for nursing matters. Persons suffering from gastric trouble are advised not to take this plant as vegetable as it increases the trouble.

### **6. *Azadirachta indica* Juss,**

**Local name:** Neem (As); Neem (Eng.), Nimdongfang (Bodo)

**Family:** Meliaceae.

**Parts used:** Root, leaf. Stem and bark.

**Medicinal uses:** Root decoction is given in diarrhoea (about 15ml thrice daily till cure) Leaf juice is given in threadworm, in body ache and in stomach ache ( about 20ml thrice daily till cure). It is said to act as blood purifier. Juice is also given as anthelmintic (about 15ml once daily in empty stomach for one week) Sometimes leaves fried in mustard

oil are eaten as anthelmintic. Root bark and stem bark is used as tonic to treat malaria.

**7. *Alocasia macrorrhiza* (L) G Don.**

**Local Name :** Kolakachu (As); Cocoyam/Arum (Eng), Thaso- gwswm (Bodo)

**Family:** Araceae.

**Parts used:** Rhizome, Tender leaf.

**Medicinal uses:** Paste of rhizome is applied on abscesses to export pus. Tender leaf with petiole-sour curry is said to prevent tonsillitis pcepta of leaves is applied on forehead for curing headache.

**8. *Bacopa monierie* (L) Penn.**

**Local Name :** BrahmiSak (As), Brahmi-megong (Bodo), Bacopa (English)

**Family :** Scrophulariaceae.

**Parts used:** whole plant.

**Medicinal uses:** Paste of whole plant is used as liniment in the chest of children in cough. Juice is given as blood purifier (about 25mL twice daily for fortnight). Decoction is prescribed in nervous disorders and used as brain tonic (about 50ml twice daily for a month) Leaf juice is given to infants in bronchitic (about 20ml twice daily for a week).

**9. *Catharanthus roseus* (L) D.Don; *Syn-Vincarosea* Linn.**

**Local Name:** Nayantora (As); Periwinkle (Eng.), Dawdai-bibar (Bodo)

**Family:** Apocynaceae.

**Part Used:** Stem and leaf.

**Medicinal uses:** Stem juice of the plant is used as remedy for diabetes Infusion of leaves is administered in menorrhagia. Juice of leaves is applied in insect bite.

**10. *Centella asiatica* (L)**

**Local Name:** Bar manimuni (As); Indian pennywort (Eng.), Gedetjat-manimuni (Bodo)

**Family :** Apiaceae

**Parts used:** Whole plant.

**Medicinal uses:** Whole plant paste is given in dysmenorrheal (about 50 mg thrice daily during the period of menses). Juice is applied on forehead in headache. Leaf juice is given in appendicitis (about 15ml twice daily for a week). Eaten in curries in peptic ulcer, diarrhea, dysentery, dyspepsia and as tonic in general weakness. The plant is used in the treatment of fever and to get relieve internal heat. It is also used to shoot headache or burns.

**11. *Curcuma longa* (L)**

**Local Name:** Halodhi (As) Turmeric (Eng), Haldai (Bodo)

**Family :** Zingiberaceae.

**Parts used:** Rhizome.

**Medicinal uses:** Juice of rhizome is given as blood purifier (about 15ml daily in empty

stomach for a fortnight). Juice mixed with lukewarm water and a pinch of common salt is given in whipping cough and also in common cold (twice daily till cure). Juice mixed with leaf juice of *Tamarindus indica* is given in pox (about 20ml twice daily for a fortnight). Juice is applied to cuts, wounds, pimples, ringworm and other skin diseases which is said to prevent septic. Powder of the dried rhizome mixed with honey and made into globules of about 50gm each and is given in peptic ulcer (twice globules twice daily for a fortnight). Dried powdered used as condiment, rhizomes are boiled and sundried' for use as condiment.

### 12. *Leucas aspera* (Roth) Spreng).

**Local Name:** Doron (As) Leucus/ Thumba (Eng) Boromphutra (Bodo)

**Family:** Lamiaceae.

**Parts used:** Leaf and root.

**Medicinal used:** Leaf juice is used as appetizer as preventive for pox and as vermicide. Root juice is applied in conjunctivitis. Leaf juice is given as colling agent (about 20ml twice daily). It is also used for sinus trouble, rheumatism and skin diseases. Deconction of 5-6 leaves of it and 10-11 leaves of *Ocimum sanctum* mixes with honey is given in hoping cough for quick recover (about 25ml. Thrice daily till cure)

### 13. *Lawsonia inermis* L. Syn; Law Sonia alba Lamk.

**Local Name:** Jetuka (As); Mehendi (Eng.), Jenthoka (Bodo)

**Family:** Lythraceae.

**Medicinal uses:** Decoction of bark is used in the treatment of jaundice and enlargement of liver (about 25ml thrice daily for a fortnight) Leaf paste with mature leave piper betel, *Punica grematum* and *Garcinia pedunculata* is applied to ingrowing of toe nail. It is also applied to itches and other skin diseases. The recipe is said to be more effective in eczema of toes. Floral paste is applied over the forehead to cure headache. Powdered seeds mixed with ghee is taken in the treatment of dysentery.

### 14. *Mimosa pudica* Linn.

**Local name:** Nilajibon (As); Sensitive plant/touch me not (Eng), Nilajihagra (Bodo)

**Family:** Mimosaceae.

**Parts used:** Leaves, stem and root.

**Medicinal uses:** Root useful for leprosy, dysentery. Paste of roots is applied in vaginal and uterine complaints, asthma, burning sensation. The leaves and stem are used for snake bite. Root juice and stem are used for, snake bite. Root juice is mixed with *Allium sativum* and 50mL of milk is added in it is given in epilepsy.

### 15. *Ocimum canum* Sims (*O. americanum* Linn)

**Local name:** Kula tuloshi (As); Basil (Eng.), Thulungshi (Bodo)

**Family :** Lamiaceae.

**Parts used:** Leaf and roots.

**Medicinal uses:** Leaf paste mixed with honey and *Zingiber officinale* is given in cough, cold and in influenza of children (about 1-5 mL depending on the age from 6 months to 10 years twice daily till cure). Paste is given in irregular menstruation (about 20ml once daily for three days starting from first day of menses). Boiled with tea and the decoction acts as preventive against malaria. The juice is given in heart disease which is said to reduce the level of blood cholesterol (about 20mL once daily for a week) A teaspoonful of fresh juice of root is applied to cure against stings or bites, paste of the fresh root is also effective in case of bites of insects and leeches. Juice is mixed with trace amount of lime used in the treatment of ringworm. Root dried in the sun and powdered and is used for brushing teeth. It is better to mix with mustard oil to make a paste and used as tooth paste.

### **16. *Phyllanthus emblica* Linn.**

**Local Name:** Amlokhi (As); Indian gooseberry/ *Emblica myrobalon* Gaertn. (Eng.), Amlai (Bodo)

**Parts used:** Fruit, leaves, roots and seeds.

**Medicinal uses:** Fruit juice is given in acidity (about 20 ml thrice daily till cure). Decoction with leaves of *Cynodon dactylon* mixed with sugar candy is given in menorrhagia (about 20 mL twice daily for a week). Juice is applied as hair wash to remove dandruff and which is said act as hair tonic. Poultice with seeds of *Seamum orientale* is given in sexual disability of men (about 10gm once daily for two month). Decoction with roots of *Solanum indicum* and stem of *Cuscuta reflexa* is given in influenza (about 20 mL thrice daily for 20 days) Decoction of seeds with shoots of *Punica granatum* is given in typhoid fever (about 25ml twice daily for a month).

### **17. *Psidium guyava* L.**

**Local Name:** Madhurium (as); Guava (Eng.) Sofari (Bodo)

**Family:** Myrtaceae.

**Part used:** Bark.

**Medicinal uses:** Bark poultice is warmed and applied to scorpion stings and other poisonous stings of plants and insects. Decoction with bark of *Punica granatum* and *Citrus medica* mixed with pinch of opium is given in acute blood dysentery (about 10ml once daily in empty stomach for five days). The young leaf juice is used gastriculcer (about 10gm thrice daily for three days), It is also used for expulsion of worms from the body especially for children.

### **Discussion**

The approach of Bodo community to the used and management of their botanical resources is determined by local traditional knowledge of the floral resources available. A total number of 44 species have been collected and identified and claimed to have remarkable curative properties. All these species belong to Angiosperms. A General

inference can also be made from the present study than some species of ethnomedicinal value have enough potential for commercial exploitation which can in turn, be instrumental for upliftment of socio-economic status of Bodo. Due to the following facts all those valuable plants are gradually going to extinct. Due to lack of knowledge of the potentialities of these plants the common people destroy them willfully for the purpose of fuel and fodder. Some are being burnt down or cut down for clearing the jungle. Some are growing and dying unused due to lack of collection. Rapid deforestation, Injudicious and unscientific collection of several rare plants by the agents of business concerns, Indiscriminate cutting down of trees by mischievous contractors enjoying political patronage and cutting down of trees by the underground markets for getting value essence or precious wood like are serious threats to our indigenous ethnomedicinal plants.

On the whole, the present study could bring to light atleast some of the invaluable ethnomedicinal information so far practiced and preserved by the Bodo people. As Bodo people have settled in remote areas, they are directly dependent on plant resources of their surrounding and as a result they have acquired enough knowledge to get benefit from wild plants in their day to day life. Besides, they preserve considerable wealth of information on many wild plants used in herbal medicine. Even for the complicated diseases ethnomedicinal counseling is offered by the "Kabirajas" or the traditional healers among them and they prefer this herbal treatment.

### Conclusion

As Bodo people have settled in remote areas, they are directly dependent on plant resources of their surroundings and as a result they have acquired enough knowledge to get benefit from wild plants in their day to day life. Besides, they preserve considerable wealth of information on many wild plants used in herbal medicine. Even for the complicated diseases ethnomedicinal counseling is offered by the "Kabirajas" or the traditional healers among them and they prefer this herbal treatment to modern medicine. The community has their own nomenclature for the plants and diseases. Besides, appropriate conservation measures for these natural wealth to be undertaken on one hand, on the other hand the wisdom of the community on the process of preparation of medicine to be given due importance.

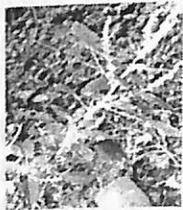


Fig 2: KhuturaSak



Fig 3: Neem



Fig 4: Kolakochu



Fig 5: Bel



Fig 6: BrahmiSak



Fig 7: Nayantora

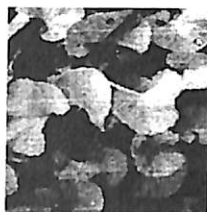


Fig 8: Bar manimuni



Fig 9: Chalkonwori



Fig10: Halodhi



Fig 11: Doron



Fig 12: Jetuka



Fig 13: Nilajibon



Fig 14: Kula tuloshi



Fig 15: Amlokhi

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# Studies on secondary metabolites of three ethno-medicinal plants in Assam, India

**Bimal Dutta<sup>1</sup> & Nayanmoni Borah<sup>2</sup>**

<sup>1</sup> Department of Botany, Sibsagar Girls' College, Sivasagar- 785640

E-mail: bimaldutta88@gmail.com

<sup>2</sup> JRF, Biotech Hub, Sibsagar Girls' College, Sivasagar- 785640

E-mail: bora.nayanmoni54@gmail.com

## Abstract

Three ethnomedicinal plants, namely, *Pogostemon benghalensis*, *Solanum spirale* and *Vitis angustifolia* of Assam, India were screened for secondary metabolites such as alkaloids, flavonoids, phenols, tannins, terpenoids and saponins, and total phenol and flavonoid contents were also studied. The present study carried out on the methyl alcoholic extract of leaves revealed the presence of most of the studied secondary metabolites in all the three species except *V. angustifolia*, where alkaloid is absent. The total phenol contents were 18.8, 13.6 and 11.2 mg/g for *Pogostemon benghalensis*, *Solanum spirale* and *Vitis angustifolia* respectively. Likewise, Total flavonoid contents were 7, 4.6 and 8.6 mg/g for *Pogostemon benghalensis*, *Solanum spirale* and *Vitis angustifolia* respectively. The phenol and flavonoid contents were well confirmed with qualitative investigations. Finding of the study provided evidences that crude extract of the plant species contain medicinally important bioactive compounds and justifies the uses of the plant in the indigenous medicine for the treatment of different diseases.

**Keywords:** Ethno-medicinal plants, secondary metabolites, *Pogostemon benghalensis*, *Vitis angustifolia* and *Solanum spirale*

## 1. Introduction

Assam, a state in the North-Eastern region of India, with a geographical area of 78, 438 sq. km. (88°25' - 96°0' E latitude and 24°5' - 28° 0' N longitudes) with hills and plains, is known for its rich flora and diverse forests and vegetations due to its unique topography, climate and altitude patterns. This region of India is also a homeland of

people belonging to more than 100 ethnic tribes and sub-tribes accordingly has been endowed with rich indigenous knowledge (Dutta & Nath, 1998). More than 500 plant species pertaining to folklore medicines have been reported from this region (Nath, 2011).

*Pogostemon benghalensis* (B.) O. Ktz., *Solanum spirale* Roxb. and *Vitis angustifolia* (Roxb.) Wall., belonging to families Lamiaceae, Solanaceae and Vitaceae respectively, are important ethno-medicinal plants of this region. *Pogostemon benghalensis* (Photo plate 1), locally known as 'Sukloti', is a semicultivated, aromatic and small shrubby plant. A curry, prepared from the tender shoot and leaves of the plant, is eaten in dysentery, diarrhea and post-delivery repairing of female (Gogoi et al. 2003). The ash of the whole plant mixed with mustard oil is used to kill worms in animal wound (Singh, 2003). *Solanum spirale* (Photo plate 2), locally known as 'Bhat-tita or Tita-kuchi', is a wild herbaceous plant. An infusion, prepared from the roots of the plant and mixed with the powder of piper fruits, is given orally in pneumonia fever (Dutta, 2014). Likewise, *Vitis angustifolia* (Photo plate 3), locally known as 'Nol-tenga', is a wild climber plant. Leaf-infusion of the plant is given orally in diarrhea and decoction of shoot is given orally in dysentery. However, these plants are less known to the literature of Indian Medicinal Plants (Kirtikar & Basu, 1935; Dastur, 1952; Chopra et al., 1956, 1969; Satyavati et al., 1976; Ambasta, 1986; Rai and Sharma, 1994).

Secondary metabolites, a group of bioactive substances, having diverse classes of organic compounds like alkaloids, flavonoids, phenols, terpenoids, tannins, etc., are produced through secondary metabolism in different plants. Medicinal value of plants lies in these chemical substances that have definite physiological action on the human body (Akinmoladun et al, 2007). Phytochemical analysis of ethnomedicinal plants for secondary metabolites is an important area of botanical research because of its relevance for the discovery of therapeutic agents and providing clues for new sources of bioactive compounds (Schultes & Raffauf, 1990; Fabricant & Fransworth, 2001). Although hundreds of plant species have been analysed phytochemically, however, works on these plants namely, *P. benghalensis*, *S. spirale* and *V. angustifolia*, have been found to be scanty. Considering the importance, a phytochemical survey for secondary metabolites, phenol and flavonoid contents, a study was conducted.

## 2. Materials and Methods

### 2.1 Collection of Plant Material

Fresh leaves of the plant species were collected from the homestead garden. Taxonomic identification was carried out with the help of regional floras and monographs (Kanjilal et al., 1934 -1940; Islam, 1989). Voucher specimens are deposited in the Herbarium of Sibsagar Girls' College, Sivasagar, Assam and India.

## 2.2 Preparation of Extract of Plant Material

The leaves were washed with running tap water, leaf materials were then air dried under shade and after complete drying the material of each species was grained in mixer. Plant extract were prepared using methanol as extracting solvent. 100g of the dried and powdered plant material (leaf) of each species was extracted with 400ml of methanol at 65°C by using Soxhlet extraction method. After filtering and evaporating to dryness, the crude methanolic extracts were obtained.

## 2.3 Phytochemical Screening for secondary metabolites

Chemical tests were carried out qualitatively on the extract following standard procedures to identify the phytochemical constituents (Edoga et al., 2005; Tiwari et al., 2011).

### 2.3.1 Test for alkaloids:

**Dragendroff's test:** In a test tube containing 1 ml of extract, few drops of Dragendroff's reagent was added and the colour developed was noticed. Appearance of orange colour indicates the presence of alkaloids.

**Mayer's test:** To 1 ml of the extract, 2 ml of Mayer's reagent was added, a dull white precipitate indicates the presence of alkaloids.

### 2.3.2 Test for flavonoids:

**Alkaline reagent test:** To the test solution, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour which turns to colourless by addition of few drops of dilute acetic acid indicated the presence of flavonoids.

**Shinoda test:** To the test solution, a few drops of concentrated HCl and a few pieces of magnesium turning were added. Development of pink or magenta red colour indicated the presence of flavonoids.

### 2.3.3 Test for phenolic compounds:

**Ferric chloride test:** To the test solution, a few drops of ferric chloride solution were added. A dark green colour indicates the presence of phenolic compounds.

### 2.3.4 Test for tannins:

**Lead acetate test:** To the test solution, a few drops of 10% lead acetate solution were added. Precipitate formation indicates the presence of tannin.

### 2.3.5 Test for terpenoids:

**Salkowski's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken well and allowed to

stand. Appearance of red colour in the lower layer indicates the presence of steroids. Formation of reddish brown colour of interface after addition of concentrated sulphuric acid to the side carefully (without shaking) indicates the presence of terpenoids.

### 2.3.6 Test for saponins:

**Foam test:** Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously then some drops of olive oil were added. The formation of stable foam was taken as an indication for the presence of saponins.

### 2.4 Determination of total phenol and flavonoid content:

Folin-Ciocalteu method, as described by Nabavi et al., 2008, was used for phenol content determination. Briefly, 100 mg (dry powder) plant sample was dissolved in 10 ml methanol of 50% (v/v with distilled water). The solution was filtered. 0.5 ml of the filtrate was mixed with 2 ml of Folin-Ciocalteu reagent (1:1 diluted with distilled water) and mixed thoroughly. After five minutes 2 ml of 10%  $\text{Na}_2\text{CO}_3$  solution was added. The solution was warmed for one minute, and then cooled. After one hour at room temperature absorbance was measured at 760 nm with UV-Visible spectrophotometer. Sample blank was concomitantly prepared containing 0.5 ml distilled water, 2 ml of Folin-Ciocalteu reagent and 2 ml of 10%  $\text{Na}_2\text{CO}_3$  dissolved in water. Total phenol content was calculated as gallic acid equivalent from a calibration curve. The calibration curve was prepared by preparing gallic acid solutions at concentration 10, 25, 50, 100, 200 and 250  $\mu\text{g}/\text{ml}$  in methanol (50%). Total phenol content is expressed in terms of gallic acid equivalent as mg/g of dry mass.

Colorimetric aluminum chloride method, as described by Nabavi et al., 2008, was used for flavonoid content determination. Briefly, 100mg (dry powder) plant sample was dissolved in 10 ml of methanol. The solution was filtered. 2 ml of the filtrate was mixed with 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The mixture was shaken and kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with UV-Visible spectrophotometer. Total flavonoid content was calculated as quercetin from a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentrations 5, 10, 25, 50, 80 and 100  $\mu\text{g}/\text{ml}$  in methanol. The blank sample was prepared in similar way by replacing aluminum chloride with distilled water. Total flavonoid content is expressed in terms of quercetin equivalent as mg/g of dry mass.

## 3. Results and Discussion

The study was carried out on the methyl alcoholic extract of leaves revealed the presence of most of the studied secondary metabolites in all the three species except *V. angustifolia*, where alkaloid is absent. The result is summarized Table 1.

Various experiments have been demonstrated that flavonoids, phenolic acids are potential antioxidant and antioxidant activity of these compounds is due to their ability to scavenge free radicals. Accumulation of free radicals can cause pathological conditions such as asthma, arthritis, inflammation, neuro-degeneration, heart disease, aging effect, etc. (Cheyner, 2005). Additionally, phenolic compounds act as (i) metal chelators, (ii) antimutagens and anticarcinogens, (iii) antimicrobial agents (Proestos et al., 2005). The growth of many fungi, yeasts and bacteria was inhibited by tannins (Chung et al., 1998). Further, tannins and terpenoids are attributed for analgesic and anti-inflammatory activities. Apart from these, tannins contribute property of astringency i.e., faster the healing of wounds and inflamed mucous membrane (Okwu and Josiah, 2006). Saponin has the potential to lower cholesterol levels in humans due to their hypocholesterolemic effect. Saponins form complexes with cholesterol to reduce cholesterol levels (Adeyemi et al., 2008). Likewise, alkaloids are a diverse group of secondary metabolites found to have antimicrobial activities by inhibiting DNA topoisomerase (Veerachari & Bopaiah, 2011).

Total amount of phenol and flavonoid contents were calculated from gallic acid ( $y = 0.011 - 0.066$ ,  $R^2 = 0.999$ ) and quercetin ( $y = 0.032 - 0.077$ ,  $R^2 = 0.999$ ) standard curves (Figure 1 & 2). The total phenol contents were 18.8, 13.6 and 11.2 mg/g for *Pogostemon benghalensis*, *Solanum spirale* and *Vitis angustifolia* respectively. Likewise, Total flavonoid contents were 7, 4.6 and 8.6 mg/g for *Pogostemon benghalensis*, *Solanum spirale* and *Vitis angustifolia* respectively. The total phenol and flavonoid contents found in their extract were expressed in terms of gallic acid and quercetin equivalent respectively (Table 2). The phenol and flavonoid contents were well confirmed with qualitative investigations. Although, the total amount of flavonoid contents (4.6, 7 and 8.6 mg/g) found to be moderate, but, amount of the total phenol contents (11.2, 13.6 and 18.8 mg/g) are comparatively higher which is encouraging and indicates the antioxidant potential of crude extract.

Table 1. Secondary metabolites constituents in the methyl alcoholic extract of leaves of *P. benghalensis*, *S. spirale* and *V. angustifolia*.

Secondary metabolites	Chemical tests	<i>P. benghalensis</i>	<i>S. spirale</i>	<i>V. angustifolia</i>
Alkaloids	Dragendorff's test	+	+	-
	Mayer's test	+	+	-
Flavonoids	Alkaline test	+	+	+
	Shinoda test	+	+	+
Phenols	Ferric chloride test	+	+	+
Tannins	Lead acetate test	+	+	+
Terpenoids	Salkowski test	+	+	+
Saponins	Foam test	+	+	+

Note: '+' for presence, '-' for absence

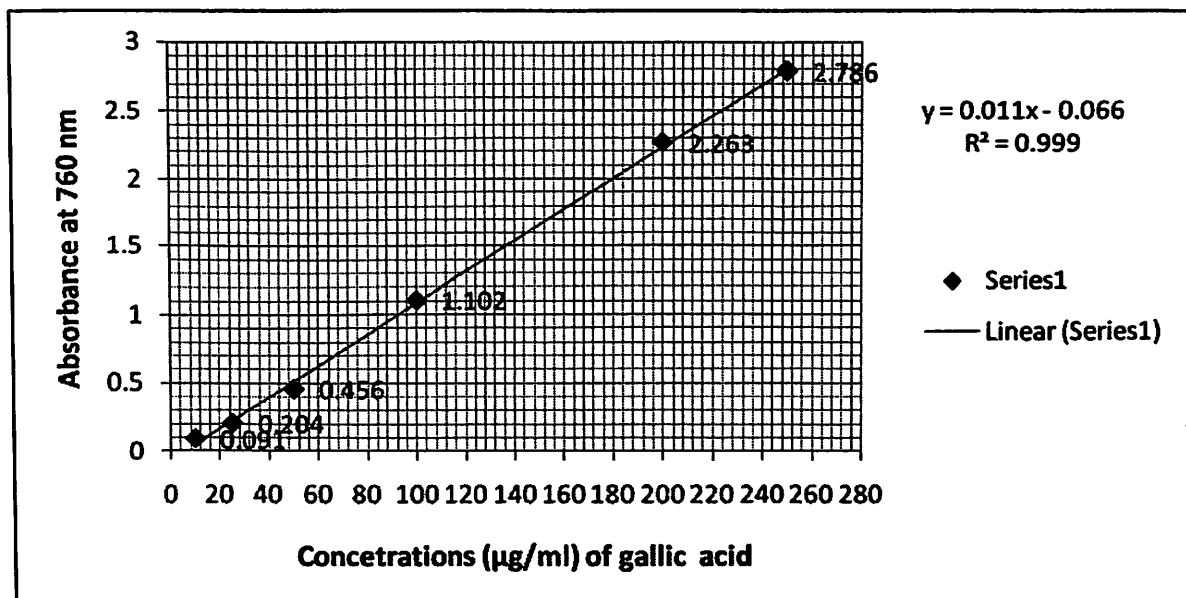


Figure 1. Standard calibration curve of gallic acid for the determination of total phenol content.

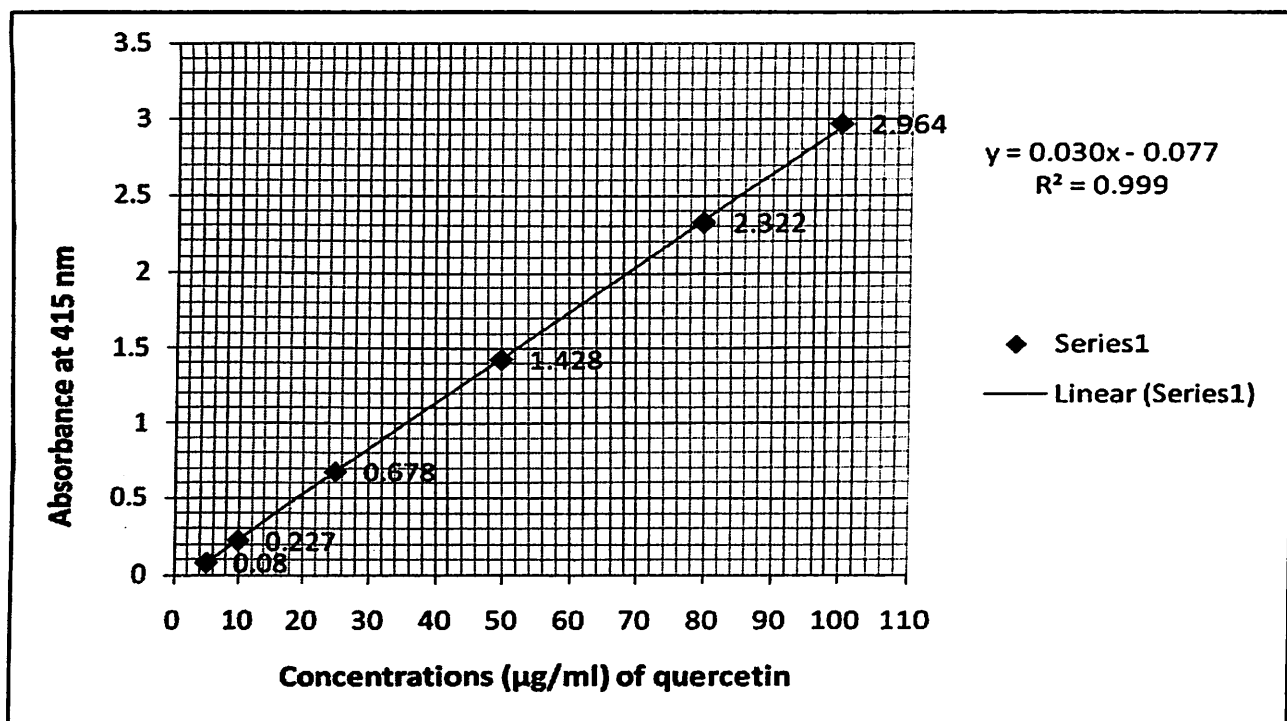


Figure 2. Standard calibration curve of quercetin for the determination of total flavonoid content.

Table 2. Total amount of phenol and flavonoid contents of *P. benghalensis*, *S. spirale* and *V. angustifolia*.

Plant name	Plant part/ Extract name	Total phenol (in mg/g, gallic acid equivalent)	Total flavonoid (in mg/g, quercetin equivalent)
<i>P. benghalensis</i>	Leaves/ Methanol extract	18.8	7
<i>S. spirale</i>	Leaves/ Methanol extract	13.6	4.6
<i>V. angustifolia</i>	Leaves/ Methanol extract	11.2	8.6

#### 4. Conclusion

The plant species screened for secondary metabolites and the total phenol and flavonoid contents seemed to have the potency to act as a source of drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. It is also suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of the plant species.

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Photo plate 1. *Pogostemon benghalensis*



Photo plate 2. *Solanum spirale*

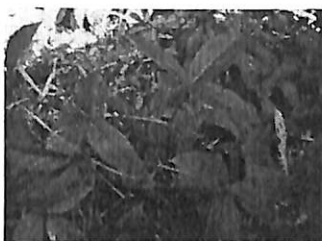


Photo plate 3. *Vitis angustifolia*

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## The sacred holy plant- tulsi (*Ocimum sanctum* L.) - Review

**Bonti Gogoi**

Subject Matter Specialist, KrishiVigyan Kendra, Simaluguri

Nagaon, Assam - 782002

Email: bonti\_gogoi@hotmail.com

### **Abstract**

India is bestowed with rich and varied heritage and cultures. And medicinal and aromatic plants are one of them which represent a consistent part of natural biodiversity. Around 70% of the total medicinal and aromatic plant species of the world are found in India. It is mostly found across the tropical forests of Western Ghats, terrain region, northeast and Himalayan region of India. Since ancient times, among the different medicinal herbs, tulsi has drawn the attention of common people due to its rich medicinal properties. The ethanol extracted from leaves has been shown to cause significant reduction in blood glucose. Tender leaves boiled with tea act as preventive medicine against malarial fever and intermittent fever. The seeds of tulsi are useful in diarrhea, constipation, piles, kidney disorder and gonorrhoea. The juice of tulsi leaves is applied to the skin in ringworm and cutaneous diseases and as drops to relieve earache. It also helps in purifying the air of the surroundings which is a major reason of planting tulsi at our courtyard and worshipping it. Tulsi is biennial/triennial shrub, erect, herbaceous, softly hairy, 30-60cm high; leaves are elliptic- oblong, pubescent on both sides, flowers are white or purplish in colour.

Other than the above uses, there are many other applications of tulsi. The plant contains mainly phenols, aldehydes, tannins, saponin and fats. The essential oil components are eugenol (about 71%), eugenol methyl ether (20%), nerol, cineole, linalool & carvacrol. Cultivation of medicinal and aromatic plants especially high value is creating a new dimension in the field of agriculture. The need for developing countries to acquire technologies and technique for programmed cultivation of medicinal and aromatic plants is of current issue. There is a need for a scientific approach for propagation of medicinal and aromatic plants and to collect relevant information regarding agro-technology,

genuine planting material, high yielding varieties etc. One has to explore wild medicinal and aromatic plants species to reduce the exploitation and conservations of our genetic stocks of tulsi.

**Keywords:** Tulsi, Chemical constituents, medicinal value, Conservation, classification of tulsi, therapeutic uses

### **Introduction**

Tulsi has been known as Holy Basil for thousands of years, as even its presence is purifying and satvic(peaceful) in nature. Tulsi is a very versatile and powerful herb, allowing its healing properties to cover a wide range of areas. The physical presence of the plant cleanses energy and the environment; but when taken internally, this herb can heal a wide range of imbalances as well. For Hindus, it is one of the most sacred plants. In fact it is known to be the only thing used in worship which, once used, can be washed and reused in pooja - as it is considered as self-purifying. Tulsi is a commonly used herb in the world of Ayurveda, and is now becoming better known in the West part of the world. Although Tulsi can be powerfully healing, it is said to be satvic(peaceful) by nature, making it suitable for all body-types in moderation (will increase Pitta in excess), with no known side effects.

In parts of India, all of the basilis are honoured as Tulsi. The leaves of Tulsi are most commonly used for their health benefits, although all parts of the plant, including the roots, stems, flowers and seeds, have significant and differing medicinal and religious symbolic properties. Tulsi beads, made from the woody stalks, are commonly strung in necklaces, bracelets, belts, and meditation malas or rosaries, which are believed by many to have spiritual as well as physical protection benefits.

### **Mythological Importance of Tulsi**

Tulsi, which is Sanskrit for "the incomparable one". In India, this sacred plant is often kept in each family's house or house yard, allowing it to bestow its blessings. The presence of tulsi is spiritually and physically cleansing, as it is known to absorb positive ions and energize negative ones; clearing the air of dust, mold spores, pollen, pet dander, odors, cigarette smoke, bacteria and viruses.

According to Brahma Vaivarta Purana, tulsi is an expression of Sita. Tulsi was the devoted wife of Shankhachuda, celestial being. She believed that Lord Krishna tricked her into sinning. So she cursed him to become a stone (shaaligraama). Seeing her devotion and adherence to righteousness, the Lord blessed her saying that she would become the worshipped plant, tulsi that would adorn his head. Also that all offerings would be incomplete without the tulsi leaf - hence the worship of tulsi. She also symbolizes Goddess Lakshmi, the consort of Lord Vishnu. Those who wish to be righteous and have a happy

family worship the tulsi. Tulsi is married to the Lord with all pomp a show as in any wedding. This is because according to another legend, the Lord blessed her to be his consort. Satyabhama once weighed Lord Krishna against all her legendary wealth. The scales did not balance till a single tulsi leaf was placed along with the wealth on the scale by Rukmini with devotion. Thus, the tulsi played the vital role of demonstrating to the world that even a small object offered with devotion means more to the Lord than all the wealth in the world.

Several medicinal properties have been attributed to the plant not only in Ayurveda and Siddha but also in Greek, Roman and Unani system of medicines. Besides oil, the plant also contains alkaloids, glycosides, saponins and tannins. The leaves contain ascorbic acid and carotene as well. The present day information about the chemical properties is based on the various studies that have been done in different parts of the world and it is likely that chemical constituents may be varying due to edaphic and geographic factors. In the ceremony of Tulsi Vivah, tulsi is ceremonially married to Krishna annually on the eleventh day of the waxing moon or twelfth of the month of Kartika in the lunar calendar. This day also marks the end of the four-month Chaturmashya period, which is considered inauspicious for weddings and other rituals, and so the day inaugurates the annual marriage season in India. The ritual lighting of lamps each evening during Kartika includes the worship of the tulsi plant, which is held to be auspicious for the home. Vaishnavas especially follow the daily worship of tulsi during Kartika.

### **Origin and distribution**

Basils are native to tropical Asia, likely to be originated in India. Tulsi varieties readily grow wild in many areas of Asia and Africa. *O. sanctum* is native throughout the world tropics and widespread as a cultivated plant and an escaped weed. It is cultivated for religious and medicinal purposes and for its essential oil [1]. In India, the plant is grown throughout the country from Andaman and Nicobar islands to the Himalayas up to 1800 meters above the sea level. It is also abundantly found in Malaysia, Australia, West Africa and some of the Arab countries.

### **Morphology**

Tulsi is an erect, much branched sub-shrub 3060cm tall, with simple opposite green or purple leaves that are strongly scented and hairy stems. Tulsi is cultivated annually from seed, although it can also be propagated from tip or root cuttings. It is usually planted (or transplanted) immediately after the rainy season ends. Much larger specimens have been noted and under special circumstances an individual plant may live for a decade or more. Leaves have petiole and are ovate, up to 5cm long, usually somewhat toothed. Leaf color ranges from light green (Vana) to dark purple (Krishna). Flowers are purplish in elongate racemes in close whorls and the tiny flowers range from white to reddish purple [2].

## Cultivation

Basil grows as a perennial in tropical climates, and is planted as an annual in temperate regions, where it may be sown directly from seed or transplanted. While other members of the basil family grow well under competitive circumstances, basil prefers little competition for sun and water. As basil is a highly frost sensitive plant, it must be protected against temperatures close to freezing. Basil prefers to be grown in full sun, however will grow better in partial shade. To avoid "damping off" disease, basil should not be overwatered. The leaves of the basil plant are most commonly used and can be increased in yield by pinching off flowers as they appear throughout the growing season. Basil may be sown outside after there is no danger of frost, or started inside and transplanted outdoors for an earlier harvest. Seeds germinate in four or five days and remain viable for years if stored in dry conditions. Opinions vary on the optimal distance between plants and between rows of basil; 8 inches between plants and rows may be used for efficient production. In colder climates and in winter, basil may be productively cultivated indoors in pots [3].

## Classification and description

Kingdom:Plantae

Class:Magnoliopsida

Order:Lamiales

Family:Lamiaceae

Genus:Ocimum

Few known important species of genus *Ocimum* which grow in different parts of the world and are known to have medicinal properties are described below.

1. *Ocimum sanctum* L. (Tulsi),
2. *Ocimum gratissimum* (Ram Tulsi),
3. *Ocimum basilicum* (Ban Tulsi),
4. *Ocimum kilimands charicum*,
5. *Ocimum americanum*,
6. *Ocimum micranthum*

*Ocimum sanctum*, known as tulsi in Hindi and holy basil in English, is an erect softy hairy aromatic herb or undershurb found throughout India. Tulsi is commonly cultivated in gardens. Two type of *Ocimum sanctum* are met within cultivation i.e. tulsi plant with green leaves known as sritulsi & tulsi plant with purple leaves known as Krishna tulsi. *Ocimum sanctum* is held sacred by Hindus and is used as medicinal plants in day to day practice in Indian homes for various ailments. It is an erect herbaceous branched, hairy , 30-75 cm. high, Leaves elliptic- oblong, acute or obtuse, entire or serrate, pubescent on both sides, minutely gland - dotted: flower purplish or crimson, in racemes , close whorled; nutlets sub-globose or broadly ellipsoid, slightly compressed

nearly smooth, pale brown or reddish, with small black markings[4].

*Ocimum gratissimum*, also known as African basil, or African Basil Wild basil in Hawaii, where it has naturalized. *Ocimum gratissimum*, also known as Clove Basil. It has pale yellow flowers, tall, branched, 1-2.5 meter high. Leaves ovate, coarsely-crenate, gland dotted, pubescent in both surface; flower pale greenish yellow, in simple or branched racemes, moderately close whorled; nutlets sub-globose, rugose, brown, with glandular depression, not mucilaginous [4]

*Ocimum basilicum* is an erect, almost glabrous herb, 30-90 cm. high, Leaves ovate-lanceolate, acuminate, toothed or entire glabrous on both surfaces, glandular; flowers white or pale purple, in simple or much branched racemes, often thyrsoid; nutlets ellipsoid, black pitted [4].

*Ocimum kilimancharicum* is an erect, sweet, pubescent herb, 30-60 cm. height, leaves elliptic-lanceolate, entire or faintly toothed, almost glabrous, gland dotted; flowers are small, leaves are ovate or oblong shaped, acute narrow at base, deeply serrated, pubescent on both surfaces; flowers in 4-6 flowered whorls on long villose racemes; nutlets ovoid to ovoid oblong, black to brown. [5]. It is an economically important medicinal perennial herb that is widely distributed in East Africa, India and Thailand. It is extensively grown in the Tropics [4].

*Ocimum americanum* (syn. *O. canum*) is a native of tropical Africa. It is known as lime, hairy or hoary basil, is an annual herb with white or lavender flowers. It is used for medicinal purposes. Plant shows antimicrobial and antioxidant activity [6]. *Ocimum americanum* contains volatile oil, flavanoids, carbohydrates, phytosterols, tannins and fixed oils [6].

*Ocimum micranthum*, or Amazonian basil, is a South American variety often utilized in ayahuasca rituals for its smell which is said to help avoid bad visions [7]. It possess antibacterial, antiprotozoal and antioxidant activity [8].

### **Biochemical composition of *Ocimum sanctum***

The leaves of *O. sanctum* contains 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpene hydrocarbon caryophyllene [9]. Fresh leaves and stem of *O. sanctum* extract yielded some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosmarinic acid and appreciable quantities of eugenol [10]. Two flavonoids, viz., orientin and vicenin from aqueous leaf extract of *O. sanctum* have been isolated. Ursolic acid, apigenin, luteolin, apigenin-7-O-glucuronide, luteolin-7-O glucuronide, orientin and molludistin have also been isolated from the leaf extract [11]. *O. sanctum* also contains a number of sesquiterpenes and monoterpenes viz., bornyl acetate,  $\alpha$ -elemene, neral,  $\alpha$ - and  $\beta$ -pinenes, camphene, campesterol, cholesterol, stigmasterol and  $\beta$ -sitosterol [12]

The chemical composition of *O. sanctum* is highly complex, containing many

nutrients and other biologically active compounds, the proportions of which may vary considerably between strains and even among plants within the same field. The various chemical constituents of *O. sanctum* are shown in Table 1.

Table 1: Chemical constituents of *Ocimum sanctum*

Plant parts	Extracts	Chemical constituents	References
Leaves / areal parts	Alcoholic extract	Aesculectin, Aesculin, Apgenin, Caffiec acid, Chlorogenic acid, Apigenin, Apigenin-oglucuronide, Triacontanolferulate, Vicenin-2, Circineol, Gallic acid, Galuteolin, Isorientin, Isovitexin, Circineol, Luteolin, Molludistin, Orientin, Procatechuic acid, Stigmasterol, Urosolic acid, Vallinin, Viceni, Vitexin, Vllinin acid	[13], [2],[15], [16]
Whole plant	Vitamin and mineral contents	Vitamin C, Vitamin A, Vitamin E, Calcium, Phosphours, Chromium, Copper, Carotene, Zink, Iron, Nickel	[16], [2],[15]
Leaves	Essential oil	Aromadendrene oxide, Benzaldehyde, Borneol, Bornyl acetate, Camphor, Caryophyllene oxide, cis- $\alpha$ -Terpineol, Veridifloro, Cubenol, Cardinene, D-Limonene, Eicosane, Eucalyptol, Eugenol, Methyl Eugenol, Farnesene, Farnesol, Furaldehyde, Germacrene, Heptanol, Humulene, Limonene, n-butylbenzoate, Ocimene, Oleic acid, Sabinene, Selinene, $\alpha$ -Camphene, $\alpha$ -Myrcene, $\alpha$ -Pinene, $\beta$ -Pinene, $\alpha$ Thujene, $\beta$ -Guaiene, $\beta$ -Gurjunene, Methyl Chavicol, Linalool, Cirsilineol, Circimaritinphytol, Isothymusin, Apigenin, Rosameric acid, Octane, Nonane, Benzene, Iedol, Cadinene, Borneol	[17], [18],[2], [19], [15], [14]
Seeds	Fixed oil	Linoleic acid, Linolenic acid, Oleic acid, Palmitric acid, Stearic acid, Sitosterol, Dilinolenol-linolins, Linodilinolin, Hexoureic acid	[20], [2],[15], [21]
Whole plant	Secondary metabolites	Alkanoids, Steroids, Tannins, Phenol compounds, Flavonoids, Resins, Fatty acids, Gums	[22]

## Pharmacological importance of *Ocimum sanctum*

Tulsi has enormous pharmacological properties. The description of all properties is yet a researchable issue. Keeping in view about the importance, some properties which are important in day to day life is described below.

**Antioxidant activity:** *O. sanctum* possess high antioxidant activity and it is mainly associated with the flavonoids present in plant parts. [23-26]. Antioxidant activity of the flavonoids (orientin and vicenin) in vivo was expressed in a significant reduction in the radiation induced lipid peroxidation in mouse liver [27]. Extracts from plants of *O. sanctum* has a significant ability to scavenge highly reactive free radicals [28]. The phenolic compounds, viz., cirsilineol, cirsimaritin, isothymusin, apigenin and rosmarinic acid, and appreciable quantities of eugenol (a major component of the volatile oil) from the fresh leaves and stems of *O. sanctum* possessed good antioxidant activity [29].

**Adaptogenic activity/antistress activity:** Tulsi is one of the most effective adaptogen which is caused by tension, emotional difficulties, poor life style habits, disease and infection, pollution and other factors. The immunostimulant capacity of *O. sanctum* may be responsible for the adaptogenic action of plant [30]. The alcoholic extract of *O. sanctum* whole plant increased the physical endurance (survival time) of swimming mice, prevented stress induced ulcers and milk induced leucocytosis, respectively in rats and mice, indicating induction of non-specifically increased resistance against a variety of stress induced biological changes by *O. sanctum* in animals [31].

**Immuno- modulator activity:** This activity helps the body to fight against antigens like disease causing agents such as bacteria, viruses, microbes, allergens etc. and helps in maintaining health. Tulsi is one of the effective immuno modulator [32]. Steam distilled extract from the fresh leaves of *O. sanctum* showed modification in the humoral immune response in albino rats which could be attributed to such mechanisms as antibody production, release of mediators of hypersensitivity reactions and tissues responses to these mediators in the target organs [33]. *O. sanctum* seed oil appears to modulate both humoral and cell-mediated immune responsiveness and GABAergic pathways may mediate these immunomodulatory effects [34].

**Anticancer activity:** The alcohol extracted from the leaves of *O. sanctum* has a modulatory influence on carcinogen metabolizing enzymes such as cytochrome P-450, cytochrome-b5, aryl hydrocarbon hydroxylase and glutathione-S-transferase (GST), which are important in detoxification of carcinogens and mutagens [35-36]. The anticancer activity of *O. sanctum* has been reported against human fibrosarcoma cells culture [37]. *O. sanctum* significantly decreased the incidence of benzo (a) pyrine induced neoplasia of stomach of mice and 3'-methyl-4-dimethylaminoazobenzene induced hepatomas in rats [38]. The alcoholic extract of the leaves of *O. sanctum* was shown to have an inhibitory effect on chemically induced skin papillomas in mice [39]. Topical treatment of *O. sanctum* leaf extract in 7,12- dimethylbenz(a)anthracene (DMBA)

induced papillomagenesis significantly reduced the tumour incidence, average number of papillomas mouse and cumulative number of papillomas in mice. Topical application of the extract *O. sanctum* significantly elevated reduced GSH content and GST activities [40]. A similar activity was observed for eugenol, a flavonoids present in many plants, including Tulsi [41]. Oral treatment of fresh leaves paste of Tulsi may have the ability to prevent the early events of DMBA induced buccal pouch carcinogenesis [42]. Leaf extract of *O. sanctum* blocks or suppresses the events associated with chemical carcinogenesis by inhibiting metabolic activation of the carcinogen [43].

**Radioprotective activity:** The flavonoids isolated from *O. sanctum* leaves, (orientin and vicenin) showed better radioprotective effect as compared with synthetic radioprotectors. They have shown significant protection to the human lymphocytes against the clastogenic effect of radiation at low, nontoxic concentrations [39]. The combination of *O. Sanctum* leaf extract with WR-2721 (a synthetic radioprotector) resulting in higher bone marrow cell protection and reduction in the toxicity of WR-2721 at higher doses, suggested that the combination would have promising radioprotection in humans [40].

**Antihypertensive and cardioprotective activities:** The transient cerebral ischemia and long term cerebral hypoperfusion (causing cellular oedema, gliosis and perivascular inflammatory infiltrate) have been prevented by *O. sanctum* [41]. The *O. sanctum* fixed oil administered intravenously produced hypotensive effect in anaesthetized dog, which seems to be due to its peripheral vasodilator action. Essential fatty acids like linoleic and linolenic acids, contained in the *O. sanctum* oil produce series 1 and 3 (PGE1 and PGE3) prostaglandins and inhibit the formation of series 2 prostaglandins (PGE2) [42]. The long term feeding of *O. sanctum* offers significant protection against isoproterenol-induced myocardial necrosis in Wistar rats through enhancement of endogenous antioxidant [43].

**Antimicrobial activity:** Aqueous extract of *O. sanctum* showed growth inhibition for Klesbiella, E. coli, Proteus and Staphylococcus aureus; while alcoholic extract of *O. sanctum* showed growth inhibition for Vibrio cholera [44]. The alcoholic extract of *O. sanctum* was also found to be active against multidrugresistant strains of S. aureus that are also resistant to common beta lactam antibiotics [45]. Similarly, *O. sanctum* was found to be active against resistant *Neisseria gonorrhoea* strains [46]. *O. sanctum* fixed oil showed good antibacterial activity against *Bacillus pumilus*, *Pseudomonas aeruginosa* and *S. aureus*. Higher content of linolenic acid in *O. sanctum* fixed oil could contribute towards its antibacterial activity [47].

**Central Nervous System (CNS) depressant activity:** The alcoholic extract of *O. sanctum* prolonged the time of lost reflex in mice due to pentobarbital (40 mg/kg, ip), decreased the recovery time and severity of electroshock and pentylenetetrazole induced convulsions. It also decreased apomorphine induced fighting time and

ambulation in "open field" trials. At high doses, *O. sanctum* extract increased swimming time suggesting a CNS stimulant and/or antistress activity. The effect was comparable to that of desipramine, an antidepressant drug. *O. sanctum* fixed oil (2-3 ml/kg, ip) has been reported to increase pentobarbitone-induced sleeping time in rats. The inhibition of hepatic metabolism of pentobarbitone / renal clearance by fixed oil could be responsible for potentiation of pentobarbitone-induced sleeping time [48].

**Analgesic activity:** The *O. sanctum* oil was found to be devoid of analgesic activity in experimental pain models (tail flick, tail clip and tail immersion methods). However, it was effective against acetic acid induced writhing method in mice in a dose dependent manner. The writhing inhibiting activity of the oil is suggested to be peripherally mediated due to combined inhibitory effects of prostaglandins, histamine and acetylcholine [48].

#### **Anti-inflammatory activity:**

Methanolic extract (500 mg/kg) and aqueous suspension of *O. sanctum* showed analgesic, antipyretic and anti-inflammatory effects in acute (carrageenan-induced pedal oedema) and chronic (croton oil induced granuloma and exudate formation) inflammations in rats [49]. The fixed oil and linolenic acid possess significant anti-inflammatory activity against PGE<sub>2</sub>, leukotriene and arachidonic acid induced paw oedema in rats by virtue of their capacity to block both the cyclo-oxygenase and lipoxygenase pathways of arachidonic acid metabolism [50-51].

**Antipyretic activity:** The antipyretic activity of *O. sanctum* fixed oil was evaluated by testing it against typhoid-paratyphoid A/B vaccine-induced pyrexia in rats. The oil on administration considerably reduced the febrile response indicating its antipyretic activity. At a dose of 3 ml/kg, the antipyretic activity of the oil was comparable to aspirin. Further, the fixed oil possessed prostaglandin inhibitory activity and the same could explain its antipyretic activity [52].

**Hepatoprotective activity:** Oral administration of hydroethanolic extract of *O. sanctum* leaves @ 200 mg/kg in male Wistar albino rats gave protection against liver injury induced by paracetamol [53]. The cold water extract (3g/100 g, orally for 6 days) of *O. sanctum* was found to be effective against carbon tetrachloride (0.2 ml/100 g, subcutaneously) induced liver damage in albino rats [54].

**Memory enhancer activity:** The Alcoholic extract of dried whole plant of *O. sanctum* ameliorated the amnesic effect of scopolamine (0.4 mg/kg) and aging-induced memory deficits in mice. Passive avoidance paradigm served as the exteroceptive behavioural model. *O. sanctum* extract increased stepdown latency (SDL) and acetyl cholinesterase inhibition significantly. Hence, *O. sanctum* can be employed in the treatment of cognitive disorders such as dementia and Alzheimer's disease [55].

**Antifertility activity:** Benzene extract of fresh *O. sanctum* leaves in male rats showed decreased total sperm count, sperm motility and weight of testis [56]. The long

term feeding (up to 3 months) of *O. sanctum* leaves (200 and 400 mg/kg) to adult male and female albino rats along with normal diet decreased sperm count, sperm motility and weight of male reproductive organs [57].

**Antidiabetic activity:** Oral administration of *O. sanctum* extract led to marked lowering of blood sugar in normal, glucose fed hyperglycemic and streptozotocin-induced diabetic rats [58]. A randomized, placebo-controlled, cross over single blind human trial indicated a significant decrease in fasting and postprandial blood glucose levels by 17.6% and 7.3%, respectively. Urine glucose levels showed a similar trend [59]. Further, *O. sanctum* has aldose reductase activity, which may help in reducing the complications of diabetes such as cataract, retinopathy, etc [60].

### Conclusion:

Tulsi has promising potential as a medicinal herb. Since it is available throughout the globe, the importance should be realized to provide additional immunity to mankind at very low cost. The chemical constituents are highly useful and its pharmacological properties can be utilized for curing various emerging human ailment. The treatment of different diseases can be possible with the proper utilization of this plant. But for the efficient utilization of this plant, it should be brought under commercial cultivation. This can bring a sustainable development to our society.

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# Traditional knowledge of Herbal practitioners and local people regarding the Ethnomedicinal uses of indigenous plants in Padumoni Tea Estate Golaghat district, Assam, India

**Dilip Tamang**

P.G. Department of Life Sciences, Debraj Roy College, Golaghat, Assam, India  
Email:dilipt23@gmail.com

## **Abstract**

A survey was conducted on the Ethnomedicinal uses of various plants in the Padumoni Tea Estate of Golaghat district, Assam. The aim of this paper is to document some traditional practices against various ailments by the people of the study area. The herbal practitioners and local people were interviewed with questionnaires regarding the use of plants against certain common diseases like Cold, Cough, Gastric, Dysentery etc. This survey work and documentation reveals 26 taxa belonging to 22 families which are used by the local community of Padumoni Tea Estate. Proper identification, and conservation of these medicinal plants are very essential.

**Key words:** Ethnomedicinal use, Traditional practices, Documentation, Medicinal plants.

## **Introduction**

Ethnobotany may be defined as an anthropocentric approach to botany and is essentially concerned with gathering information on plants and their use. (Rao and Henry 1997). A large number of Ethnomedicinal information remained endemic to certain regions or people due to lack of communication. India is the second largest country in the world in respect of population. Over 550 tribal communities are covered under 227 ethnic groups residing in about 5000 villages in India in different forests and vegetation types. The ethnic and rural people of India have preserved a large bulk of traditional knowledge of medicinal uses of plants growing around them. This knowledge is handed down to generations through word of mouth and is extensively used for the treatment of

common diseases and conditions. (Mishra, Dwivedi, Shashi and Prajapati, 2008). Herbal medicine even today plays an important role in rural areas and various locally produced drugs are still being used as household remedies for different ailments. (Qureshi & Ghufra 2005). As the medicinal plants in nature are easily available and cheap, so the people from rural areas of developing countries use traditional knowledge against various ailments. The World Health Organization (W.H.O.) reported that 80% of the world populations rely chiefly on indigenous medicine and that the majority of traditional therapies involve the use of plant extracts or of their active constituents. (Rahman, Sultana, Islam and Zaman 2013). The local inhabitants have enormous knowledge about medicinal uses of plants is mostly undocumented and transmitted orally from generation to generation. Therefore, it is urgent to explore and document this unique and indigenous, traditional knowledge, before it diminishes with the knowledgeable persons. Further, documentation of indigenous and traditional knowledge is very important for future critical studies which lead to sustainable utilization of natural resources. (Lokho and Narasimhan 2013). However, one of the greatest difficulties confronting the research workers is the inadequate authentic information on the identification of the plants as recorded in those ancient literatures (Barukial and Sarmah 2011). This present study is concerned with the survey regarding the use of various plants with Ethnomedicinal value in the selected study site and to document them with the plant parts used against common ailments.

## **Materials and method**

### **Study area**

The Golaghat district is located in between 26°0'-27°1' N and 93°0'-94°18' E latitude and longitude respectively. It stretches an area of 3502 sq.km. Area under urban sector is 31.52 sq. Km. while the area under rural sector is 3470.48 sq.km. Golaghat constitutes 4.46% of the total area of Assam state in terms of area. The Golaghat District has extreme climate with large variation of temperature from season to season as also from day to night. The highest temperature recorded is 38°C and lowest at 14°C. The study site, Padumoni Tea Estate is situated 5.8 km. away from Golaghat on the Golaghat-Jorhat road. It is situated on a distance of 5.8 km from Golaghat. The latitude and longitude are 26°54'74"N and 93°97'83"E respectively. The local people of the area are basically the tea garden community and other community such as Ahom, Kalita etc. There are around 300 houses of Tea garden community people. The people are dependent on surroundings including the plants for their basic needs.

### **Methods of information and data collection**

The current study was carried out as a survey work in the locality of Padumoni Tea Estate of Golaghat district during the month of October 2016. The local people,

mainly the age-old people of the locality and the experienced Herbal practitioners were interviewed with certain questionnaires. They were consulted for the use of various locally available plants, plant parts against different kinds of common diseases. The vernacular name, plant part used etc. were collected and enlisted. The identification of the plants were done in their natural habitats. Moreover, the plants were collected as a voucher specimen and identified by consulting with the experienced people from taxonomy background. With the help of available literature, the medicinal use of the plants were cross checked and verified.

## Result

The survey work carried out throughout the study area revealed certain plant species which are medicinally very important. Among them there are mostly angiosperm and only one species is pteridophyte. (Indicated by \*). The plants were enlisted in table 1.

Table 1: Ethnobotanical aspects of the plants used by the people of Study area

Sl. No	Botanical Name	Family	Common Name (Assamese)	Plant part used	Ethnomedicinal aspects
1	<i>Acorus calamus</i> L.	Araceae	Boch	Underground rhizome	Cold, cough, pneumonia, Fever
2	<i>Ageratum conyzoides</i> L.	Asteraceae	Gundhua-bon	Leaves and tender shoots	Cuts and wounds
3	<i>Allium sativum</i> L.	Amaryllidaceae	Nohoru	Bulb	Cough and bronchitis
4	<i>Alstonia scholaris</i> . (L.) R. Br.	Apocynaceae	Sotiyona	Bark	Malaria
5	<i>Amphireurono pulatum</i> *	Thelypteridaceae	Biholongoni	Leaves	Possesses insecticidal properties
6	<i>Azadirachta indica</i> A. Juss.	Meliaceae	Mohaneem	Leaves and fruits	Skin diseases
7	<i>Bryophyllum Calycinum</i> Salisb.	Crassulacaceae	Duportenga	Leaves	Kidney stone
8	<i>Calotropis gigantea</i> (L.) Dryand.	Asclepiadaceae	Akon	Leaves	Against dog bite
9	<i>Carica papaya</i> L.	Caricaceae	Omita	Fruit	Against Gastric
10	<i>Coccinia grandis</i> (L.) Voigt	Cucurbitaceae	Kunduli	leaves	Ear problem

11	<i>Colocasia esculenta</i> (L.) Schott	Araceae	Kochu	Petiole	Juice is used in cuts and itching
12	<i>Costus speciosus</i> (J.Koenig) Sm.	Zingiberaceae	Jomlakhuti	Rhizome	Jaundice and Diabetes
13	<i>Curcuma longa</i> L.	Zinziberaceae	Halodhi	Rhizome	Juice is Pain killer
14	<i>Euphorbia nerifolia</i> L.	Euphorbiaceae	Hiju	Leaves	Latex is used in swelling of nails
15	<i>Lantana camera</i> L.	Verbinaceae	Gu-phool	Leaves	Insect repellent
16	<i>Lawsoni aintermis</i> L.	Lythraceae	Jetuka	Leaves	Dandruff and skin diseases
17	<i>Leucas aspera</i> (Willd.) Link	Lamiaceae	Durun Bon	Leaves	Appetite and digestion
18	<i>Nicotiana tabacum</i> L.	Solanaceae	Dhopaat	Leaves	Insect, Leech repellent
19	<i>Ocimum sanctum</i> L.	Lamiaceae	Tuloshi	Leaves	Cough and cold
20	<i>Paederia foetida</i> L.	Rubiaceae	Bhedailota	Leaves	Diarrhoea and rheumatism
21	<i>Piper nigrum</i> L.	Piperaceae	Jaluk	fruit	Cough
22	<i>Psidiumguajava</i> L.	Myrtaceae	Modhuri	Leaflet	Against dysentery
23	<i>Solanumindicum</i> L.	Solanaceae	Bhekuri-tita	Fruit	Effective in cough
24	<i>Spondia spinnata</i> (L. f.) Kurz	Anacardiaceae	Omora	Fruit	Dysentery and possesses antifungal properties
25	<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Am.	Combretaceae	Arjun	Bark	Heart disease
26	<i>Vigna mungo</i> (L.) Hepper	Fabaceae	Maati-maah	seed	Against Rheumatism along with zinger

## Discussion

The plants used by the local people against various ailments belongs to different families. Total 26 genus of plants belonging to 22 families were recorded having crucial medicinal properties. Among them the families, Lamiaceae, Zingiberaceae, Solanaceae and Araceae contains 2 genera of plants. The other remaining families contains 1 genus each (Graph 1).

The medicinal plants were not wholly used in the ailment treatment. The leaves, bark, Seed, fruits rhizome, bulb and petiole were the used plant parts by the local people. 52% of the total plants enlisted they use leaves as a medicine which is the highest used plant part among the all followed by fruit which is 18%.

Graph 1: Number of plants belonging to the respective family

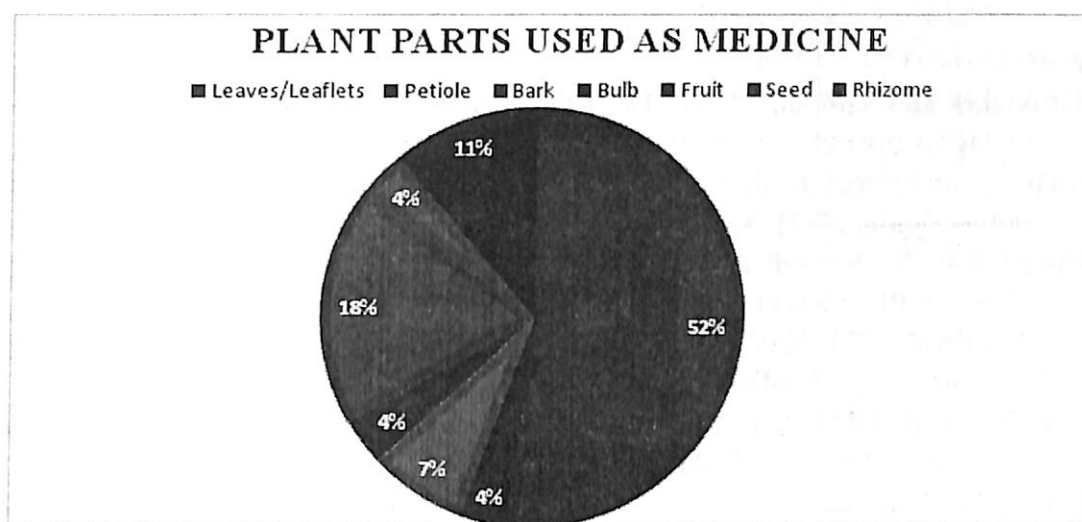
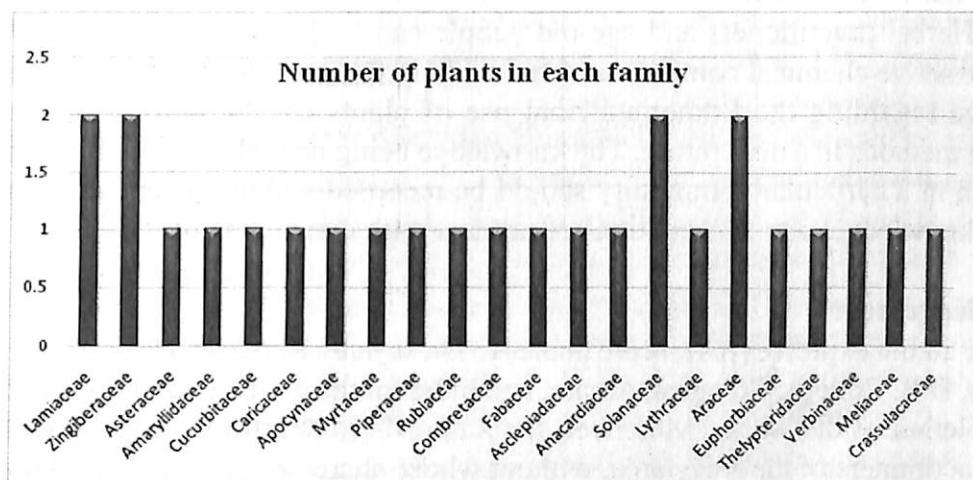


Fig 1: Percentage of plant parts used as ethnomedicine.

The extensive use of plants were recorded and enlisted against various ailments by the local people. It was observed people use the Ethnomedicinal plants as the first line of defence against the ailments. Moreover, some people directly go to the medical treatment as soon as the disease appears. Some local people were also experienced with very little knowledge of Ethnomedicinal plants. This may occurred due to the oral information without proper documentation of the age-old traditional knowledge and lack of awareness.

## Conclusion

The present study reveals that the people in the study area utilizes plants against various health related problems. The data received during the interactions and interviews with the Herbal practitioners and age-old people can be further used to identify and isolate the active chemical compound of the medicinal plant. Moreover, the traditional knowledge regarding the Ethnomedicinal use of plants can be helpful in the drug designing methods in a near future. The knowledge being passed on from generation to generation in a particular community should be recorded and documented so that the valuable knowledges and information are not lost with due course of time.

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# Effect of methanolic root extract of *Careya arborea* Roxb. on normal fertility in albino mice

Evarani Kalita<sup>1</sup> & J.C.Kalita<sup>2</sup>

<sup>1</sup> Assistant Prof., Dept. of Zoology, Handique Girls' College, Ghy-1

<sup>2</sup> Associate Prof., Dept. of Zoology, Gauhati University

Email: evaranikalita@yahoo.com

## Abstract

Nature has been a source of medicinal agents for thousands of years. An impressive number of modern drugs have been isolated from nature. Numerous plants have been used historically to control fertility, and modern scientific research has confirmed anti-fertility effects in at least some of these plants tested. Though modern medicine has provided several preventive methods of contraceptives but none of this is very safe. Nowadays medicinal plants have been screened for contraceptive potential and anti-fertility effects, because of their little or no side effects. To study the effects of *Careya arborea*.Roxb. on pregnancy, methanolic root extracts of this plant was administered orally to normal cyclic mice at different doses and duration. The results show that the minimum effective dose of the root extract to prevent pregnancy was 500 mg/kg bw. The root extract induced strong activity to prevent pregnancy at the dose level 1000 mg/kg bw. The extract showed significant result after treatment of 14 consecutive days. Interestingly the effects were seen to be reversed. All the treated non pregnant mice resume their normal fertility after 44 days of withdrawal of treatment.

**Keywords:** Medicinal plants, root extract, antifertility, pregnancy, reversible effects.

## Introduction

Nature has been a source of medicinal agents for thousands of years. Plants and plant-based products have been used as a valuable and safe natural source of medicines for treating various ailments. An impressive number of modern drugs have been isolated from nature. Numerous plants have been used historically to control fertility, and modern scientific research has confirmed anti-fertility effects in at least some of these plants tested. Though modern medicine has provided several preventive methods of

contraceptives but none of this is very safe. Nowadays medicinal plants have been screened for contraceptive potential and anti-fertility effects, because of their little or no side effects.

Choudhary et al. (1990) observed the anti-implantational and abortifacient effects in rats treated with the ethanolic leaf extracts of *Beaumontia grandiflora*. For antifertility effects, evaluation of herbs has been in progress to identify effective and safe substances. For women who can't use modern forms of contraception, herbs can offer alternatives; and reducing fertility would be better than no birth control (Umadevi et al., 2013). Montasert et al. (2007) observed that administration of alcoholic extract of *Physalis alkekengi* on days 1-5 of pregnancy, significantly decreased the number of implantation sites, number and weight of neonates.

*Rumex steudelii* Hochst is one of the traditionally used antifertility plants in Ethiopia. Studies showed that the methanolic root extract of the plant had reversible antifertility effect in experimental animals. The extract significantly decreased the number of healthy small antral, graffian follicles and corpora lutea with concomitant significant increase in the number of atretic follicles of the same stage in dose dependent manner (Solomon et al., 2010). Roop (2015) reported considerably reduced fertility index in female albino rats after administration of methanol fraction of *Melia azedarach* Linn. seed extract.

In view of current interest in the field of phyto-chemicals with anti-fertility activity, there is an urgent need to investigate the estrogenic activity of traditionally used plant. Therefore the objectives of the present study were to investigate the effects of *Careya arborea* Roxb. on normal fertility in female albino mice to make sure of its efficacy in terms of pregnancy. The root of this plant has been used traditionally as oral contraceptive agent after delivery to prevent pregnancy, by a group of tribal people of Nalbari district, Assam (personal survey report).

## **Materials and Methods**

### **Experimental animals**

All the animals used in the present study were of healthy adult Swiss female albino mice approximately 3 months of age and weighing 20-25gm, taken from the Animal House of the Department of Zoology, Gauhati University.

### **Preparation of root extract**

The methanolic root extract of *Careya arborea* Roxb. was prepared after the method described by Kholkute et al. (1978a). For oral administration the required amount of extracts were suspended in vehicle Tween-80 (Polyoxyethylenesorbitan Mono-oleate: Himedia) @ 0.1 ml/ml normal saline. In our experiment we used 17 $\alpha$ -oestradiol (E2) as positive control. For oral administration, 17 $\alpha$ -oestradiol (Sigma-Aldrich Corporation.

St. Louis, Missouri, USA) was prepared in analytical grade 100% ethanol as 1mM-stock solution and diluted with normal saline containing 10 alcohols for the required concentration. Test materials were orally administered via gastric intubations (Kholkuteet al., 1978a) in a volume of 0.5ml. Treatments were carried out every morning at 24 hr interval for 14 consecutive days.

### **Preparation of vaginal smears**

Vaginal smears were prepared to study the changes associated with estrous cyclicity and duration of estrus in the treated mice. Vaginal smears were prepared as per the method described by Emmens (1941). Using Allen-Doisy (1923) test, vaginal smears were evaluated by scoring the number of cornified cells relative to the population of all cell types present as per the method described by Terenius (1971).

### **Evaluation of lethal dose ( $LD_{50}$ )**

The method of Omkar (1994) was followed for the evaluation of  $LD_{50}$  of the root extract. A range of doses of extracts (50mg/kg bw to 5000mg/kg bw) was administered to the experimental animals (number of animals per group were 10). Animals were exposed to the doses and observed for 96hrs to determine the  $LD_{50}$ .

### **Experimental doses**

In the present study the  $LD_{50}$  value could not be determined as mortality rate was not to the optimum. Depending on the ranges of doses used to obtain  $LD_{50}$  a number of doses of the plant extract (i.e. 50mg, 100mg, 250mg and 500mg, 750mg and 1000mg/kg bw) were used in the present studies for different experimental purposes. In all the experiment, E2 was used as positive control (10 g/kg bw). Two control groups, one untreated and a vehicle treated receiving Tween-80 and normal saline at the ratio 1:10 were accompanied all experiments.

### **Experimental design**

The experiment was designed as per the method described by Sharpe et al. (1995). To conduct the experiment, animals were divided into seven groups, each group having 6 animals. Group I and II were control (untreated) and vehicle control (tween-80 mixed with normal saline). Group III was positive control, receiving 10 $\mu$ g/kg bw of E2. Group IV, V, VI and VII received 250mg, 500mg, 750mg and 1000mg/kg bw of the root extract. At the end of the exposure period, animals were allowed for mating (on getting estrus) with sexually experienced healthy adult mice in the ratio 2:1 (female: male) and kept under good husbandry condition. Mating was confirmed by the presence of vaginal plug. After that males were removed and vaginal smear were studied daily up to day 22 of mating to ascertain the pregnancy. Animals were health checked for any clinical sign.

If they did not deliver by the end of the period of 22 days, it was once again observed for cycles and allowed to mate. For comparisons, animals of the control and vehicle control group were also subjected to the same process. In this way, animals were subjected up to 3rd mating in those animals that did not conceive by the end of 1st and 2nd mating.

After determination of minimum effective dose of the root extracts to prevent pregnancy in adult cyclic female mice, further studies were carried out only with two doses (250mg and 500mg) of the root extract.

Duration of different stages of normal estrous cycle, body weight and clinical sign of the animals were recorded on daily basis throughout the period of experiments.

## Results

The root extract did not show any sign of acute toxicity or mortality over the observation period of 96 hrs up to the dose level of 5000mg/kg bw. Therefore it was assumed that the extract is devoid of any acute toxic effect proving their wide margin of safety up to this dose level.

In present studies, Tween-80 mixed with normal saline at the ratio 1:10 was used as vehicle for different experimental purposes. Vehicle control animals did not reveal any significant difference with control animal for all variables. But, data are shown only for the purpose of clarity.

Determination of minimum effective dose of the root extracts to prevent pregnancy

The minimum effective dose of the root extract to prevent pregnancy was 500 mg/kg bw. The root extract induced strong activity to prevent pregnancy at the dose level 1000 mg/kg bw (results are shown in Table 1).

The root extract had a significant dose-dependent pregnancy inhibitory activity. After 1st mating, in group IV receiving 250mg/kg bw of root extract only 40% pregnancy was inhibited. Animals in groups V, VI and VII receiving 500mg, 750mg and 1000mg/kg bw of the root extract respectively, showed 100% inhibition of pregnancy.

By the end of the repeated 2nd mating (i.e. after 22 days of 1st mating), in group IV (250mg/kg bw), the animals which were not pregnant after 1st mating became pregnant. In group V and VI, 40% and 50% of the treated animals respectively did not conceive after 2nd mating. On the other hand, group VII (1000mg/kg bw) exhibited 100% inhibition of pregnancy even after the repeated 2nd mating.

By the end of the repeated 3rd mating (i.e. after 22 days of 2nd mating), in group V (500mg/kg bw) and VI (750mg/kg bw), the animals which were not pregnant after 2nd mating became pregnant. But the animals of group VII (1000mg/kg bw) exhibited 80% pregnancy inhibitory activity even after this repeated 3rd mating. Therefore, group VII exhibited strong inhibitory activity in comparison with other two groups.

Table 1. Effect of E2 and root extract on pregnancy inhibition.

Experimental groups	No. of animal	% Pregnancy inhibition		
		After 1st mating	After 2nd mating	After 3rd mating
I (Control)	6	*	NN	NN
II (Vehicle control)	6	*	NN	NN
III (10µg E <sub>2</sub> )	10	100	60	**
IV (250mg extract)	10	40	**	NN
V (500mg extract)	10	100	40	**
VI (750mg extract)	10	100	50	**
VII (1000mg extract)	10	100	100	80

\* (Single Asterisk) denote all pregnant

\*\* (Double Asterisk) denote rests were pregnant, NN=No need

E<sub>2</sub> (10µg/kgbw) exhibited 100% inhibitory activity after 1st mating. In this group 60% of the treated animals did not conceive even after 2nd mating. But, by the end of the repeated 3rd mating (i.e. after 22 days of 2nd mating), the animals that were not pregnant after 2nd mating became pregnant. In contrast, all the animals of group I (control) and II (vehicle control) became pregnant immediately by the end of 1st mating.

Effect of the crude extract on food intake and body weight in adult cyclic female mice:

Throughout the period of treatment, the amount of food intake was reduced significantly (Results are shown in figure 1) in the animals of positive control group and the extract treated groups relative to the control groups (13.00±0.19gm and 13.13±0.24gm for control and vehicle control group respectively). Though not statistically significant within treated groups, reduction in food intake was higher in positive control group (11.32±0.31gm) than extract treated groups (11.67±0.34gm and 11.67±0.34gm for group IV and V respectively).

At the end of the treatment, extract treated groups IV (21.90±0.37gm) and V (21.67±0.34gm) showed no change in their body weights relative to the controls. But the positive control group had a significantly reduced body weight (19.95±0.36gm) relative to the controls and extract treated groups. Control and vehicle control group had the values of 21.75±0.20gm and 21.42±0.21gm respectively.

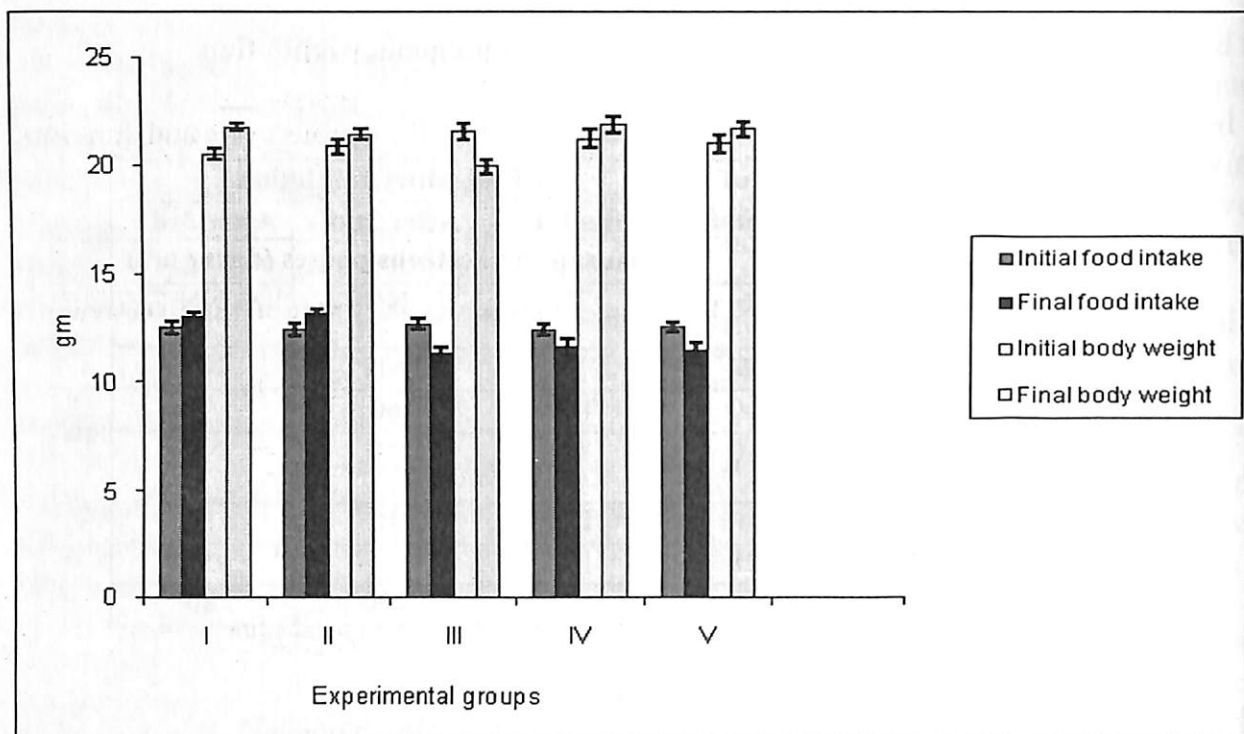


Figure 1. Changes in food intake and body weight before (initial) and after (final) treatment in adult cyclic female mice. Data are presented as mean  $\pm$  SEM (n=10). Where no error bars are visible, the errors were smaller than the symbols. (Here experimental groups I, II, III, IV and V are denoted for control, vehicle control, positive control, lower and higher dose of the extract respectively).

### Effect of the crude extract on the normal reproductive cycle of adult female mice:

The root extract of the plant induced a dose dependent effect on the normal cyclicity. In normal adult cyclic mice the total duration of the estrous cycle was 4-5 days (Table 2). In these animals the estrus was last for about 12-18 hours and the diestrus was last for about 57-62 hrs. Proestrus and metestrus were last for about for 12-15 hrs and 21-25 hrs respectively. In all animals of extract treated group IV, the diestrus phase of the estrous cycle was prolonged (up to 5/6 days) during the treatment period where as the length of estrus (6-8 hrs), metestrus (7-8 hrs) and proestrus (4-5 hrs) were last for only few hrs. Therefore the duration of the cycle was 6-7 days. But in group V, the estrus was prolonged (up to 5/6 days) in all animals. In this group the length of proestrus (5-6 hrs), metestrus (10-12 hrs) and diestrus (14-18 hrs) were last for only few hrs. In this group the duration of the cycle was 6-7 days.

On the other hand,  $17\beta$ -oestradiol induced persistent estrus in all animals from 2nd day onwards till the end of the experiment. Interestingly in this group proestrus,

metestrus and diestrus were not found during the treatment period.

Table 2. Effect of E<sub>2</sub> and root extract on the length of the estrous cycle and duration of each of its phases (n=6/gr).

Experimental groups	Length of the cycle (days)	Duration of estrous phases (days / hrs)			
		Proestrus	Estrus	Metestrus	Diestrus
I (Control)	4-5	12-15hrs	12-18 hrs	21-25 hrs	2-3 days
II(Vehicle control)	4-5	12-15 hrs	14-17 hrs	23-25 hrs	58-62hrs
III (10µg E <sub>2</sub> )	-	-	Persistent estrus	-	-
IV (250mg extract)	6-7	4-5 hrs	6-8 hrs	7-8 hrs	5-6 days
V(500mg extract)	6-7	5-6 hrs	5-6 days	10-12 hrs	14-18hrs

## Discussion

In the present study, the crude methanolic root extract of *Careya arborea* Roxb. induced a strong antifertility activity (pregnancy inhibition) in 3 months old Swiss albino mice at the dose level 1000mg/kg bw, when administered orally for 14 consecutive days (Table 1). This dose induced 100% sterility even by the end of the repeated 2nd mating (after 22 days of 1st mating). The results of this study on the antifertility activity of the root extract were consistent with earlier published data on many crude extracts and the active principles from medicinal plants that were evaluated in different laboratory for their antifertility effects in different animal model. Kholkuteet al. (1978a) reported dose dependent antifertility activity of methanolic extract of dried berries of *Embelia ribes* Burm. in rats. They reported a graded dose related effect of methanolic extract.

Though not statistically significant within treated groups (Figure 1), reduction in food intake was higher in positive control group (11.32± 0.31gm) than extract treated groups (11.67±0.34gm and 11.67±0.34gm for group IV and V respectively). This result was consistent with the findings of Dubuc (1985), who observed estrogen-mediated reductions of growth in male and female mice. He also reported that the body weight changes correlated with daily food intakes.

In the present study, the average duration of estrus was prolonged at higher dose (500mg) of extract treated animal (Table 2). But, in the animals exposed to lower dose (250mg) of extract, the diestrus phase was prolonged. Taking into consideration, the length of diestrus and estrus between control and treated animals, it may expect that that the root extract of the plant somehow affected the normal reproductive cycle. There are

some evidences that plant compounds alter the menstrual cycle in women. Dietary supplements of isolated soy protein or soymilk have both increased and reduced cycle length in women (Cassidy et al., 1994; Lu et al., 1996; Petrakis et al., 1996). Plant compound phytoestrogen can induce irregular estrus and anestrus in cattle (Adams, 1995). Benzene and chloroform extract of *Piper longum* fruits prolonged the diestrus phase by 5-10 days in female rats (Kholkuteet al., 1978b). But, Ghosh and Bhattacharya (2004) observed a decrease in the duration of diestrus phase with concomitant increase in the duration of estrus phase after administration of the seed extracts of *Thespesia populnea* to rats leading to significant inhibition of implantation sites in uterine horns.

Comparing the duration of every phase of the estrous cycle between treated and control mice, it may consider that in extract treated mice, and each phase of the cycle was influenced by the extract. It may be due to the imbalance in hormonal status as suggested by other workers (Cassidy et al., 1994; Lu et al., 1996). In rat, vaginal smears display the cell pictures characteristic of each hormone, estrogen or progesterone (Montes and Luge, 1988). Therefore ovarian hormones regulate the cytological changes that take place in vaginal smears. Montes and Luge (1988) reported that during estrus phase, presence of cornified cells depends on the availability of estrogen. In the present study, it was observed that administration of the root extract increased the length of the diestrus phase in the animals exposed to lower dose, but increased the length of the estrus phase in the animals exposed to higher dose of the root extract. Therefore the alteration in the estrous cycle was dose dependent. In the present study prolongation in the duration of diestrus phase indicate less availability of estrogen whereas prolongation in the duration of estrus phase indicate more availability of estrogen in extract treated mice, as suggested by Luge (1988). Animals exposed to E2 exhibited the positive response for persistent estrus.

## Conclusion

The present studies have established that the root of the plant *Careya arborea* Roxb. is a potential source of antifertility activity. However, a number of important issues remain to be investigated. There should be a thorough investigation on the oestrogenic nature of the plant. Moreover, further studies are also needed to confirm the hormonal alteration induced by the plant that is responsible for the changes leading to inhibit pregnancy. As the herbal preparations are safe, comparing with the synthetic steroidal contraceptives, the plant *Careya arborea* Roxb. can brings a new dilemma as contraceptives for better development of mankind.

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## Evaluation of Phytochemical composition, in vitro antioxidant and antibacterial activity of two types of *Averrhoa carambola* L. fruit

\*Farhana Sultana<sup>1</sup>, Raihana Shobnam<sup>2</sup>

Jayanta Barman<sup>3</sup>, Mousmi Saikia<sup>2</sup>

<sup>1</sup>Biotech-Hub, ADP College, Nagaon

<sup>2</sup>Department of Herbal Science and Technology, ADP College, Nagaon

<sup>3</sup>Department of Physics, ADP College, Nagaon

\*Corresponding author. Tel.: 9707779150

E-mail address: farhana\_miss@ymail.com

### Abstract

Herbal products are gaining much popularity now-a-days. There is a revival of interest with plant based medicines due to increasing realization of the side effects and health hazards associated with the arbitrary use of synthetic products. Phytochemical screening of *Averrhoa carambola* L. fruit reveals it as a valuable medicinal plant with numerous medicinal properties. During the preliminary phytochemical analysis, the methanol and water extracts of two types of *Averrhoa carambola* L. fruit was screened for the presence of carbohydrates, proteins, alkaloids, flavonoids, saponins, glycosides and terpenoids. Antioxidant activity was measured using total phenolics content, flavonoid content and DPPH free radical scavenging assays. The methanol extract of sour type of fruits shows higher antioxidant activity than the sweet type of fruit. This may be due to the presence of higher amount of phenolics and flavanoid content. Phenolic compounds which acts as natural antioxidant and antibacterial agents. The antimicrobial activity was tested in vitro using well diffusion assay on four bacterial species such as *B.subtilis*, *S.aureus*, *E.coli* and *K. pneumonia*. Methanolic extract of sour type fruit was the most active against the bacterial species.

**Keywords:** *Averrhoa carambola*, Antioxidant activity, Antibacterial activity, phenolic content, Methanolic extract.

## 1. INTRODUCTION

*Averrhoa carambola* L. generally called star fruit is an attractive, slow growing evergreen tree that belongs to the oxalidaceae family. There are two types of *Averrhoa carambola* L. are where type one (tart type) is smaller in size, sourtest, richlyflavored with less oxalic acid content the second type is larger in size, mildflavored with less oxalic acid and known as honeytype (Noor Asna and Noriham 2014). *Averrhoa carambola* is mainly found in Malaysia, Taiwan, with smaller concentrations in Thailand, Israel, Florida, Brazil, Philippines, China, Australia, Indonesia, in the warmer parts of India, Bangladesh and other areas of the world with the same climate (Ghani, 2003). *Averrhoa carambola* tree is a slow growing, short trunked with a much branched, bushy, broad, rounded crown and reaches up to 6-9m in hight. It grows on its best if the climatic condition is wet, humidand distinctdry. It likes a well-drained, clay-loam soil with pH of 5.5-6.5 and can withstand some water logging. It cannot tolerate drought conditions or salt. Carambolas have compound leaves 6 to 12 inches (15 to 30) long that are arranged alternately onbranches. Each leaf has 5 to 11 green leaflets 0.5 to 3.5 inches long (1.5 to 9cm long) and 0.4 to 1.8 inches (1 to 4.5) wide (Crane, 1994). The flowers are arranged in small clusters and each cluster is attached to the tree with red stalks. Theflowers are small, about 6mm wide, pedicellate with 5 petals (having curved ends and sepals).the color of flowers are purple to bright purple (Dasgupta et al, 2013). Thefruits are green when small and turn yellow or orange when ripe. The fruits are frshy five lobed, ovate to ellipsoid that ranges from 5-8cm long and 9cm wide. The skins of fruits arethin, light to dark yellow and smooth with waxy cuticle. The fruit is cruncy, and has a slightly tart, acidic, sweet taste, reminsiscent of pears and apples. The fresh is light yellow, translucent, crisp and very juicy without fiber (Dasgupta et al, 2013). There are 2 distinct classes of carambola fruit-the smaller, very sour type, richly flavored, with more oxalic acid; the larger, so-called "sweet" type, mild-flavored, rather bland, with less Oxalic acid (Gaurav et al, 2012).

*Averrhoa carambola* is a very good source of natural antioxidants like L-ascorbic acid,epicatechin and Gallic acid in gallotanin forms. The ascorbic acid content of the star fruit is different in sweet and sour type of the fruit. (Dasgupta et al, 2013). The ripe fruit of carambola is used in ayurveda as digestive, tonic and strengthening. The dried fruit is also used in fever it has cooling and antiscorbicproperties. Roots of these plants are also used to treat eruption from chickenpox and hence help to allay the outcome of fever. It is also known as the one of the best Indian cooling medicine. Fruits are used as antioxidant and astringent. The ripe fruits are known for curing bleeding piles, particularly for internal piles. In Chinese meteria medica it is used to quenchthirst, increase the salivarysecretion and hence to ally fever. InBrazil, the carambola is recommended as diuretic in kidney and bladder complaints. Fruits are useful in diarrhea, vomiting, hyperdipsia, haemorrhoids, intermittent fever, scavis and general debility. Also, the fruit

is sour, astringent to the bowels and it is very much useful in elimination of internal worms. The leaves are antipruritic, antipyretic, antihelminthic and are also helpful in scabies, fractured bones and various types of poisoning, intermittent fever and elimination of intestinal worms (warrier et al, 1994).

Therefore, the present study was aimed to perform phytochemical screening, evaluation of anti-oxidant and antimicrobial property of the two types of fruit (sour type and sweet type) of *A. carambola*.

## **2. Materials and Method**

**2.1 Collection and identification of plant material:** the plant material of both type is collected from northern part of Nagaon district, Assam.

### **2.2 Preparation of crude powder:**

Fresh mature and healthy fruits are collected. These were thoroughly washed and kept them in clean area. The fresh fruits are then cut into small pieces and these were subjected to drying at 40°C in a hot air oven for 3 days. Then the fruit pieces were taken out of the oven when they are fully dried enough to crush. The dried material was crushed in mixture grinder to coarse powder. The dried powder was stored in airtight bottles at room temperature for further extraction.

### **2.3 Phytochemical investigation:-**

The preliminary phytochemical investigations were carried out for different phytoconstituents by two solvents: methanol and water of *A. carambola* fruit with different chemical tests and reagents (Niloferet. Al., 2013 and minor modification).

### **2.4. Estimation of total phenolic content:-**

The concentration of phenolics is determined using the Folin-Ciocalteu method. 1 mg of each dried extract was dissolved in 1 ml of methanol and then mixed by vortexer at constant rotation till fully dissolved. Methanolic solution of the extract in the concentration 1 mg/ml was analyzed. The reaction mixture was prepared by mixing 1 ml of each methanolic solution of each extract at different dilutions (30, 50, 70 & 90 µl/mg) with 5 ml Folin-Ciocalteu's reagent. After 3 minutes, 4 ml of 20% Na<sub>2</sub>CO<sub>3</sub> was added to the above solution and shaken vigorously. Blank was concomitantly prepared, containing 1 ml methanol and 5 ml Folin-Ciocalteu's reagent and 4 ml of 20% Na<sub>2</sub>CO<sub>3</sub>. The absorbance was determined using a spectrophotometer at 650 nm wavelength. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure is followed for the standard solution of gallic acid and the calibration line was constructed based on the measured absorbance. The concentration of phenolics was read (µg/ml) from the calibration line. Then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

### **2.5 Estimation of total flavanoid content:**

The content of flavanoid in the examined plant extracts was determined using the aluminium chloride method. Extract solution was prepared by dissolving 1 mg of each

dried extract in 1ml methanol separately and then missed with vortexer at constant rotation. each fruit extract in the concentration of 1mg/ml of different dilution(30,50,70&90µl/mg).0.5 ml of each methanolic solution is pipette out separately and mixed with1.5ml methanol,0.1ml of 10%alluminium chloride ,0.1 ml of 1M potassium acetate and then 2.8 ml of distilled water. The reaction mixtures were then incubated for 30 minutes at room temperature. The absorbance was determined using spectrophotometer at 415nm.the same procedure was applied for the standard solution of quarcetin and the calibration line was constructed. Based on the measured absorbance, the concentration of flavanoids was read µg/ml on the calibration line. The content of flavanoid in extracts is expressed in terms of quarcetin equivalent (mg/g) of extract.

## **2.6 Invitroantioxidant activity:**

### **2.6.1 DPPH method:**

The antioxidant activity of plant extract and standard can be measured in vitro by hydrogen donating or free radical scavenging ability by using (DPPH) diphenyl -2 picrylhydrazyl radical. DPPH free radical scavenging method is an easy, rapid and sensitive way to survey the antioxidant activity of specific compound or plant extract. DPPH is a purple colored stable free radical. When reduced it becomes the yellow colored diphinylpicryl hydrazine. The ability of the both plant extract to scavenge DPPH free radicals was assessed by the standard method. The stock solution of each 1mg dried extracts of both sample were prepared in methanol to achieve the concentration of 1mg/ml. Dilutions were made to obtain concentrations of 100,200,300 and 400µg/ml. Diluted solutions (0.5 ml each extracts) were mixed with methanol and an aliquot of 2.5 ml of 75µM DPPH solution. After 90 minutes incubation in darkness at room temperature (230C), the absorbance was recorded at 517 nm using UV-VIS spectrophotometer. Control sample contain all the reagents except the sample. The DPPH solution in methanol was prepared daily before the absorbance measurements. The same procedure was prepared for the standard solution of gallic acid. The data were presented as mean value ±standard deviation of scavenging activity was calculated as follows-

Scavenging % = (absorbance of control - absorbance of test) / absorbance of control

### **2.6.2 Ferric Reducing Activity Power (FRAP assay):**

The reducing power of extracts were determined by the FRAP assay method according to Benzie and Strain (1996) and minor modification. The stock solutions included was 300mM acetate buffer (3.1g C<sub>2</sub>H<sub>2</sub>NaO<sub>2</sub>, 3H<sub>2</sub>O and 16 ml C<sub>2</sub>H<sub>4</sub>O<sub>2</sub>) at pH of 3.6, 10Mm TPTZ (2,3,6- tripyridyl-s-triazine) solution in 40mM HCl and 20mm FeCl<sub>3</sub>.6H<sub>2</sub>O solution. At the beginning of experiment 1mg of each extracts were dissolved in 1ml of methanol to make the concentration of 1mg/1ml and then mixed by vortexer at constant rotation. The fresh working solution of each extract was prepared using different dilution (50,100,150,200, 250 and 300µg/ml) of FeCl<sub>3</sub>.6H<sub>2</sub>O solution. The

standard Gallic acid was prepared by same procedures by adding the stock solution at appropriate concentration except the plant extracts. The sample mixture and standard was then incubated at 37°C for 1 hour. Readings of the colored mixtures were then taken at 593 nm by UV-VIS spectrophotometer. The experiment was performed thrice for each of the extracts and results were averaged.

### **2.7 Antibacterial Assay:**

The tested both gram negative and gram positive bacteria species were supplied by MTCC, Chandigarh, Punjab, India. Antibacterial studies were performed using agar well diffusion method. 20mg extracts of different solvent (methanol and water) taken in 1ml eppendorf tube and were dissolved in 1ml of dimethylsulfoxide (DMSO) to give a concentration of 0.02g/ml and mixed by vortexer at constant rotation for 1-2 hours. The UV light for 30 minutes before starting the experiment. The assay method was carried out by preparation of 3 wells of 5mm diameter for each extract using a sterile cork borer in agar plate aseptically. The agar cylinders were removed using a sterile loop. The wells were grouped as the test well and the control wells. The test well was filled with 25, 30 and 50µl from the stock solution of the test material and the control well was filled with the same amount of the solvent i.e. DMSO. A marketed antibacterial drug, chloramphenicol was used as the positive control. The wells were kept for 15 minutes for drying by passing the blower in LAF. The plates were then taken out from the LAF and incubated at 37°C overnight. The inhibition zones were recorded in the test well as well as the control well.

### **3. Results:**

Phytochemical screening of the extracts showed presence of phytoconstituents like alkaloid, phenol, flavanoid and saponin where as glycoside and resin was found absent.

#### **3.1 Total phenolic content:**

Total phenolic content of the different extracts of *Averrhoa carambola* were determined by using the Folin Ciocalteu reagent and were expressed as Gallic acid equivalents (GAE) per gram of plant extract. The total phenolic contents of the test fractions were calculated using the standard curve of Gallic acid. Methanol extract of sweet type of *Averrhoa carambola* L. was found to contain the highest amount of phenols (Table 1). Phenol contents of the extracts were found to decrease in the following order: methanol sour extract > methanol sweet extract > water sour extract > water sweet extract (Figure 1).

#### **3.2 Total flavanoid content:**

Aluminium chloride colorimetric method was used to determine the total flavanoid contents of the different extracts of *Averrhoa carambola*. Total flavanoid contents were calculated using the standard curve of quercetin and was expressed as quercetin equivalents (QE) per gram of the plant extract. Flavanoid content of the extracts were

found in the order: Methanol extract of Sour Fruit>Methanol extract of Sweet Fruit>Water extract of Sour Fruit>Water extracts of Sweet Fruit(Figure1).

**Table 1: Phenol and flavanoid contents in two types of *Averrhoa carambola* fruit extract.**

compound	EXTRACT			
	Sour type		Sweet type	
	methanol	water	methanol	water
TPC in GAE (mg/g)	56.2	50	50.11	46
TFC in GAE (mg/g)	45.22	41.11	44.22	33

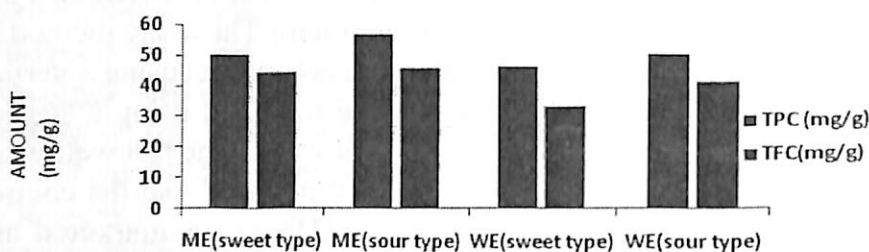


Fig 1: Total phenolic and flavanoid content in *Averrhoa carambola* fruits (sour type & sweet type)

### 3.3 Antioxidant activity of fruit extracts of *Averrhoa carambola*:

#### 3.3.1 DPPH Method:

The assay is based on the measurements of the antioxidant ability to scavenge the stable radical DPPH. DPPH is a stable nitrogen centered free radical which produces violet colour in methanol solution. The solutions progressively reduced to yellow colored product diphenylpicryl hydrazine with the addition of the extracts in a concentration dependent manner. The inhibition percentages were shown in the table 2.

**Table 2: Inhibition percentage of two types of *Averrhoa carambola* fruit**

Concentration µg/mg	% of inhibition ±SD				
	METHANOL		WATER		ASCORBIC ACID
	Sour type	Sweet type	Sour type	Sweet type	
50	20.88±.94	11.07±.99	18.208±2.5	10.46±1.5	51.53±1.5
200	54.441±1.51	52.14±2.5	51.22±2.014	50.018±2.5	71.33±.47
300	65.525±1.52	60.22±.25	62.44±2.005	58.66.66±1.9	86.66±.94
400	76.487±2.12	72.59±2.5	73.55±2.2	68.151±5.55	96.32±1

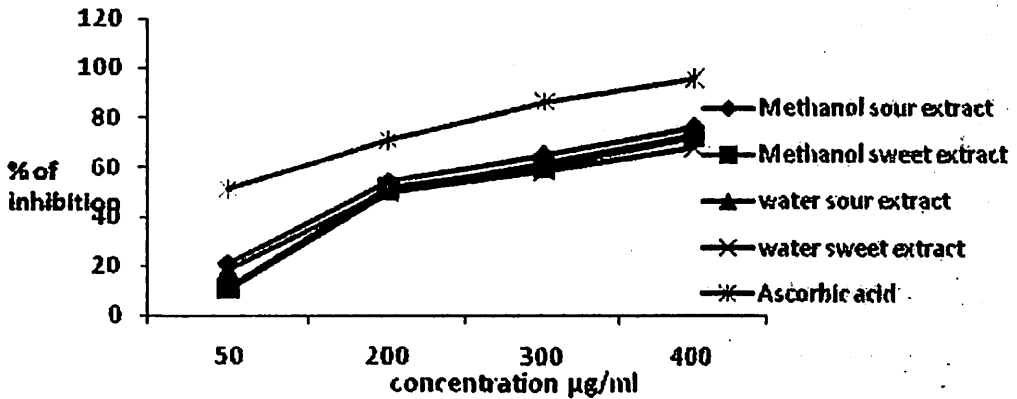


Fig 2: DPPH free radical scavenging activity of two types of *Averrhoa carambola* fruit

### 3.3.2 Free Radical Reducing Activity Power (FRAP Assay):

The reducing power ability of Fe<sup>3+</sup> was carried out by varying the concentration of extracts. The result of this analysis as shown in Figure 3 indicates that reducing activity increases as the amount of extracts increased.

Table 3: Reducing power activity of *Averrhoa carambola*

Concentration µg/ml	% of inhibition				
	Sour type		Sweet type		Trolox
	Methanol	Water	methanol	water	
100	35.12±.012	27.123±.146	25.143±.167	25.196±.887	53.33±.984
200	43.45±.887	40.23±.149	37.11±.456	34.18±1.22	71.33±0.47
300	55.12±1.12	52.18±0.192	50.332±1.2	50.36±2.5	81.22±1.41
400	67.12±1.1	65.11±1.2	59.22±1.1	62.12±1.1	92.11±1.1

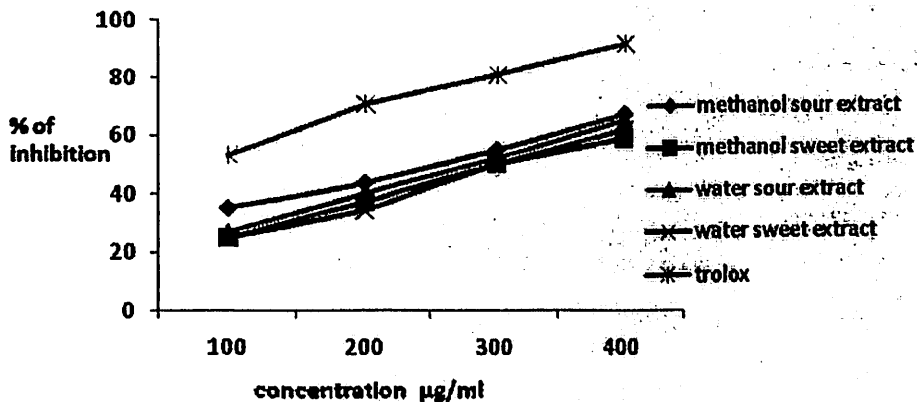


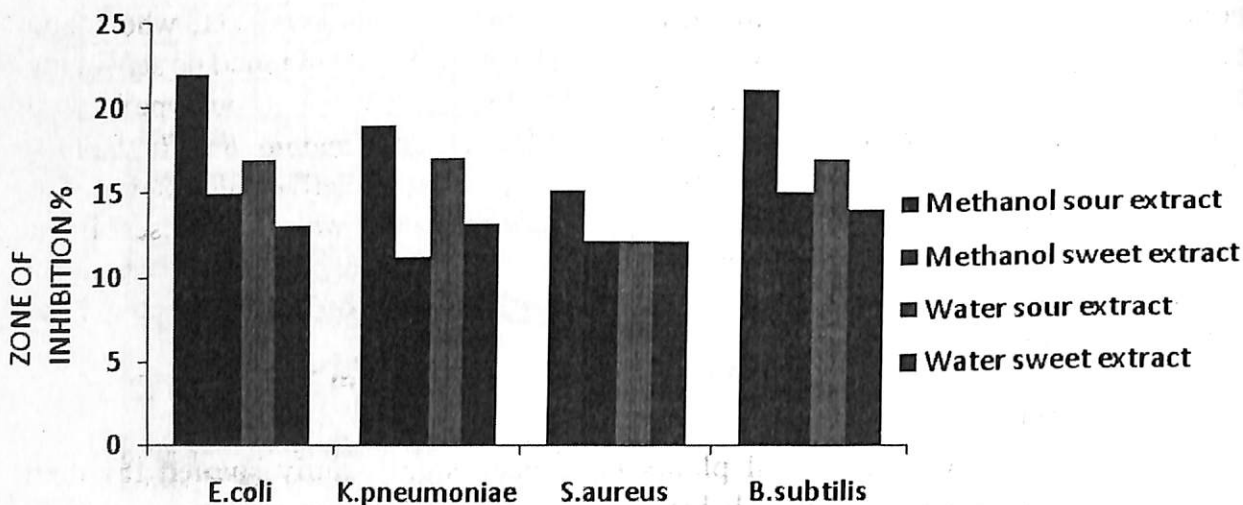
Fig 3: Free radical reducing power by FRAP assay of star fruit

#### 4.6 Antibacterial evaluation:

The antimicrobial potential of different extract of two types of *A. carambola* fruit (sour type and sweet type) were screened by disc diffusion method and the zone of inhibition were measured in mm diameter. From the stock solution of 100mg/ml different concentrations of fruit extract were put against the microorganisms, among those concentrations the inhibition zones measured in 50µl is shown in the table-4.

**Table 4: zone of inhibition of *A. carambola* fruit**

Tested organism	Zone of inhibition in diameter(mm)				Chloramphenical
	Sour type		Sweet type		
	Methanol	Water	Methanol	Water	
<i>Escherichia coli</i>	22	17	15	13	26
<i>Klebsiella pneumoniae</i>	19	17	11	13	22
<i>Staphylococcus aureus</i>	15	12	12	12	22
<i>Bacillus subtilis</i>	21	17	15	14	20



**Fig 12: Zone of inhibition of two types of *Averrhoa carambola* fruit extract**

#### 4. Discussion

The antioxidant property of two types (sour type and sweet type) of *Averrhoa carambola* fruit extracts have been determined by DPPH free radical scavenging method and FRAP assay. The antioxidant activity studies of two types of *A. carambola* fruit extracts reveals that methanol extracts of both type of fruits (sour and sweet) have more antioxidant activity than water extract. In the comparison between sour and sweet type of fruit extract it is observed that sour type have more antioxidant activity than sweet type. Lim and Lee, (2013) reported that the antioxidant capacities of star fruit were increased significantly with ripening, except for the total ascorbic acid content, our study reveals that the antioxidant activity of *A. carambola* fruit is different according to their ripening stage. The FRAP values obtained from this finding are slightly lower than values reported by Yan et al, 1996 and Wong et al, 2006. The difference may be due to the types and the quantity of phytochemical and the food matrix presence in the sample. Environmental factors such as climatic growth condition, ripening stage, temperature, duration of storage and thermal treatment may have influenced the antioxidant activity. Compared to the studies mentioned above, it can be said that, with the fruits containing significant antioxidant properties, which can be further screened to find out a probable source of medicine.

In the antimicrobial assay, the methanol extract of sour type fruit showed a good range of activity against micro-organisms *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Escherichia coli* in concentration of 50 µg. In a previous study Rahman et al., 2014, found that Star fruit showed antimicrobial activity against the micro-organisms, *Staphylococcus aureus*, *Klebsiella pneumonia* in ethanolic extract, but aqueous extract showed no result. In another finding Wakteet al. 2011, who found sensitivity against *Staphylococcus aureus* in methanol extracts and found no sensitivity in case of aqueous extract. But in the present study, aqueous extract of sour type showed more effective results against *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis*. The results indicate the test samples contain biologically active ingredients. The knowledge of exact mode of inhibition of specific compounds which are present in the plant extract may contribute to the successful utilization of such natural compounds for further research and to find out the treatment of infectious disorders like bacterial and fungal diseases

#### CONCLUSION:

In recent years, medicinal plants have been significantly studied for their phytomedicinal properties which bring known and unknown medicinal virtues. Phytochemical screening of *A. carambola* reveals it as a valuable medicinal plant. The presence of different phytoconstituents makes this plant a potent source for modern drugs and may help to perform higher research to develop natural drug therapy. Future

research is needed to design this herb as a drug after completing the molecular level research work. Further investigations are needed to explore individual bioactive compounds responsible for these pharmacological effects and the mode of actions.

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# The Medicinal Plant: Tulsi, its Properties and Benefits (Review)

**Gayatree Das**

Department of Mathematics, ADP College, Nagaon

E-mail: das\_gayatri20@yahoo.in

## **Abstract**

Tulsi an Indian holy plant is found in almost every Indian household. This holy Tulsi is also known as the "queen of the Herb". It has been used in India for around 5000 years and is acclaimed for its healing properties of the mind, body and spirit. Tulsi belongs to the lamiaceae family. It is also called by names like Manjari/Krishna Tulsi (sanskrit), Trittava (Malayalam), Tulsi (Marathi) and Thulsi (Tamil & Telegu). It is called holy basil in English. Three main forms are generally recognised: i) Rama Tulsi with stems and leaves of green ii) Krishna Tulshi with stems and sometimes also leaves of purple iii) Vana Tulsi which is unmodified from its wild form. Tulsi has very potent germicidal, fungicidal, anti-bacterial and anti-biotic properties that are great for resolving fevers, common cold, cough, headaches etc. tulsi is a beneficial herb for the entire body and regular usage of the herb could result in a healthier and longer life. In this paper we discuss about the properties of holy Tulsi and its benefits so that after knowing all these properties and benefits of this herb one can try for home remedy which works wonder on life and living habits.

## **Introduction**

The medicinal use of plants is very old. According to Ayurvedic system of medicines a large number of plant are employed in the traditional medicines for the treatment of several diseases. Among these medicinal plants one of the important plant is Tulsi. Tulsi is a Sanskrit word which means "matchless one". Scientifically it is called as "*Ocimum Sanctum*" belongs to the Lamiaceae family. *Ocimum Sanctum* (Tulsi or holy basil) has a very special place in the Hindu culture. The natural habitat of tulsi varies from sea level to an altitude of 200 m. It attains the height of about 75 - 90 cm. It has round oval shaped leaves which are up to 5 cm long. The leaves are 2- 4 cm in length. Its seeds are flat. Its flowers are purple - creamish in colour. Its seeds are yellow to reddish in colour. Leaves of Tulsi contains very essential oil.

It is found growing naturally in the moist soil nearly all over the globe. variations in soil type and rainfall may also equate to a difference in the size and form of the plants as well as their medicinal strength and efficiency. Several medicinal properties have been attributed to the tulsi plant not only in Ayurveda and Siddha but also in Greek, Roman and Unani system of medicines. The tulsi plant is even known to purify or de-pollute the atmosphere and also works as are repellent to mosquitoes , flies and other harmful insects.

Many Hindus have tulsi plant growing in front of or near their home, often in special pots. Hindus regard it as an earthly manifestation of the goddess Tulsi. It is regarded as a great worhipper at the god Vishnu and his forms like Krishna and Vithoba. There are three variants of Tulsi; Rama Tulsi, Krishna Tulsi, and Vana Tulsi. Each of the variants has its own distinctive taste. The parts of Tulsi generally used are its leaves, seeds and dried roots.

The extract obtained from Tulsi plants is used to cure various diseases such as common cold, inflammation, malaria, heart diseases, and many more. Tulsi contains hundreds of beneficial compounds and possesses strong anti-oxidant, anti-bacterial, anti-viral, adaptogenic, and immune enhancing properties. It has been used for centuries as an important component of Ayurveda for its diverse healing properties and regarded in Ayurveda as an "elixir of life" and is well known to promote longevity.

#### Nutritional Value of Tulsi/Basil:

Before progressing to its benefits the nutrient facts of Tulsi are given below:

Principle	Nutrient Value	RDA %(recommended Dietary allowance)
Energy	23KCal	1%
Carbohydrates	2.65g	2%
Protein	3.15g	6%
Total Fat	0.64g	2%
Cholesterol	0 mg	0%
Dietary Fiber	1.60g	4%
<b>Vitamins</b>		
Folates	68	17%
Niacin	0.902 mg	6%
Pantothenic Acid	0.209 mg	4%
Pyridoxine	0.155 mg	12%

Riboflavin	0.076 mg	6%
Thiamin	0.034 mg	2.5%
Vitamin A	5275 IU	175%
Vitamin C	18 mg	30%
Vitamin E	0.80 mg	5%
Vitamin K	414.8	345%
<b>Electrolytes</b>		
Sodium	4 mg	0%
Potassium	295 mg	6%
<b>Minerals</b>		
Calcium	177 mg	18 %
Copper	385 mg	43%
Iron	3.17 mg	40%
Magnesium	64 mg	16%
Manganese	1.15 mg	57 %
Zinc	0.81 mg	7%

### Properties and Benefits of Tulsi:

Not only from a religious point of view, but from a medicinal perspective too, Tulsi has endless value. In Ayurveda, it is considered as the destroyer of all the doshas. The Tulsi benefits are many and people also chew raw leaves regularly to reap these benefits. Tulsi leaves also re-energize and rejuvenate the body of a person. It keeps one fresh mentally and stimulates the thought process. The holy basil is a homemade remedy for a lot of common ailments. Its anti-bacterial and anti-fungal properties are very effective in preventing breakout on acne prone skin. This herb can cure difficult skin conditions like those caused due to ring worms and even leucoderma. Tulsi is a tonic for the entire body. Its roots, stems and seeds have also medicinal value like its leaves. Here we discuss some main properties and benefits of tulsi leaves.

**Cures a fever:** Tulsi has very potent germicidal, fungicidal, anti-bacterial and anti-biotic properties that are great for resolving fevers. It has the potential to cure any fever right from those caused due to common infections to those caused due to malaria as well.

**Beats diabetes:** leaves of holy basil are packed with anti-oxidants and essential oils that produce eugenol, methyl eugenol and caryophyllene. Collectively these

substances help the pancreatic beta cells (cells that store and release insulin) function properly. This in turn helps increase sensitivity to insulin. Lowering one's blood sugar and treating diabetes effectively. An added advantage is that the anti-oxidants present in the leaves help beat the ill effects of oxidative stress.

**Protects the heart:** Tulsi has a powerful anti-oxidant component called Eugenol. This compound helps protect the heart by keeping one's blood pressure under control and lowering his/her cholesterol levels. Chewing a few leaves of tulsi on an empty stomach everyday can both prevent and protect any heart ailments.

**Beats stress:** According to a study conducted by the Central Drug Research Institute, Lucknow, India, tulsi helps to maintain the normal levels of the stress hormone - cortisol in the body. The leaf also has powerful adaptogen properties (also known as anti-stress agents). It helps sooth the nerves, regulates blood circulation and beats free radicals that are produced during an episode of stress. People who have high stress jobs can chew about 12 leaves of tulsi twice a day to beat stress naturally.

**Dissolves kidney stones:** The holy basil being a great diuretic and detoxifier is great for the kidneys. Tulsi helps reduce the uric acid levels in the blood (one of the main reasons for kidney stones is the presence of excess uric acid in the blood), helps cleanse the kidneys, the presence of acetic acid and other components in its essential oils helps in breaking down kidney stones and its painkiller effect helps dull down the pain of kidney stones. To relieve kidney stones one must take the juice of tulsi leaves with honey, every day for six months to help wash out the stone from the kidney.

**Beats cancer:** With strong anti-oxidant and anti-carcinogenic properties tulsi has been found to help stop the progression of breast cancer and oral cancer (caused due to chewing tobacco). This is because its compounds restrict the flow of blood to the tumour by attacking the blood vessels supplying it. One can take the extract of tulsi every day to keep these conditions at bay.

**Helps to quit smoking:** Tulsi is known to have very strong anti-stress compounds and is great to help one quit smoking. It helps by lowering the stress that may be involved in trying to quit smoking, or stress that leads to the urge to smoke. It also has a cooling effect on the throat just like menthol drops and helps control the urge to smoke by allowing the person to chew on something. Ayurveda relies heavily on tulsi leaves as a smoking cessation device. chew some leaves whenever the urge to smoke arises. Another plus is that the antioxidant property of the leaves will help fight all the damage that arises out of years of smoking.

**Keeps skin, hair healthy and glowing:** The holy basil has powerful purifying properties. When eaten raw, it purifies the blood giving the skin a beautiful glow, and prevents the appearance of acne and blemishes. Its anti-bacterial and anti-fungal properties are very effective in preventing breakouts on acne prone skin. Ayurvedic doctors say that this herb can cure difficult skin conditions like those caused due to ring

worms and even leucoderma. Apart from all this, it helps in reducing itchiness of the scalp and helps to reduce hair fall. Mix the powder in coconut oil and apply regularly to the scalp to prevent hair fall. Eating tulsi leaves, drinking the juice, or adding its paste to a face pack can help cure skin and hair conditions.

**Heals respiratory conditions:** Tulsi has immunomodulatory (helps to modulate the immune system), antitussive (suppresses the cough center, reducing the amount of cough) and expectorant properties (helps expel phlegm from the chest), that make it a great relief for coughs, cold, and other respiratory disorders including chronic and acute bronchitis. Another great property of this leaf is that it has anti-bacterial and anti-fungal properties that help to beat the infection causing the respiratory problem. It also relieves congestion since it contains potent components like camphene, eugenol and cineole in its essential oils. Its anti-allergic and anti-inflammatory properties also help to treat allergic respiratory disorders.

**Cures a headache:** Tulsi helps to relieve headaches caused due to sinusitis, allergies, cold or even migraines. This is because it has pain relieving and decongestant properties, that help relieve the pain and resolve the root cause of the condition. If one suffering from a headache, can take leaves or tulsi extract..

These are just some benefits of the plant. Apart from these other benefits including treatment for common colds are itchiness of the skin, treatment for insect bites, curing common conditions of the eye and as a herbal remedy for bad breath.

## Conclusion

Current scientific research offers substantial evidence that tulsi protects against and reduces stress; enhances stamina and endurance; increases the body's efficient use of oxygen; boosts the immune system; lessens aging factors; enhances the efficacy of many other therapeutic treatments; and provides a rich supply of antioxidants and other nutrients. Overall tulsi is a premier adaptogen, helping the body and mind to adapt and cope with a range of physical, emotional, chemical and infectious stresses. One can try for home remedy with this medicinal plant-tulsi which works wonder on life and living habits.

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# Medicinal plantwealth of Wetlands of Barpeta District Assam, India and it's conservation

**Jitu Das and Manoj Kr. Rajbangshi**  
P.G. Department of Zoology, Bajali College  
Email:jitudasblog@gmail.com

## Abstract

The study was carried out in the wetlands of Barpeta district namely Kapla, Sikhroita, Kapilimara, sarangpuriya during 2015-16 to document the medicinal plants used by the people with the help of structured format of questionnaires and interviews with the local people and fisherman of the study area. During the investigation period, 40 common medicinal plant were found in the study area of which 28 species of plants are aquatic and others are semi aquatic belongs to 25 families and 19 orders. An attempt has been made to discuss the mode of application of the plants in various ailments along with the conservation for sustainable use of the medicinal plants.

**Key words:** Medicinal plant, Kaplabeel, Sikhroitabeel, Barpeta district, Conservation

## Introduction

The Northeastern region is one of the richest bio-diversity areas since time immemorial. The state of Assam harbors a varied number of medicinal and aromatic plant species with enormous potentiality. Barpeta district lies in the lower reach of the river Brahmaputra and is situated in the coordinates 26.3216° N, 90.9821° E and bounded by Bhutan in North, Brahmaputra river in South, Nalbari district in east and Goalpara and Bongaigaon district in west. It experiences medium to high rainfall throughout the year. Literacy rate of Barpeta in 2001 census are 57.35 % and population rate of Barpeta in 2001 was 1394755 and in 2011 was 1693622 with various ethnic tribes such as Bodo, Kachari, Muslim, Hindus etc. The inhabitants of the area mostly depend on the forest for food, shelter, medicine and other basic necessities. Documentation, systematic and through investigation of plant species of the area are of paramount importance before these are lost forever. Comprehensive research for this area is required to preserve the germplasm of medicinal and aromatic plants species. Floristic composition works in

the State of Assam have been carried out by some workers, while many others have worked on medicinal uses of plants of Assam and Meghalaya, Kamrup district of Assam, among tribes of Mikir Hills, Bodo, Karbi, Mishing and Rajbongshis tribes, for family planning and birth control, aquatic and marsh plants 1-15. The paper enumerates medicinal uses of the herbaceous plants of North-Kamrup district or Rangia sub-division of Kamrup district, Assam.

Wetland of India was explored by Biswas & Calder (1937), Subramanyam (1962), Cook (1996) and Fassett (2000). An account of Uttar Pradesh was given by Sen (1959) Sahai & Sinha A.B. (1968) Srivastava et.al. (1987), Malaya & Singh (2004), and Saini (2010). The study was undertaken in the beels of Barpeta district to document medicinal plant used by the people in various ailments and along with their conservation status.

### Study area :

The study was carried out in the wetlands of Barpeta district namely Kapla (station 1) Sikhroita (station 2), Kapilimara (station 3), sarangpuriya (station 4) for 1 year in the 2015-16. (fig.1). The "KaplaBeel" is located in and around the village "Baniakuchi" near "Sarthebari" under Barpeta district of Assam. The total area of the "KaplaBeel" is about 642 Bigha (91 hectares). Sikhroitabeel is situated in the east of Barpetadistrict in the Bajali subdivision area. It covers an area of 50 hectre. The average depth of the beel is 1.4 m. Kapilimarabeel covers an area of 64 hectre and it's depth is 5.5 m in summer and 1.5-2 m in winter. Sarangpuriya beel lies near Pathsala town, Nijisariah railway station, in coordinates 26.5119° N, 91.1809° E and it covers an area of 150bigha. (19.2 hectre)



Fig :1 Map of Barpeta district and Kaplabeel showing study area

## Materials and methods

During the survey in 2016, the medicinal plants used in primary healthcare system of different localities of Beel area of Barpeta district were documented. Ecological habits of each and every species were studied. The collected specimens were identified in Botany department, Bajali College following Kanjilal ( ). Standard methodologies for gathering information on medicinal uses of the species are followed was followed.

## Result and Discussion

Present studies revealed the occurrence of 40 species under 19 order and 18 families in the wetlands of Barpeta District ( Table 1). Out of these, 35 species families are medicinally important. The enumeration embodies alphabetically arranged list of plant species priding correct botanical names of species followed by local name part use and uses. Plant part uses in different problems like skin problems including wounds, eczema, stomach problems gastro-intestinal, diarrhea, dysentery, fracture of bone, speematorrhoea, blood dysentery, and use as a tonic in different forms such as juice, extract, paste, etc. On the other hand water is the prime requisite of the vegetation of the wetland and any alteration in the availability of water affects their presence as well as distribution. But due to anthropogenic activities these wetlands are disappearing at a very fast rate. Most of the area of the wetland has been converted to agriculture fields and residential colonies an urgent need of the time is to conduct a detailed survey of the wetlands of this region.

Table 1: Medicinal plant from the wetlands of Barpeta District, Assam

Order	Family	Species	Local name	Abundance	IUCN status
Acorales	Acoraceae	<i>Acorus calamus</i>		++	LC
Apiales	Apiaceae	<i>Centella asiatica</i>		++	LC
Alismatales	Araceae	<i>Lemna minor linn</i>	Saru-puni	+++	LC
		<i>Colocasia antiquorum sachott</i>	Pani -kasu	++++	LC
		<i>Pistia stratiotes</i>	Jalkumbi	+++	LC
	Solanaceae	<i>Alisma plantago</i>		++	LC
Asterales	Asteraceae	<i>Eclipta prostrata</i>	Kehraj	+++	LC
Boraginales	Boraginaceae	<i>Heliotropium indicum</i>		+++	LC
Campanulales	Campanulaceae	<i>Spencoclea zeylanica</i>	Pani-leheti	+	LC
Caryophyllales	Polygonaceae	<i>Ageratum conyzoids</i>		++++	LC
	Amaranthaceae	<i>Alternathera sessilis</i>		++	LC
		<i>Pistia stratioteslinn</i>		+++	

Commelinales	Pontederiaceae	<i>Monochoria vaginalis</i>	Mateka	++++	LC
		<i>Monochoria hastata</i>		++	LC
	Commelinaceae	<i>Commelina diffusa</i>		++	LC
		<i>Commelina benghalensis</i>	Kana-simalu	++	LC
Fabales	Fabaceae	<i>Aeschynome neasperalinn</i>	Bar-kuhila	+++	LC
Lamiales	Lanviaceae	<i>Leucas cephalata</i>	Doron-ful	++	LC
	Lentibulariaceae	<i>Utricularia wallichiana wight</i>	Pani-kotli	+	LC
	Planaginaceae	<i>Bacopa monnieri</i>		+++	LC
Liliales	Pontederiaceae	<i>Monochoria hastata</i>		++	LC
Myrtales	Lythraceae	<i>Trapa natans Linn</i>	Singari	++	LC
	Onagraceae	<i>Ludwigia adscendens</i>	Pani-Keseru	+	LC
		<i>Ludwigia parviflora</i>	Pani-jalakia	++	LC
Nymphaeales	Nymphaeaceae	<i>Nymphaea alba linn</i>	BagaVetful	++	LC
		<i>Nymphaea rubra Roxb</i>	RangaVetful	++	LC
		<i>Nymphaea luteum Linn</i>	Haladhiyavetful	++	LC
		<i>Euryale ferox</i>	Nikari	++	LC
Nostocales	Oscillatoriaceae	Algae	Selai	++++	LC
Proteals	Nelumbonaceae	<i>Nelumbo nuciferagaerth</i>	Padumful	+++	LC
Polygonales	Polygonaceae	<i>Polygon barbatum</i>		++	LC
		<i>Polygonum glabrum</i>	Pani-jalakia	++	LC
		<i>Polygonum hydropiper</i>	Pani-jalakia	++	LC
Ranunculales	Ranunculaceae	<i>Raninculus scleratus</i>	Jal-dhan ia	++	LC
Solanales	Menyanthaceae	<i>Nymphoides indica</i>		++	
Ukinanam	Uglyhuridthis	<i>Bryphyllum pinnatumlinn</i>	Pategaja	++	LC

Table 2: Medicinal plants with their uses

Sl. No.	Scientific name	Local name	Medicinal uses
1.	<i>Acorus calamus</i>	Bach	To treat cough, asthma
2.	<i>Alisma plantago L.</i>		Used as stomachic and as a digestive.
3.	<i>Ageratum conizoids</i>		Leaf and shoot used as antiseptic on cuts and wounds and healing to check bleeding
4.	<i>Alpina nigra</i>	Tara gas	To treat Stomach disease, Bronchitis, Headache
5.	<i>Alternanthera sessilis</i>		
6.	<i>Aeschynomenea speralinn</i>	Bar Kuhila	To treat diabetes
7.	<i>Bacopa monnieri</i>	Brahmi	Used as cardiac and nervetonic,
8.	<i>Cobocasia antiquorum sachott</i>	Panikasu	To treat anemia, goiter and increase eyesight
9.	<i>Colocasia esculenta Linn</i>	Kala kasu	To treat Rheumatism, Adinites, anemia
10.	<i>Commelina benghalensis</i>	Kehraj	Used as astringent, demulcent, laxative and mucilaginous
11.	<i>Commelina diffusa</i> Burm		The leaves are diuretic and febrifuge, the crushed leaves and stems are used as a remedy for irregular menstruation
12.	<i>Cyperus aromaticus</i>	keyabon	Tubers are medically used in skin disease.
13.	<i>Centella asiatica</i>		Used in chronic dysentery, poultice is applied on carbuncle, cuts, as antiseptic in wounds
14.	<i>Clinogyne dichotoma</i>	Patidoi	To stop bleeding
15.	<i>Euryale ferox</i>	Nikari	To treat breathing problems. Increases digestion
16.	<i>Eclipta prostrata L.</i>	Kehraj	In ayurvedic medicine, the leaf extract is considered a powerful liver tonic, rejuvenative, and especially good for the hair.
17.	<i>Heliotropium indicum</i>		To treat wounds, skin ulcers and furuncles
18.	<i>Ipomoea aquatic</i> Forsk	Kolmou	To treat Diabetes, Jaundice
19.	<i>Lemna minor</i>	Sarupuni	To treat skin disease
20.	<i>Leucas cephalotes</i>	Doron	Insect bites, Jaundice, Liver disease
21.	<i>Ludwigia adscendens</i>	Pani-Keseru	Whole plant is used as a poultice in ulcers and other skin diseases
22.	<i>Ludwigia parviflora</i>	Panijalakia	To treat dysentery and fever
23.	<i>Nelumbo nucifera</i> Gaertn	Padumful	To treat insomnia, indigestion, skin disease, headache
24.	<i>Monocharia hastifolia</i>	Mateka	To treat Pneumonia, toothache
25.	<i>Monocharia hastata</i>		Used as a stomachic
26.	<i>Nymphaea alba</i>	Baga Vetful	A decoction of the root is used in the treatment of dysentery or diarrhea
27.	<i>Nymphaea arubra</i>	Ranga Vetful	To treat blood impurity and vitiation disorders such as acne, skin diseases, bleeding disorders etc
28.	<i>Nymphaea alatum</i>	Haladhuyavetful	To treat burning sensation, as in gastritis, neuropathy, burning sensation in eyes
29.	<i>Nymphoides indica</i>		An emollient plaster is made from the stems, leaves and flowers when extracting small shot from wounds of a hunting accident

1.	<i>Oscillatoria</i>	Selai	To treat skin disease, cancer
2.	<i>Physalis minima</i> linn	Kapalfuta	To treat Ascites, Bronchitis, cold
3.	<i>Pistia stratiotes</i>	Jalakumbi	To treat Anemia, leprosy, bladder cancer
4.	<i>Polygonum barbatum</i> u L. ( <i>Polygonaceae</i> )		Used as Stimulant, diuretic, diaphoretic.
5.	<i>Polygonum glabrum</i>	Pani-jalakia	Crushed leaves are taken in pneumonia.
6.	<i>Polygonum hydropiper</i>	Pani-jalakia	Five tender shoots ground with 10 cloves of <i>Alliun sativum</i> , 5 dried flower buds of <i>Syzygium aromaticum</i> mixed with 25 ml water and given in chest pain.
7.	<i>Ranunculus sceleratus</i>	Jaldhaniya	useful in constipation It is indicated in, infested wounds
8.	<i>Sphenoclea zeylanica</i>	Panikheti	To treat stomach disease
9.	<i>Schoenoplectus grossu</i> Palla	Keheru	Treating Vomiting, Eyes, Diarrhea
10.	<i>Trapa natans</i> Linn	Singari	To treat pyoria, anemia, cough, leprosy
11.	<i>Utricularia wallichiana</i> wight	Panikotli	To treat skin disease

Some major threats to the medicinal plant during investigation period were also recorded. Shrinkage of the area of the beel is occurring mainly due to conversion of the wetland for agriculture purpose by the local community. In Kapilmarabeel, crop field expansion was observed as highest along with fishery. In Sikhroitebeel, there was some natural area of wetland and fishery and piggery was also seen. In Kaplabeel too, wetland conversion to crop field and fishery was observed. The sarangpuriyabeel is highly shrinking due to agricultural use. When wetland is converted to crop field, the tractors are used, which destroys the medicinal plant in the wetland along with fish and other aquatic organisms.

For the conservation of medicinal plant along with biodiversity of wetland, the development of community awareness, respect, values for the benefits of wetlands is very much in need in most of the wetlands of Barpeta District. The lack of proper management committee is observed in all the wetlands, which is also required for the conservation of wetlands.

## Conclusion

Wetlands supports a rich number of medicinal plant and maximum medicinal plant was recorded in Kaplabeel and minimum was recorded in Kapilmarabeel. In India majority of medicinal plant are found in the North Eastern region. There is very little awareness about the commercial prospects of medicinal plants in NE India for recording, conservation, trade and cultivation even though the prospect is high. People's participation is need of hour to conserve the medicinal plant in the habitat for sustainable use.

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# Study of Some Medicinal Aquatic Macrophytes of Wetlands of Dhing, Nagaon, Assam

**Manikongkona Kataki, Sanjeeb Kumar Nath & Anju Deka Bora**

Department Of Botany, Dhing College Dhing, Nagaon, Assam.782123

Email: sanjeebkumarnath@gmail.com

## Abstract

Wetlands are treasure house of several medicinal plants. The commercial value of these plants are still needs special attention, in addition the local people utilize these plants for various curative purposes. A number of these plants are very sensitive to the differences in the normal physico-chemical parameter of these wetlands. Slight alteration of the normal physico-chemical parameter wetland may result in the partial or complete disappearance of these plants. This in turn may result in economic loss in terms of medicinal value. The loss of these medicinal plants will also result in the traditional knowledge of these medicinal properties which very often can't be retrieved. Thus in this paper an attempt has been made to document some medicinal plants of wetlands of Dhing area of Nagaon district of Assam.

**Keywords:** Wetland's plants, medicine, Dhing area, aquatic macrophytes.

## Introduction

Wetlands are considered to be the most biologically diverse form of ecosystem that exists on earth's surface. Wetlands are part of aquatic ecosystem that plays a major role on the biogeochemical cycle of the earth affecting the atmosphere, the climate and the hydrological cycle of a particular area. The aquatic macrophytes of the wetlands are known for its commercial value; in addition some of these plants are widely used by the local people for its medicinal value. Wetlands are very important in terms of socio-economy, biology, ecology and aesthetic view point of any area. Wetlands are the transitional or 'Ecotonal' zones between permanently aquatic and dry terrestrial ecosystems. Ramsar convention (1971) has defined wetlands as "Areas of marsh, fen, or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt including areas of marine water the depth of which at low tide does not exceed six metres".

Dhing is a small town located at the North-West part of Nagaon district in the state of Assam, India. It is in Nagaon sub-division. Dhing is located at a distance of around 25 kilometres from Nagaon town. The road from Nagaon town is the main road through which Dhing is connected. Dhing is connected with Guwahati by a broad-gauge railway track. Dhing is located at 26.47°N 92.47°E. It has an average elevation of 58 metres (190 feet). The present investigation has been conducted to assess the medicinal use of these aquatic plants by the people living in and around Dhing area. This paper deals with 10 medicinal plants (aquatic macrophytes) under 9 families, which are commonly used by the people residing in and around the area for the treatment of some common diseases and are described along with their local names, scientific names and mode of usage.

The wetlands of Dhing are very rich in plant resources which are being used by the local people for their day to day necessities and also as medicine for various diseases, the present investigation has been carried out to record the macrophytes species of the wetlands Dhing, Nagaon district of Assam, which provide valuable herbal medicine to the rural people for treatment against various common diseases.

Several floristic work has been carried out and a number of research papers have appeared in different journals on medicinal and ethno botanical property of plants by Fassett (1940), Chopra et al (1956), Jain (1965, 1975, 1994, 1995, 2000), Chopra et al (1968), Kapoor & Mitra (1979), Maheshwari & Singh (1979), Ahuja (1986), Asolkar (1992), Kareiva (1994), Cook (1996), Chamberlian (1998), Maliya & Singh (2003) and Maliya (2004)

### **Methodology**

Dhing is located at 26.47°N 92.47°E. Dhing is a small town located at North-west part of Nagaon district in the state of Assam, India. To study and record the flora and its medicinal properties found in the Dhing area an adequate field tours were undertaken. The collected specimens were pressed and dried. After proper chemical treatment and were identified by consulting and comparing herbarium sheets at Gauhati University Herbarium and Regional Herbarium of the Botanical Survey of India, Eastern Circle at Shillong. To gather the adequate knowledge the inhabitants of Dhing area were interviewed to find out the traditional knowledge regarding herbal medicine as used by the people living in and around the study area. Sufficient numbers of people from different communities were interviewed for the purpose.

### **Results and Discussion**

During the study it has been found that most of the inhabitants living in and around the Dhing area are from economically poor section. Ethnomedicinal studies among different people of Dhing in the treatment of various diseases as discussed may not be a

mere coincidence, but may be an indication of some useful properties of these aquatic macrophytes. The chemicals present in these aquatic macrophytes and its reported use for the treatment of various diseases may be a direct relationship between them. But proper investigations are required on these ethnomedicinal plants. Efforts should be made and inhabitant should be encouraged for the conservation and cultivation of these medicinal plants and their extinction can be prevented and people may also get an inexpensive remedy.

Since, the wetlands are rich in plant resources, people of its vicinity often visited to collect their traditional medicinal plant species from the wetlands..

### **Some Medicinal aquatic macrophytes of wetlands of Dhing:**

**1. *Acorus calamus* L:** *Acorus calamus* belongs to family Acoraceae and locally known as Boss. It is a Perennial wetland monocot herb with aromatic rhizome leaves linear, long, spadix sessile, cylindrical and dense flowered, fruit are reddish and few seeded berries.

**Medicinal uses:** Both leaves and root of *A. calamus* have shown antioxidant, antimicrobial and insecticidal activities. Rhizome is used in diarrhea, inflammation, fever, bronchitis, and epilepsy of children. It has also proved to be very effective against cattle tick.

**2. *Alternanthera sessilis* (L) DC:** *Alternanthera sessilis* belongs to family Amaranthaceae, locally known as Mati-kaduri. *Alternanthera sessilis* is an aquatic plant known by several common names, including sessile joyweed and dwarf copperleaf. It is used as an aquarium plant. The plant occurs around the world. The leaves are used as a vegetable. Young shoots and leaves are eaten as a vegetable in several places of the world. Occasionally it is cultivated for food or for use in herbal medicines. This is a perennial herb with prostrate stems, rarely ascending, often rooting at the nodes. Leaves lanceolate, linear-oblong and opposite. Small white flowers are borne in axillary clusters. Fruits are broad.

**Medicinal uses:** Twigs are used in jaundice and liver problem

**3. *Commelina benghalensis* L.:** *Commelina benghalensis* belongs to Commelinaceae family and locally known as Kona simolu. It is a Perennial, branched, diffuse herb of marshes, rootstock with self pollinating flowers. Leaves are ovate-oblong, acute, and base rounded, flower is blue.

**Medicinal uses:** Stem juice applied to stop bleeding of cuts, burns and eyelid sore. It is also used as diuretic, febrifuge and anti-inflammatory

**4. *Eclipta prostrata* (L) L.:** *Eclipta prostrata* belongs to family Asteraceae locally known as kehraj. This plant has cylindrical grayish roots. This species are grown commonly in moist places as a weed in warm temperate to tropical area world wide. Flower is white and axillary. Fruit achene.

Medicinal uses: Fresh leaves are given to elephantiasis; trouble of liver and in jaundice

**5. *Ipomoea aquatica* Forsk.:** *Ipomoea aquatica* belongs to Convolvulaceae family, locally known as kolmou. *Ipomoea aquatica* is a semiaquatic, tropical plant grown as a vegetable for its tender shoots and leaves. *I. aquatica* grows in water or on moist soil. These are perennial, aquatic prostrate herb. Rooted with floating shoot. Leaves alternate, long stalked, elliptic pointed. Stem is hollow creeping, jointed, floating. Flower regular, pink in colour. Fruit berry

**Medicinal uses:** Twigs are consumed as vegetable and juice of the leaf is given for blood purification.

**6. *Monochoria hastata* (L) Solm:** *Monochoria hastata* belongs to family Pontederiaceae, locally known as Kar meteka. It occurs on permanently wet swamps, freshwater pools, mudflats in rivers, ditches and rice fields, and along canal banks. It is perennial, anchored emergent, erect robust herb with creeping rootstock, rooting in mud. Leaf triangular, ovate with hastate base, mostly with a narrowed apex. Flower pale blue in raceme. Fruit a membranous capsule.

**Medicinal uses:** Leaf juice is used for curing boils.

**7. *Nelumbo nucifera* Gaertn:** *Nelumbo nucifera* is one of two species of aquatic plant in the family Nelumbonaceae. *Nelumbo nucifera*, also known as Indian lotus and locally known as Podum. The roots of lotus are planted in the soil of the pond or river bottom, while the leaves float on top of the water surface or are held well above it. The flowers are usually found on thick stems rising several centimeters above the leaves. It is giant, perennial, aquatic herb with stout, creeping, underground rhizome. Leaves are, large, alternate, orbicular, margin upturned prominently veined from the centre. Flower large, Pinkish red or white.

**Medicinal uses:** Roots are given in small pox and dysentery

**8. *Pistia stratiotes* L:** *Pistia stratiotes* L belongs to family Araceae locally known as Bor puni. It is a perennial monocotyledon with thick, soft leaves that form a rosette. It floats on the surface of the water, its roots hanging submerged beneath floating leaves. It is stoloniferous aquatic herb with a short stem bearing a rosette of sessile leaves. Root adventitious. Vegetative propagation by offsets. Leaves ovate. Inflorescence spadix. Fruit green, ovoid.

**Medicinal uses:** Leaf juice boiled in coconut oil and use in chronic skin diseases.

**9. *Polygonum hydropiper* L.:** *Polygonum hydropiper* is a plant of the family Polygonaceae. Locally known as *Pothorua bihlongoni*. It is annual, anchored emergent, erect herb. Stem is glabrous, node below swollen. Leaf is lanceolate, acute. Each leaf base has stipules which are fused into a stem-enclosing sheath that is loose and fringed at the upper end. Flower pink. Fruit nut, trigonous.

**Medicinal uses:** Leaf juice is used in skin diseases and in uterine disorder

**10. *Vallisneria natans* (Lour) Hara:** *Vallisneria natans* belongs to family Hydrocharitaceae locally known as Pata ghah. It is submerged rooted, perennial, tufted, stemless, stoloniferous herb. Root fibrous. Leaves are linear, entire, narrow, ribbon shaped, apex obtuse. Flower white, fruit oblong

**Medicinal uses:** It is used as a stomachic and for leucorrhoea

### **Conclusion**

People residing in Dhing area derived maximum health care benefits from the plant resources of the wetlands. As the communication difficulties, the easily available indigenous herbal medicines are the first level of contact for the people when they require medical care. It is very essential to take immediate steps to introduce the use of traditional herbal medicine to supplement primary health care. Health education should be given to the rural people especially concerning the use of indigenous herbal remedies. Inventory and documentation of various medicinal aquatic herbs, which are used to treat common diseases, should be developed. For preservation of such medicine plants, the natural wetlands should be kept in safe that are the only suitable habitat for most of the valuable aquatic plants. As most of these natural wetlands ('beel') have been converted to commercial fisheries and clearing of vegetation cover of the wetlands is a regular practice, proper management programmes should be adopted also to conserve the valuable indigenous aquatic medicinal plants, which serve as substitute of costly drugs of present days.

Useful information of 10 important species is present which are used to treat some common disease. The plants are medicines against various diseases. There is need for further investigations on these ethnomedicinal plants. These plants should be screened and scientifically verified for determining the true therapeutics and pharmacodynamic properties. It can be further utilized in health care needs. Efforts should be made for their conservation.

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# Traditional Medicinal System Practised By The Tea-Tribes Of Sakmuthi Tea Estate, Nagaon District, Assam

**Kaushik Jyoti Gohain<sup>1</sup>, Sourav Goswami<sup>1</sup> & Rajib Kagyung<sup>2</sup>**

<sup>2</sup>Department Botany, A.D.P College, Nagaon, Assam

<sup>1</sup>M.Sc. 3rd semester, Herbal Science and Technology

A.D.P. College, Nagaon, Assam

Email: gohainkaushik071@gmail.com

## Abstract

The Tea-Tribes people possess the knowledge of a number of herbal remedies which are prepared from wide range plant species. Some remedies are reported to have high ethno-medicinal value against some important diseases. The present study is conducted among Tea-Tribe communities in Kaliabor area of Nagaon District. Their knowledge of religions and spiritual treatment of diseases and ailments has been practiced from generation to generation. This paper recorded 15 plant species of medicinal importance used by the tribe in preparing different type of polyherbal formulations, such as *Cuscuta reflexa* Roxb., *Calotropis gigantea* (L.) Dryand., *Acorus calamus* L., *Curcuma longa* L., *Mangifera indica* L., *Averrhoa carambola* L., *Momordica charantia* L., *Psidium guajava* L. are used by Tea-Tribes for curing frequently occurring potent diseases like Jaundice, Malaria and T.B etc..

**Key-words:** Polyherbal, Tea-Tribes, Ethno-medicinal plants, Jaundice, Malaria, T.B.

## Introduction

The history of Herbal medicine is very old as human civilization. The practice of herbal medicine system flourished with the increasing use for curing various types of ailments. In developing countries like India the indigenous system of medicine with folklore medicine plays and significant role in health care system of the population. As per WHO estimates 80% of the world population is still dependent on traditional medicine [3].

The tea-tribes of Assam are the conglomeration of descendants of both tribal and backward class hindus who were brought by the British colonial planters as indentured

labourers from the predominantly tribal and backward castes dominated regions of present day Jharkhand, Odisha, West- Bengal, Telangana, and Chattisgarh into Assam during 1860-90s in multiple phases for the purpose of being employed in the tea gardens industry as labours. They are found mainly in the districts of Kokrajhar, Udalguri, Sonitpur, Nagaon, Golaghat, Jorhat, Sivasagar, Dibrugarh, Tinsukia and almost all the districts of Assam in India.[7]

The main aim of the study is to evaluate the plants used by The Tea-Tribes of Nagaon District of Sakmuthi tea-estate located in kaliabor sub-division of Assam for various purposes to record the new and the less known uses of the plants by them.

## Methodology

### 1. Studyarea

Geographically, Nagaon district of Assam is situated between the  $25^{\circ}47'$  to  $26^{\circ}42'$  N and longitude  $92^{\circ}25'$  to  $93^{\circ}19'E$  in the flood plains of river Brahmaputra and covers a geographical area of 4,435.3 sq km and has an altitude of 60.6 meter above sea level.

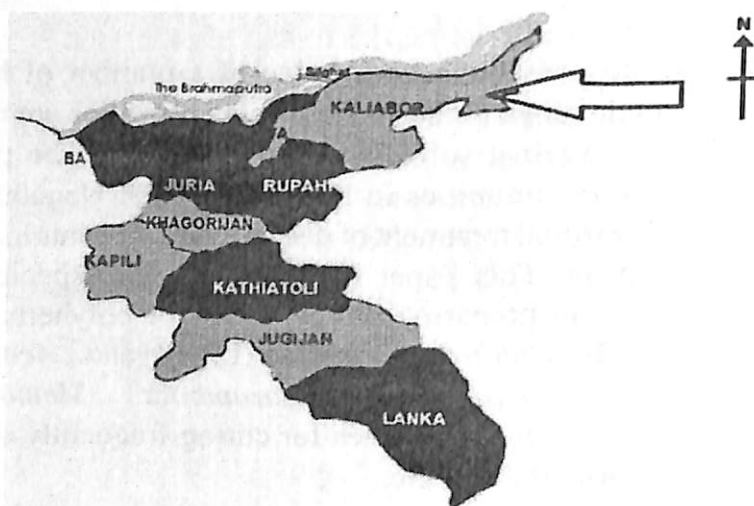


Fig 1: The arrow mark indicates the study area of Kaliabor subdivision located in Nagaon district

### 2. Methods

The field work has been carried out intensively during the year (2015-2016) among the tea-tribes belonging to the Sakmuthi tea-estate of Kaliabor Area of Nagaon district. The Tea-estate is present in the foothills bordering Karbianglong. It was established in the year 1875 by the Britishers. The information related to medicinal uses of different plants were collected after consulting with the old age people and experienced herbal practitioners.[6]

Medicinal plant specimens were collected from those study area that was identified by the local herbal practitioners. Later we prepared the herbarium of those specimens and were deposited in the Department of Herbal Science & Technology, ADP College.

### Results & Discussion:

A total of 15 species belonging to 15 Genus and 15 families were identified. For each species local name, scientific name, habit, parts used and medicinal uses were recorded and enumerated in Table 1.

Table 1: Observation of Data Analysis

Sl No	Local Name	Scientific Name	Family	Habit	Parts Used	Method of Preparation	Disease Treated
1	Dharbhaag	<i>Acorus calamus</i> L.	Acoraceae	Herb	Rhizomes tem	Leaves and rhizomes are crushed and the juice is taken orally	Jaundice
2.	Akashi Iota	<i>Cuscuta reflexa</i> Roxb.	Convolvulaceae	Climber	Tendrils	The tendril is crushed and mixed with the juice of the ginger and 1 spoon of sugar is added and taken orally for 1 week.	Jaundice
3.	Temras	<i>Psidium guajava</i> L.	Myrtaceae	Tree	Leaves	The leaves are crushed and the juice is taken orally	Dysentery
4.	Lousun	<i>Allium sativum</i> L.	Amaryllidaceae	Herb	Bulb	Hot-milk is mixed with paste of garlic and is taken orally	Tuber-culosis
5.	Nimbu	<i>Citrus limon</i> L. Burm. f	Rutaceae	Tree	Fruit	It is heated in the fire and the juice of the lemon is squeezed out and sugar is added to it.	Dysentery
6	Kmarenga	<i>Avarrhoa carambola</i> A. Juss	Oxalidaceae	Tree	Fruit	The fruit is eaten	Jaundice
7	Neem	<i>Azadirachta indica</i> A. Juss	Meliaceae	Tree	Leaves	The neem leaves are crushed and paste is prepared and added with turmeric paste	Fungal infection
8	Akan Paat	<i>Calotropis gigantea</i> L. Dryand	Apocynaceae	Tree	Leaves	The leaves are heated and then mustard oil is applied on the leaf and tied on the area of pain in the joints and bones	Arthritis
9	Hadjod	<i>Cissus quadrangularis</i> L.	Vitaceae	Herb	Stem	The stem is cut and tied up in the fractured area of the bones	Fractured bones of humans and animals, torn muscles and ligaments toothache
10	Bhendri	<i>Jatropha gossypifolia</i> L.	Euphorbiaceae	Shrub	Stem	The stem is used in the form of brush	
11	Duportenga	<i>Bryophyllum pinnatum</i> (Lam.) Oken	Crassulaceae	Herb	Leaves	The leaves are crushed and juice is taken out	Gall-bladder
12	Haldhi	<i>Curcuma longa</i> L.	Zingiberaceae	Herb	Rhizome	Haldhipaste is prepared and mixed with lime and onion paste	Pain Killer
13.	Tita-Kerala	<i>Momordica charantia</i> L.	Cucurbitaceae	Climber	Leaves and fruit	The leaves and fruits are crushed and made into a paste form	Inflammation, Ear pain
14	Dhotura	<i>Datura metel</i> L.	Solanaceae	Shrub	Leaves, flowers	Five dhotura leaves are taken crushed mixed with 100ml of milk and taken orally	Dog bites
15	Chirata	<i>Andrographis paniculata</i>	Acanthaceae	Herb	Stem	The stem is soaked in water and then taken in empty stomach early in the morning	Malaria

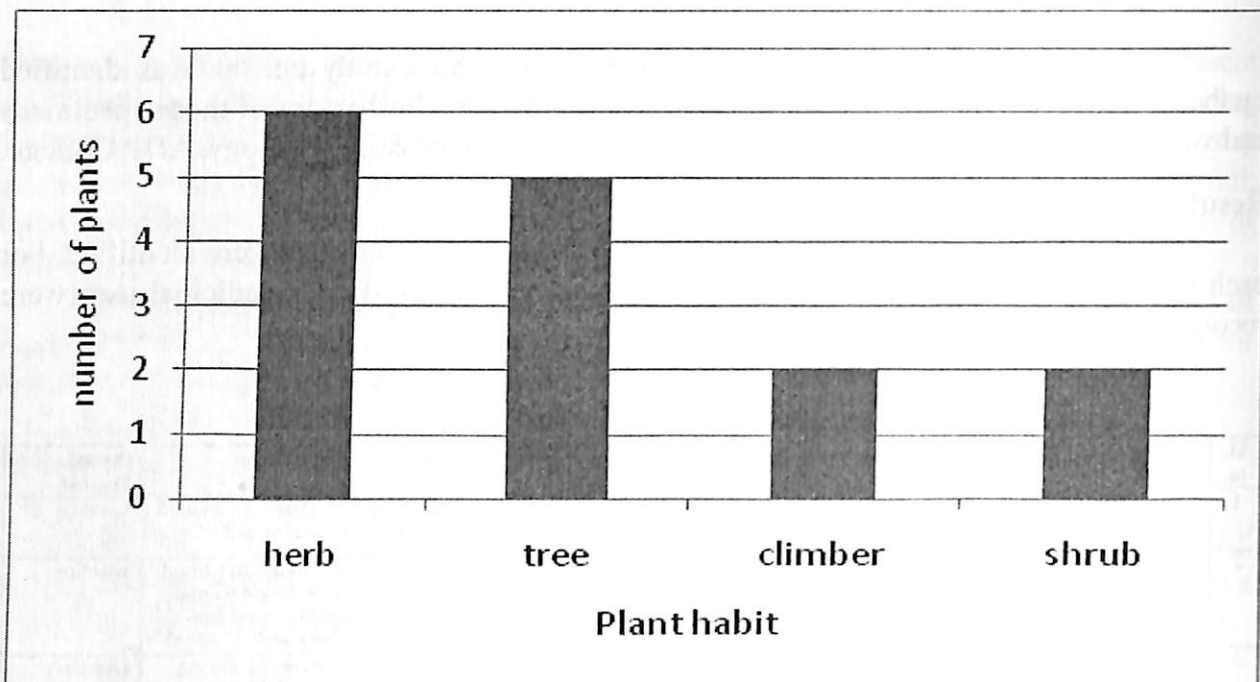


Fig 2: Different types of plants used by the tea-tribes of sakmuthi T.E

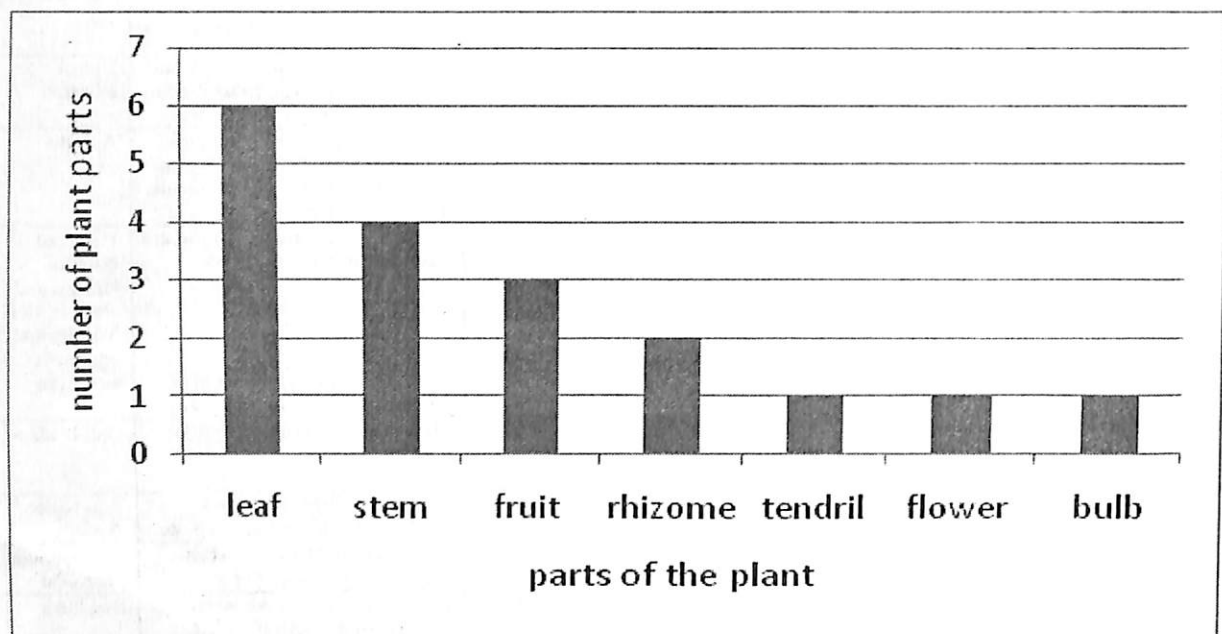


Fig 3: Different plant parts used for preparing polyherbal formulation by the tea tribes

The ethno-medico-botanical investigations reveals that the Tea-Tribes of Sakmuthi Tea-estate are primarily dependant on the plant resources to cure various ailments and diseases. Leaves and stem are found as the mostly used plant parts (6 species) followed by stem (4 species), fruit (3 species), rhizome (2 species), bulb (1 species), tendril (1 species) and flower (1 species) . Most of the plants are taken orally in the form of extract or applied to the body in the form of paste. Different additives such as sugar, milk and mustard oil are also added to some formulation.[4]

Various types of diseases such as Jaundice, Dysentery, Malaria, Arthritis, Bone-fracture, toothache, dog-bites, tuberculosis, fungal-infection was seen in the study area and accordingly the herbal formulations were prepared from the plant resources by the community for curing those disease



A: *Curcuma longa*



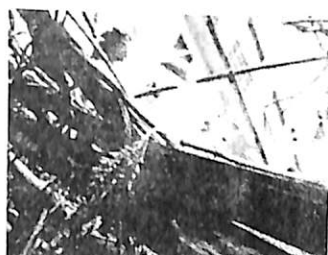
B: *Acorus calamus*



C: *Cissus quadrangularis*



D: *Citrus limon*



E: *Momordica charantia*



F: *Avarrhohoa carambola*



G: *Calotropis gigantea*



H: *Psidium guajava*



I: *Bryophyllum pinnatum*

Fig 4: Different types of plant species used by the Tea-Tribes

Proper identification, documentation and conservation of the traditional knowledge about the medicinal plants which might give us some modern drug if clinical and biochemical test have to be done. Inventory and documentation of medicinal plants at local level is important for the development of Indian system of medicine and homeopathy drugs at Pharmaceutical Industry.

### **Acknowledgement**

Authors greatly acknowledge to Tea-tribes community herbal practitioners Jayshankar Tirkey, Pintu Dubey and my friend Harendra Kumar Ojha. Authors also acknowledge Principal of A.D.P College and HOD of Deptt of HST, A.D.P College for the permission to undertake the study. Authors are also hardly thankful to the tribal people of the study area for their help and support during their study.

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# Comparative study of the hydroxylation of phenol over Zr-MFI

**Kishor Kr. Shah**

Department of Chemistry, ADP College, Nagaon

e-mail ID: kishoreshah14@gmail.com

## Abstract

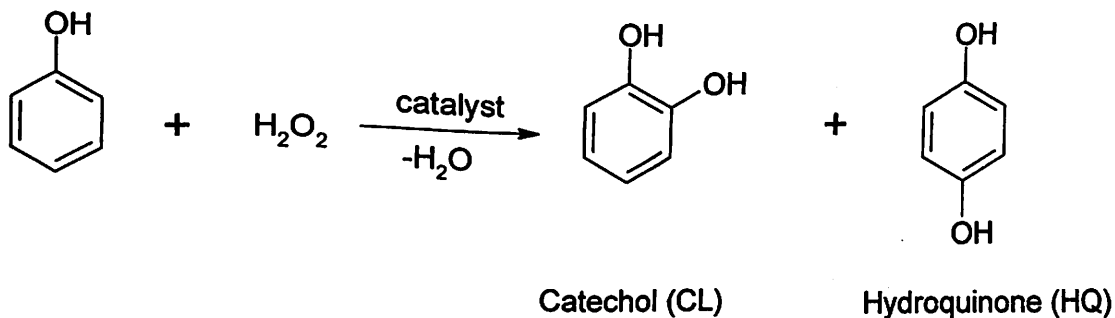
Phenol oxidation into Catechol (CL) and hydroquinone (HQ) is an industrially very important process because CL and HQ are widely used as starting materials for medicine, perfume and many fine chemicals. Catechol and hydroquinone are two high value phenolic derivatives widely used as photography chemicals, antioxidants, polymerization inhibitors and also in pesticides, flavoring agents, and medicine.

In this work, the catalytic behavior of MFI and Zr-MFI for the partial oxidation of phenol with H<sub>2</sub>O<sub>2</sub> has been reported. Special emphasis is given to the influence of Zr content and Si/Al ratio on its activity. The conversion was found to increase with the increase in the solvent polarity.

**Key-words:** MFI, Catechol, Zirconium.

## Introduction:

Oxidation of phenol by hydrogen peroxide should produce catechol (CL) and hydroquinone (HQ) as shown in Scheme 4.1.



Scheme Hydroxylation of phenol into diphenols.

The reaction is very important process because CL and HQ are widely used as starting materials for preparation of medicine, perfume and many fine chemicals [1]. Phenol hydroxylation has been studied over various catalysts, which include both homogeneous and heterogeneous catalysts. Though metal ions in homogeneous phase are active for diphenols production, the reaction rate and selectivity to diphenols are not appreciable [2,3]. Other than simple metal ions, metal complexes, have also been used. Though these metal complexes exhibit better catalytic activity and selectivity than metal ions, these complexes are not desirable for industrial applications due to shortcomings of handling and recovery of these homogeneous catalysts. Homogeneous catalysts have also been immobilized in the cavities of well-defined microporous zeolites and used as catalysts for phenol hydroxylation. However, the results are disappointing due to the narrow pores of the host species. Zeolites containing transition metal ions in their framework exhibit unique redox properties in hydroxylation of organic substrates with peroxides as hydroxylating agents.

The catalytic activity of H-MFI and Zr-MFI for phenol hydroxylation with 30 % H<sub>2</sub>O<sub>2</sub> has been investigated in the present study. Phenol hydroxylation has been carried out primarily using acetonitrile as solvent. The reaction has also been carried out in different temperatures taking water as solvent. It has been observed that all the catalysts are active for phenol hydroxylation and in all the cases, a mixture of catechol and hydroquinone are formed. The formation of catechol and hydroquinone could be more likely due to electrophilic substitution of (-OH) on the benzene ring of phenol at ortho and para positions.

### **Experimental:**

Phenol hydroxylation was carried out in liquid phase in a three-necked round bottom flask equipped with reflux condenser. The reaction was performed in the temperature range of 333 to 353 K. Prior to use in the reaction, all the catalysts were activated at 393 K for 4 h. The temperature of the reaction was maintained by a thermostated oil bath. After each experiment the catalyst was separated by filtration and then dried at 383 K for 12 h. The dried catalysts were then calcined in air at 753 K for 6 h and reused. It was observed that the performance of the catalyst was not significantly affected even after two runs. The reaction products were identified by GC-MS (Perkin Elmer, Clarus - 500). Two major products (catechol and hydroquinone) were obtained with few minor products which were not identified.

Hydroxylation of phenol reaction was carried out over S100 (simple MFI with SAR 100), S200 (simple MFI with SAR 200) and S100Zr3 (simple MFI with SAR 100 and Al to Zr ratio 3) catalysts at temperature 353 K with mole ratio of the reactants (Phenol: H<sub>2</sub>O<sub>2</sub>) 1:1 and with catalyst amount 5 (W/W) % with respect to the total substrate both under organic solvent free condition and using additional water as solvent.

In order to study the effect of reaction time, the products were withdrawn at 2, 4, 6 and 8 h intervals and were analyzed by GC-MS. The results of phenol hydroxylation over different catalysts are presented in Tables 1 to 6 and corresponding plots are shown in Figures 4.1-4.3 and 4.6- 4.8.

**Table 1 Effect of reaction time on phenol hydroxylation on S100**

Reaction conditions

Catalyst: H-MFI zeolite (S100),

Temperature: 353 K

Mole ratio of the reactants = phenol: H<sub>2</sub>O<sub>2</sub> (30%) = 1: 1

Catalyst amount: 5 (W/W) % with respect to total substrate

Reaction Time (h)	Conversion of phenol (%)	Product selectivity (%)		Ratio (CL/HQ)
		Catechol	Hydroquinone	
2	18.6	74.8	22.8	3.28
4	24.8	76.3	22.1	3.45
6	32.6	76.8	21.7	3.54
8	34.4	76.4	21.6	3.54

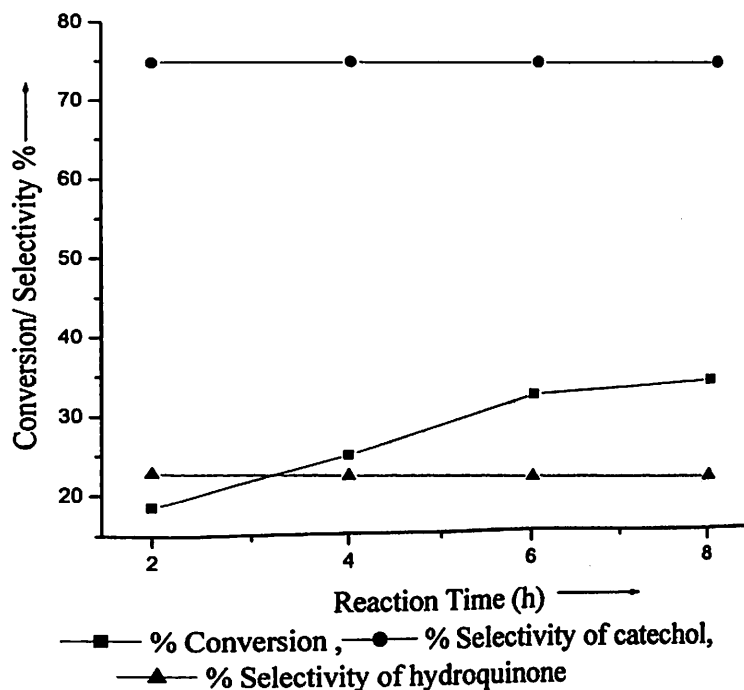


Fig.4.1 Effect of reaction time on hydroxylation of phenol

**Table 2 Effect of reaction time on phenol hydroxylation on S200**  
Reaction conditions

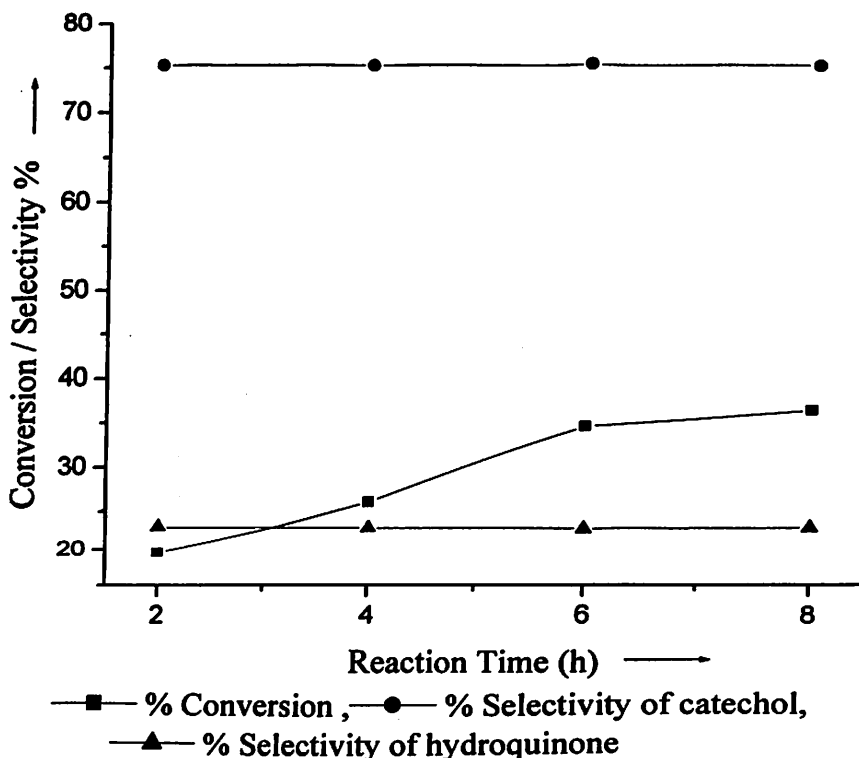
Catalyst: MFI zeolite (S200),

Temperature: 353 K

Mole ratio of the reactants = phenol: H<sub>2</sub>O<sub>2</sub> (30%) = 1: 1

Catalyst amount: 5 (W/W) % with respect to total substrate

Reaction Time (h)	Conversion of phenol (%)	Product selectivity (%)		Ratio (CL/HQ)
		Catechol	Hydroquinone	
2	19.6	75.3	23.2	3.25
4	25.4	75.4	22.4	3.37
6	33.8	76.8	21.6	3.56
8	35.4	75.4	21.1	3.57



**Fig.4.2 Effect of reaction time on hydroxylation of phenol on S200**

**Table 3 Effect of reaction time on phenol hydroxylation on S100Zr3**  
Reaction conditions

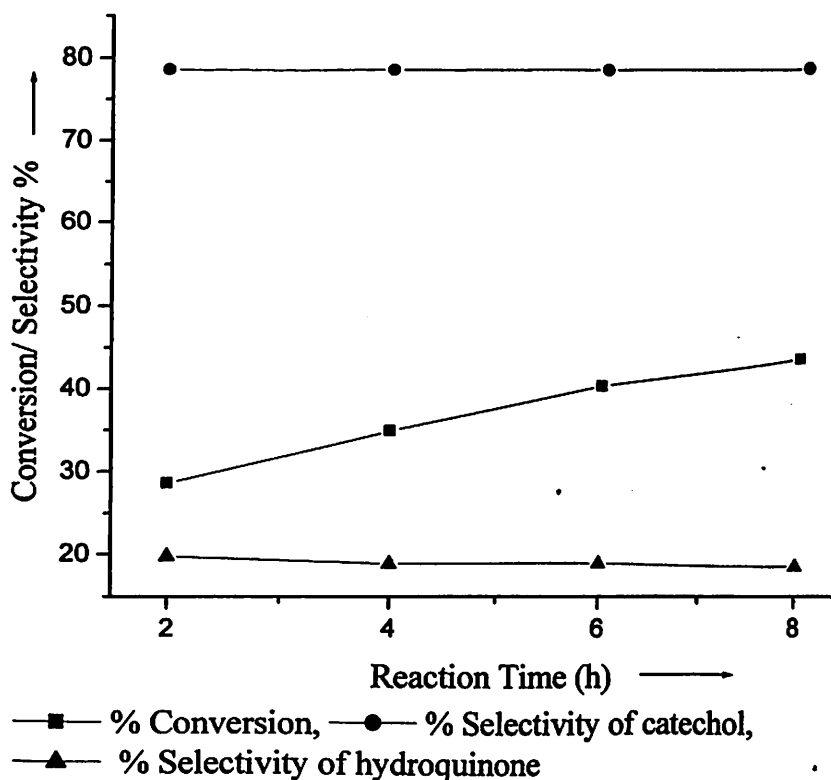
Catalyst: MFI zeolite (S100Zr3),

Temperature: 353 K,

Mole ratio of the reactants = phenol: H<sub>2</sub>O<sub>2</sub> (30%) = 1: 1

Catalyst amount: 5 (W/W) % with respect to total substrate

Reaction Time (h)	Conversion of phenol (%)	Product selectivity (%)		Ratio (CL/HQ)
		Catechol	Hydroquinone	
2	28.6	78.6	19.8	3.97
4	34.8	78.2	18.9	4.13
6	40.4	78.3	18.9	4.14
8	44.2	79.7	18.7	4.26



**Fig. 4.3 Effect of reaction time on hydroxylation of phenol on S100Zr3**

Effect of reaction time on phenol hydroxylation with 30 % H<sub>2</sub>O<sub>2</sub> and additional water

**Table 4 Effect of reaction time on phenol hydroxylation in additional water on S100**

Reaction conditions

Catalyst: H- MFI zeolite (S100), Temperature: 353 K,

Mole ratio of the reactants = phenol: H<sub>2</sub>O<sub>2</sub> (30%): solvent = 1: 1:10,

Catalyst amount: 5 (W/W) % with respect to total substrate

Reaction Time (h)	Conversion of phenol (%)	Product selectivity (%)		Ratio (CL/HQ)
		Catechol	Hydroquinone	
2	26.5	80.2	16.9	4.74
4	32.2	79.9	16.8	4.76
6	46.7	80.8	16.9	4.78
8	48.5	81.7	15.9	5.14

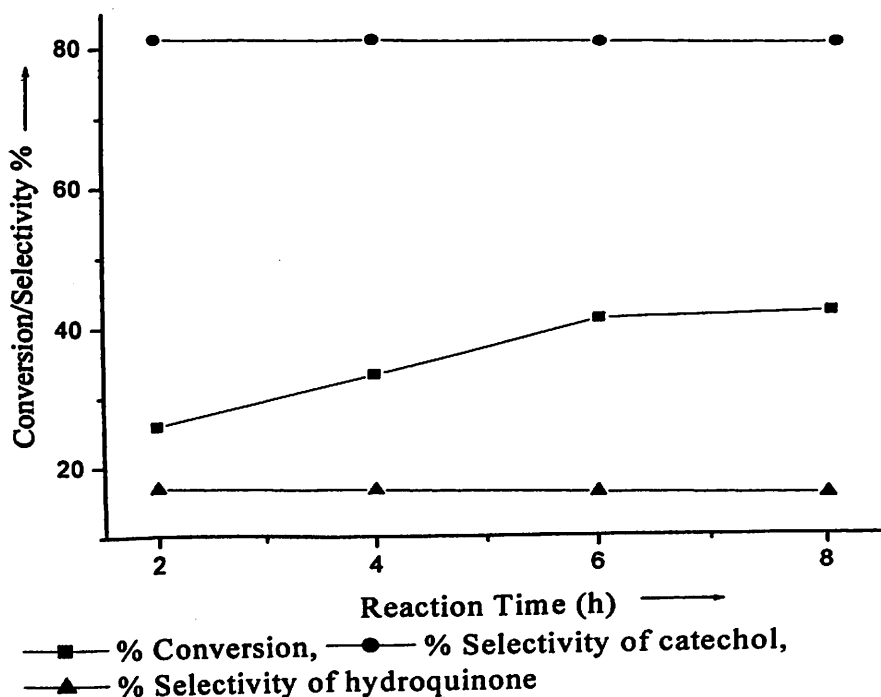


Fig. 4.6 Effect of reaction time on hydroxylation of phenol in water on S100

**Table 5 Effect of reaction time on phenol hydroxylation in additional water on S200**

Reaction Conditions

Catalyst: H- MFI zeolite (S200)

Temperature: 353 K

Mole ratio of the reactants = phenol: H<sub>2</sub>O<sub>2</sub> (30%): solvent = 1: 1:10

Catalyst amount: 5 (W/W) % with respect to total substrate

Reaction Time (h)	Conversion of phenol (%)	Product selectivity (%)		Ratio (CL/HQ)
		Catechol	Hydroquinone	
2	25.8	81.3	16.8	4.83
4	33.6	82.1	16.7	4.92
6	41.3	81.1	16.2	5.01
8	42.4	81.4	15.9	5.12

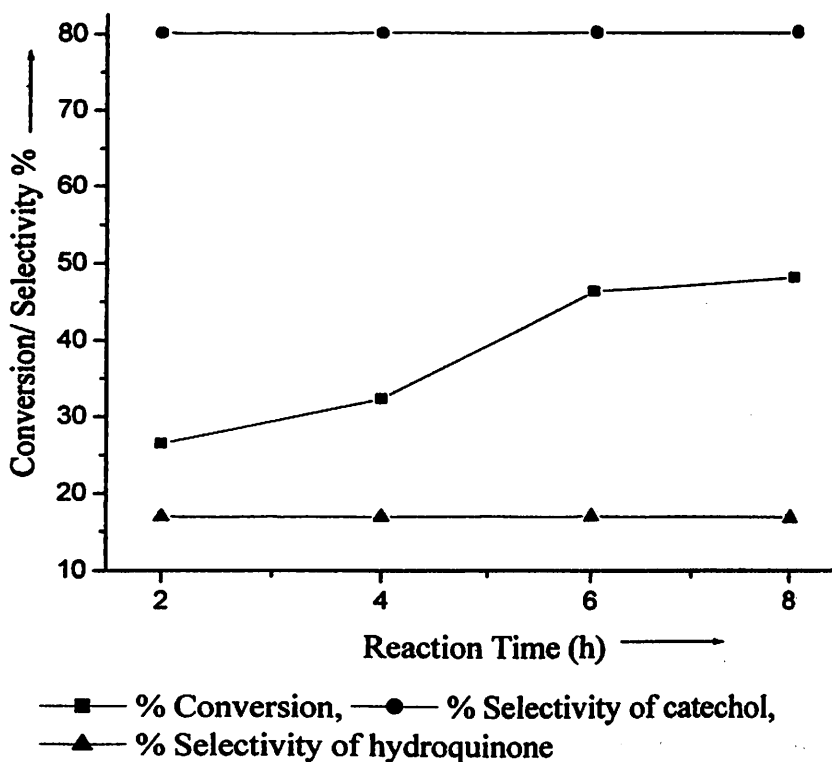


Fig. 4.7 Effect of reaction time on hydroxylation of phenol in water on S200

**Table 6 Effect of reaction time on phenol hydroxylation in additional water on S100Zr3**

Reaction conditions

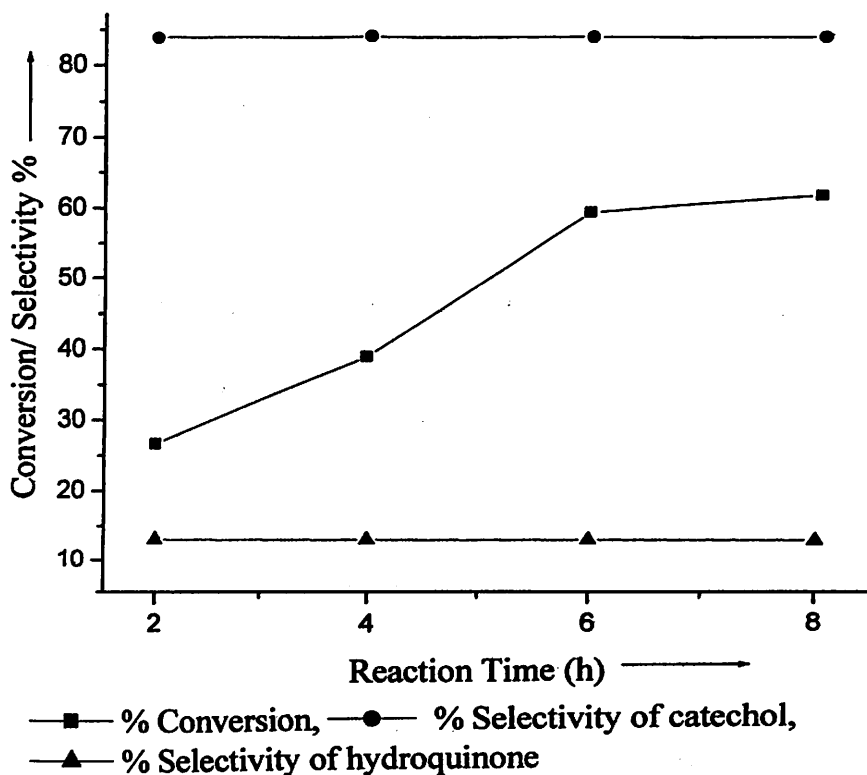
Catalyst: MFI zeolite (S100Zr3)

Temperature: 353 K

Mole ratio of the reactants = phenol: H<sub>2</sub>O<sub>2</sub> (30%): solvent = 1: 1:10

Catalyst amount: 5 (W/W) % with respect to total substrate

Reaction Time (h)	Conversion of Phenol (%)	Product selectivity (%)		Ratio (CL/HQ)
		Catechol	Hydroquinone	
2	26.8	83.9	13.2	6.36
4	39.8	85.7	13.0	6.59
6	60.2	85.3	12.8	6.66
8	62.4	85.4	12.3	6.94



**Fig. 4.8 Effect of reaction time on hydroxylation of phenol in water on S100Zr3**

## Results and Discussion:

In the present studies, conversion of phenol was found to be better when 30%  $H_2O_2$  was used with additional water as solvent in all cases. The influence of solvent can be interpreted on the basis of its polarity. Phenol conversion increases with solvent polarity. This may be due to the fact that phenol and  $H_2O_2$  can reach the active sites more easily in water medium than in organic solvents. We believe that the proximity of the hydroxylating agent and the substrate molecule on or near the active catalyst site is essential for driving the reaction. In water, both phenol and  $H_2O_2$  dissolve simultaneously and approach the active center, thereby generating hydroxy radicals, thought to be the active species involved in the hydroxylation reaction. Moreover, such an electrophile is easily produced and stabilized in water than in organic solvents. Possibly, the lack of a hydroxylated nature for the other organic solvents may be responsible for the non-occurrence of this reaction in some cases [4].

It is observed that under all reaction conditions catechol selectivity was higher than the hydroquinone selectivity. Protic solvents have been seen to favour the formation of HQ whereas aprotic solvents have been seen to favour the formation of CL.

## Conclusion

Hydroxylation of phenol was successfully carried out at the temperature range 333-353 K with 30 %  $H_2O_2$  and 30 %  $H_2O_2$  + additional water with phenol to hydrogen peroxide mole ratio of 1:1 using parent and modified Zr-MFI Zeolite. Under the present reaction conditions, catechol was the predominant product. When the progress of the reaction was monitored with time during the hydroxylation of phenol, it was observed that in all cases conversion was found to increase with increase of reaction time. In case of parent MFI samples, it is observed that conversion increases with increase in the silicon to aluminium ratio while it is less than that in case of Zr-MFI samples. Conversion of phenol was found to be better when 30%  $H_2O_2$  was used with additional water as solvent in all cases. The influence of solvent can be interpreted on the basis of its polarity. Phenol conversion increases with solvent polarity.

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# Medicinal uses of some NTFP's of Arunachal Pradesh, India

**M. K. Singh<sup>1</sup> and B.R. Gogoi<sup>2</sup>**

Wood Science and Forest Products Laboratory, Department of Forestry,  
North Eastern Regional Institute of Science and Technology,  
Nirjuli- 791109, Arunachal Pradesh, India  
Email: singh.malti978@gmail.com

## Abstract

The main aim of the present study was to highlight the medicinal uses of some NTFP's of Arunachal Pradesh using consensus analysis. A total of 35 species belonging to 30 families were studied. An informant consensus (FIC) analysis revealed a highest consensus factor for dermatological disorder (0.80%) followed by pain (0.71%), gastrointestinal disorder (0.61%) and lowest in respiratory system disorder (0.50%). Among different plant parts leaves were used highest in treatment of various diseases followed by whole plant, fruit, stem and roots.

**Keywords:** NTFP's, Informant consensus, Dermatological disorder, Stem

## Introduction

Forests are multi-functional ecosystem; those are the richest natural resources of the World and provide an often complex array of goods that includes timber as well as non-timber. The use of Non-Timber Forest Produce (NTFP) is as old as human existence. In sustenance and rural economics, the role and contribution of NTFPs are crucial because of their versatile nature, as source of food, fodder, fibre, herbal products, cosmetics and cultural products. Non-timber forest products (NTFP) are attracting more attention in recent years. NTFP plays a vital role in augmentation of tribal household income, especially in the remote and isolated pockets near and around the fringe of forest. The NTFP's possess important part of the traditional life style in Arunachal Pradesh and proper utilization has been making substantial contribution to the local livelihood.

## Materials and methods

The present study was carried out on the taxonomic survey of the NTFP's of Arunachal Pradesh. The survey was based on random selection of different villages and

the information was collected from direct interaction with the local people. Over 80 informants were chosen among different age groups. Herbarium were made of the collected species as per standard herbarium techniques (Jain and Rao, 1977) and housed in the NERIST herbarium, Department of Forestry. The plant specimen was identified by standard taxonomic procedure.

To evaluate the variability of the use of NTFP plants and to determine homogeneity on the informant's knowledge, the informants consensus factor (FIC) was calculated (Heinrich et al., 1998) given as below:

$$FIC = (N_{ur} - N_t) / (N_{ur} - 1)$$

Where,

Nur = Number of use reports by informants for usage of particular illness.

Nt = Number of species used for particular illness category by all informants.

This factor ranges between 0 and 1, where a high value means a good indicator for high rate of informant consensus.

## Results and Discussion

The present study reveals the uses of different plant parts for treatment of various diseases in the state. A total of 35 species belonging to 30 families are recorded. The highest number of plants was obtained from family Acanthaceae, followed by Lamiaceae, Asteraceae, Euphorbiaceae, Piperaceae, Rutaceae, Rubiaceae and Apocynaceae. (Fig.1). Among all plants used leaves were used mostly (54%) followed by whole plant (19%), fruit (18%) and stem (10%) (Fig.2). Informants consensus factor (FIC) was carried out and revealed a highest consensus factor for dermatological disorder (0.80%) followed by pain (0.71%), gastrointestinal disorder (0.61%) and lowest in respiratory system disorder (0.50%) (Table 2). Detailed studies of the collected species are summarized below (Table 1 and Fig.1).

Table 1: Plant species used in the treatment of various diseases

Sl. No.	Illness category	Diseases	Scientific name	Family	Local name	Parts used
1	Dermatological Disorder	Skin diseases	<i>Aloe barbadensis</i> Mill.	Aloaceae	Ghrit- Kumari (N)	Leaves
			<i>Azadirachta indica</i> A.Juss.	Meliaceae	Noem	Leaves
			<i>Drymaria cordata</i> Willd.exSchult.	Caryophyllaceae	Ropsik-Romnik(N)	Whole plant
			<i>Polygonum alatum</i> Dulac	Polygonaceae	Uyushayan (A)	Leaves
		Wound healing and Cuts	<i>Solanum torvum</i> Sw.	Solanaceae	Byakta(N)	Fruit, seed
			<i>Ageratum conyzoides</i> L.	Asteraceae	Pasho(N)	Leaves
			<i>Colocasia esculenta</i> (L.) Schott	Araceae	Annyi (A)	Juice of leaves
		Allergy	<i>Oxalis corniculata</i> L.	Oxalidaceae	SajangHabo (N)	Whole plant
			<i>Artemisia indica</i> Willd.	Asteraceae	Tapin(N)	Leaves
		Relief burning	<i>Paederia foetida</i> L.	Rubiaceae	TapinRimin (N)	Leaves and stem
<i>Aloe barbadensis</i> Mill.	Aloaceae		Ghrit- Kumari (N)	Leaves		
2	Gastrointestinal Disorder	Dysentery	<i>Acorus calamus</i> L.	Acoraceae	Bass, boch (Ass)	Rhizome
			<i>Andrographis paniculata</i> Nees	Acanthaceae	Kaalmegh	Whole plant

		<i>Garcinia morella</i> Desr.	Clusiaceae	Mibia(N)	Fruit
		<i>Garcinia pedunculata</i> Roxb. ex Buch. Ham.	Clusiaceae	Prejangbizi(A)	Fruit
		<i>Houttuynia cordata</i> Thunb.	Saururaceae	Machandari (P)	Whole plant
		<i>Psidium guajava</i> L.	Myrtaceae	Madhuri	Leaves
		<i>Tinospora cordifolia</i> (Willd.) Mierr. ex Hook. f. & Thomson.	Menispermaceae	Amar lota (Ass)	Stem
		<i>Urena lobata</i> L.	Malvaceae	Borival(N)	Root
	Diarrhoea	<i>Acorus calamus</i> L.	Acoraceae	Bass, boch (Ass)	Rhizome
		<i>Paederia foetida</i> L.	Rubiaceae	TapinRimin (N)	Leaves and stem
	Stomach pain	<i>Acorus calamus</i> L.	Acoraceae	Bass, boch (Ass)	Rhizome
		<i>Clerodendrum colebrookianum</i> Walp.	Lamiaceae	Poto(N)	Leaves, shoot
		<i>Hydrocotyle rotundifolia</i> Roxb.	Apiaceae	Barung(N)	Whole plant
		<i>Cannabis sativa</i> L.	Cannabaceae	Bhang	Leaves
		<i>Houttuynia cordata</i> Thunb.	Saururaceae	Machandari (P)	Whole plant
		<i>Terminalia bellerica</i> ( Gaertn. ) Roxb.	Combretaceae	Bahid (N)	Fruit
		<i>Terminalia chebula</i> Retz.	Combretaceae	Hilikha	Fruit
		<i>Zanthoxylum armatum</i> DC.	Rutaceae	Onier (N)	Fruit
	Liver problem	<i>Murraya koenigii</i> (L.) Spreng.	Rutaceae	Norsinghach	Leaves
	Digestive problem	<i>Aegle marmelos</i> (L.) Corrèa	Rutaceae	Bel	Leaves
		<i>Centella asiatica</i> Urb.	Apiaceae	Narang (N)	Whole plant
		<i>Garcinia pedunculata</i> Roxb. ex Buch. Ham.	Clusiaceae	Prejangbizi(A)	Fruit
		<i>Leucas aspera</i> Link	Lamiaceae	Dhub	Leaves
		<i>Mentha spicata</i> Crantz	Lamiaceae	Pudina (As)	Leaves
		<i>Phyllanthus emblica</i> L.	Euphorbiaceae	Amlakighoss	Fruit
		<i>Terminalia bellerica</i> ( Gaertn. ) Roxb.	Combretaceae	Bahid (N)	Fruit
		<i>Terminalia chebula</i> Retz.	Combretaceae	Hilikha	Fruit
	Gastric	<i>Centella asiatica</i> Urb.	Apiaceae	Narang (N)	Whole plant
		<i>Mentha spicata</i> Crantz	Lamiaceae	Pudina (As)	Leaves
		<i>Paederia foetida</i> L.	Rubiaceae	TapinRimin (N)	Leaves and stem
		<i>Psidium guajava</i> L.	Myrtaceae	Madhuri	Leaves
	Acidity	<i>Murraya koenigii</i> (L.) Spreng.	Rutaceae	Norsinghach	Leaves
		<i>Urena lobata</i> L.	Malvaceae	Borival(N)	Root
	Intestinal worm	<i>Andrographis paniculata</i> Nees	Acanthaceae	Kaalmegh	Whole plant
		<i>Nyctanthes arbor-tristis</i> L.	Oleaceae	Evahgach	Leaves, flower
	Urinary problems	<i>Averrhoa carambola</i> L.	Oxalidaceae	Rohdoi (Ass)	Fruit
		<i>Bryophyllum pinnatum</i> (Lam.) Oken	Crassulaceae	Pate goja	Leaves and stem
		<i>Paederia foetida</i> L.	Rubiaceae	TapinRimin (N)	Leaves and stem
3	Respiratory system disorder	<i>Acorus calamus</i> L.	Acoraceae	Bass, boch (Ass)	Rhizome
	Cough	<i>Adhatoda vasica</i> Nees	Acanthaceae	Vasak(P)	Leaves
		<i>Andrographis paniculata</i> Nees	Acanthaceae	Kaalmegh	Whole plant
		<i>Houttuynia cordata</i> Thunb.	Saururaceae	Machandari (P)	Whole plant
		<i>Ocimum sanctum</i> L.	Lamiaceae	Eulochi (A)	Leaves
		<i>Phyllanthus emblica</i> L.	Euphorbiaceae	Amlakighoss	Fruit
		<i>Piper longum</i> Blume	Piperaceae	Pipli	Inflorescence
		<i>Piper mullesua</i> Buch.-Ham. ex D. Don	Piperaceae	Ahoma (T)	Inflorescence
		<i>Spilanthes paniculata</i> Wall.	Asteraceae	Byadhi(N)	Leaves
		<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Arn.	Combretaceae	Arjungoch	Bark
		<i>Terminalia chebula</i> Retz.	Combretaceae	Hilikha	Fruit
		<i>Zanthoxylum armatum</i> DC.	Rutaceae	Onier (N)	Fruit
	Asthma	<i>Adhatoda vasica</i> Nees	Acanthaceae	Vasak(P)	Leaves
		<i>Andrographis paniculata</i> Nees	Acanthaceae	Kaalmegh	Whole plant
		<i>Artemisia indica</i> Willd.	Asteraceae	Tapin(N)	Leaves
		<i>Ocimum sanctum</i> L.	Lamiaceae	Eulochi (A)	Leaves
		<i>Piper longum</i> Blume	Piperaceae	Pipli	Inflorescence
		<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Arn.	Combretaceae	Arjungoch	Bark

4	Fever	Fever	<i>Adhatoda vasica</i> Nees	Acanthaceae	Vasak(P)	Leaves		
			<i>Aegle marmelos</i> (L.) Corrèa	Rutaceae	Bel	Leaves		
			<i>Bryophyllum pinnatum</i> (Lam.) Oken	Crassulaceae	Pate goja	Leaves and stem		
			<i>Carica papaya</i> L.	Caricaceae	Omita	Leaves		
			<i>Impatiens latifolia</i> Wall.	Balsaminaceae	Riong(N)	Leaves		
			<i>Nyctanthes arbor-tristis</i> L.	Oleaceae	Evahgach	Leaves		
			<i>Ocimum sanctum</i> L.	Lamiaceae	Eulochi (A0)	Leaves		
			<i>Oroxylum indicum</i> (L.) Benth. ex Kurz	Bignoniaceae	Batghila(P)	Bark		
			<i>Paecleria foetida</i> L.	Rubiaceae	TapinRimin (N),	Leaves and stem		
			<i>Piper longum</i> Blume	Piperaceae	Pipli	Inflorescence		
		<i>Piper mullesua</i> Buch.-Ham. ex D. Don	Piperaceae	Ahoma (T)	Inflorescence			
	Malaria		<i>Adhatoda vasica</i> Nees	Acanthaceae	Vasak(P)	Leaves		
			<i>Andrographis paniculata</i> Nees	Acanthaceae	Kaalmegh	Whole plant		
			<i>Azadirachta indica</i> A. Juss.	Meliaceae	Neem	Leaves		
			<i>Nyctanthes arbor-tristis</i> L.	Oleaceae	Sewaliphul	Leaves		
			<i>Ageratum conyzoides</i> L.	Asteraceae	Pasho(N)	Leaves		
5	Pain	Body pain	<i>Artemisia indica</i> Willd.	Asteraceae	Tapin(N)	Leaves		
			<i>Rauwolfia serpentina</i> Baill.	Apocynaceae	Sarpagandha (Ass)	Root		
			<i>Rauwolfia tetraphylla</i> L.	Apocynaceae	Sarpagandha (Ass)	Root		
			<i>Ricinus communis</i> L.	Euphorbiaceae	Rockrom(N)	Seed		
			<i>Impatiens latifolia</i> Wall.	Balsaminaceae	Riong(N)	Leaves		
		Headache	Toothache	<i>Solanum torvum</i> Sw.	Solanaceae	Byakta(N)	Fruit, seed	
				<i>Aloe barbadensis</i> Mill.	Aloaceae	Ghrit- Kumari (N)	Leaves	
		6	Diabetes	Diabetes	<i>Andrographis paniculata</i> Nees	Acanthaceae	Kaalmegh	Whole plant
					<i>Averrhoa carambola</i> L.	Oxalidaceae	Rohdoi (Ass)	Fruit
					<i>Azadirachta indica</i> A. Juss.	Meliaceae	Neem	Leaves
<i>Catharanthus roseus</i> (L.) G. Don	Apocynaceae				Sadabahar	Leaves		
<i>Scoparia dulcis</i> L.	Scrophulariaceae				Mithasem	Leaves		
<i>Clerodendrum colebrookianum</i> Walp.	Lamiaceae				Poto(N), Oin(A)	Leaves		
7	General health	High blood pressure	<i>Rauwolfia serpentina</i> Baill.	Apocynaceae	Sarpagandha (Ass)	Root		
			<i>Rauwolfia tetraphylla</i> L.	Apocynaceae	Sarpagandha (Ass)	Root		
			<i>Scoparia dulcis</i> L.	Scrophulariaceae	Mithasem	Leaves		
			<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Arn.	Combretaceae	Arjungoch	Bark		
			<i>Tinospora cordifolia</i> (Willd.) Miers ex Hook.f. & Thomson.	Menispermaceae	Amar lota (Ass)	Stem		
			<i>Phyllanthus emblica</i> L.	Euphorbiaceae	Amlakighoss	Fruit		
		Mouth ulcer	Blood purifier	<i>Spilanthes paniculata</i> Wall.	Asteraceae	Byadhi(N)	Leaves	
				<i>Aloe barbadensis</i> Mill.	Aloaceae	Ghrit- Kumari (N)	Leaves	
		Blood deficiency	Sinus treatment	<i>Andrographis paniculata</i> Nees	Acanthaceae	Kaalmegh	Whole plant	
				<i>Azadirachta indica</i> A. Juss.	Meliaceae	Neem	Leaves	
<i>Centella asiatica</i> Urb.	Apiaceae			Narang (N)	Whole plant			
<i>Melastoma malabathricum</i> L.	Melastomataceae			Daidassa(N)	Fruit, leaves.			
Swollen muscles		<i>Drymaria cordata</i> Willd. ex Schult.	Caryophyllaceae	Ropsik-Romnik(N)	Whole plant			
		<i>Tinospora cordifolia</i> (Willd.) Miers ex Hook.f. & Thomson.	Menispermaceae	Amar lota (Ass)	Stem			

Figure 1:1 (1-12): Gross morphology of collected plant specie



Fig. 1 : *Andrographis paniculata*



Fig. 2 : *Aloe barbadensis*



Fig. 3 : *Bryophyllum pinnatum*

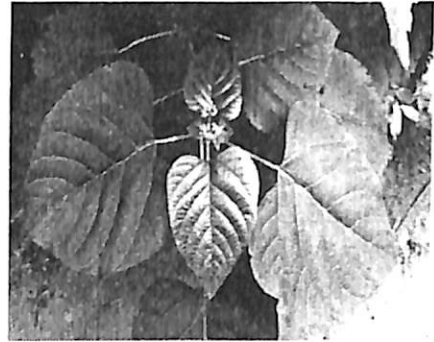


Fig. 4 : *Clerodendrum colebrookianum*

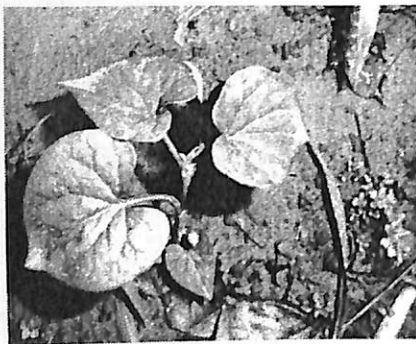


Fig. 5 : *Houttuynia cordata*



Fig. 6 : *Murraya koenigii*



Fig. 7 : *Nyctanthus arbortristis*



Fig. 8 : *Ocimum sanctum*



Fig. 9 : *Piper mullesua*



Fig. 10 : *Rauwolfia serpentina*

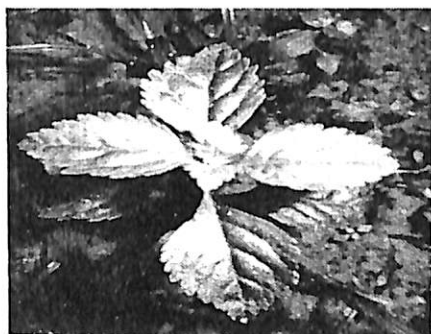


Fig. 11 : *Scoparia dulcis*

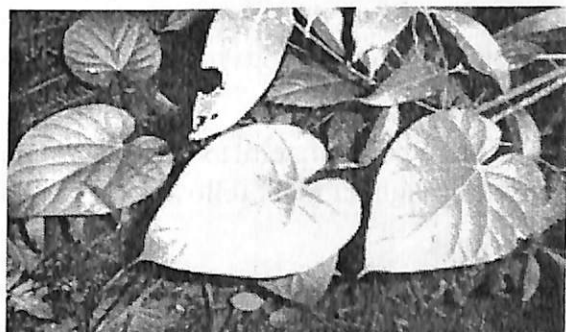


Fig. 12 : *Tinospora cordifolia*

**Table 2: Informant consensus of NTFP's with medicinal importance of different ailment categories**

Sl. no	Illness category (Diseases and disorders)	Number of taxa (N <sub>t</sub> )	Number of use reports (N <sub>ur</sub> )	Informant's consensus index factor (F <sub>ic</sub> )
1.	Dermatological disorder (Skin diseases, woundhealing and Cuts, Allergy, Relief burning)	11	51	0.80
2.	Gastrointestinal disorder (Dysentery, Diarrhoea, Stomach pain, Stomach disorder, Liver problem, Digestive problem, Gastric, Acidity, Intestinal worm, Urinary problems)	26	65	0.61
3.	Respiratory system disorder (Cough, Asthma)	13	25	0.50
4.	Fever (Fever, Malaria)	13	30	0.59
5.	Pain (Body pain, Headache, Toothache)	7	22	0.71
6.	Diabetes	6	12	0.55
7.	General health (High bloodpressure, Hair fall, Mouth ulcer, Blood purifier, Blood deficiency, Sinus treatment, swollen muscles)	15	30	0.52

### Conclusion

The present study has brought to concern for the use of various plant species in treatment of different ailments. As these plants usually occur in wild habitat, their habitats are severely threatened and are in way of extinction due to anthropogenic activities in the area. Therefore there is an urgent need for the conservation of this valuable NTFP's of the state through various training programmes and awareness among the local people.

### Acknowledgments

The author is grateful to University Grant Commission, India for providing financial support through national fellowship for OBC students.

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# Medicinal Plants Used by the Koch Rajbongshi Traditional Healers of Dhemaji District Assam, India

**\*Manash Pratim Dutta, Pranjal Boruah & Mousmi Saikia**  
Dept. of Herbal Science and Technology, ADP College, Nagaon  
\*Email: dmanashpratim6@gmail.com

## Abstract

The Koch Rajbongshi is one of the major tribe of Assam with colourful culture and tradition. Indigenous herbal medicine forms an important part of their traditional culture. However, the knowledge has remained largely unexplored. The Dhemaji district is rich in diverse vegetation including medicinal plant species. In the present study, it has been found that about 27 species of plants belonging 19 families have been used traditionally by the Koch Rajbongshi people of study area. The Koch Rajbongshi prefer plants as home remedy against various disease like jaundice, pneumonia, piles, malaria etc. They have used different parts of the plants like whole plants, roots, rhizomes, seed flower, stem bark, fruit etc. for curing several kinds of illness.

**Keyword:** Koch Rajbongshi, Dhemaji, Medicinal Plants, Traditional healers

## Introduction

Since time immemorial, mankind has used plant extracts from different plants to cure many diseases and thus relieve him from physical agony. Plants plays significant role not only in economy but also in traditional medicines. Indian people (both rural and urban) care for medicinal plants as they know so much about them and have done significant work on its applications. Probably no other medical culture has such an extensive, detailed and deep understanding about the value of medicinal plants in the globe[6]. Since historical time there is never ending relation between ethnic communities and use of medicinal plants for herbal treatment in the region[8]. It plays an important role in Koch Rajbongshi traditional medicine. The Koch Rajbongshi is an ancient tribe originally from the ancient Koch kingdom. The word Rajbongshi literally means "Rajar Bongxo" or "royal community". The Koch Rajbongshi tribe has their own customs and

tradition, languages and beliefs. The homelands of this ancient tribe include West Bengal, Assam, Arunachal Pradesh, Meghalaya and various North Eastern parts of India..[1]The Rajbongshi economy was mainly based on agriculture. The people of these villages have maximum dependency on the forest resources lying close to its proximity for fulfillment their daily needs.

Koch Rajbongshi is a dominant indigenous tribes of the district of Dhemaji. The Koch Rajbongshi is one of the major tribe of Assam with colourful culture and traditions. Indigenous herbal medicine forms an important part of their traditional culture. The practice of using indigenous plants for the treatment of various health ailments has been an old practice. But the ethnic tribes of this region still use several plants for their primary health problems, which are commonly found in this region. The plants *Tinospora cordifolia*, and *Oroxylum indicum*,*Momordica charantina* etc. are used as medicine by the local Koch Rajbongshipeople.[3]

The main objective of the present study was to discover and record the medicinal plants and their medicinal value used by Koch Rajbongshi traditional healers Dhemaji district, Assam and their conservation.

## Methods

**Data Collection:** The above data was prepared after Interviewing with local people and traditional healers. Literature Study, Personal interview and Survey was carried out at different villages of Dhemaji District.

**Data Analysis:** The data was tabulated according to their medicinal values which is used for treating various diseases.

## Area of the study

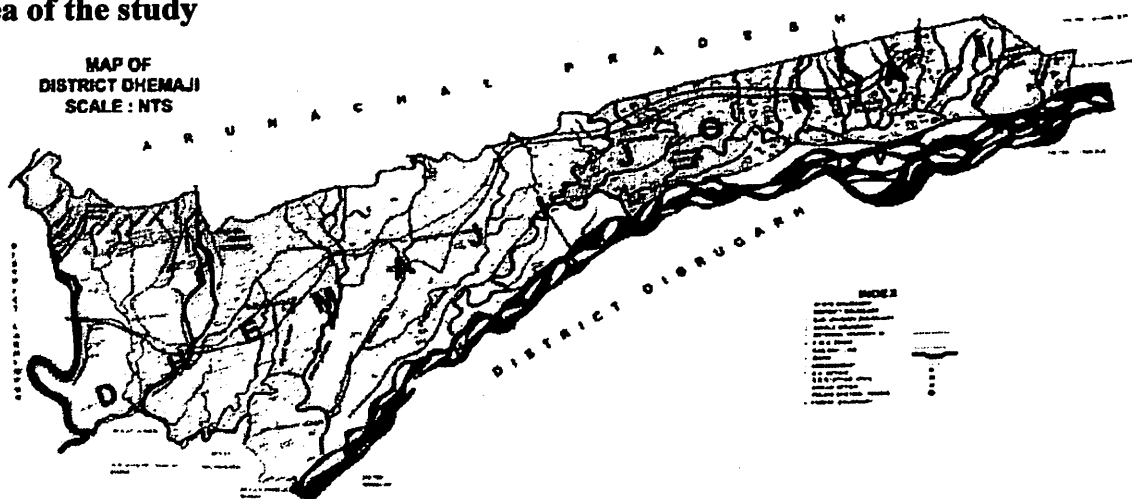


Fig: Map of Dhemaji District

Dhemaji district is situated in the remote corner of northeast India on the north Brahmaputra. Geographically, Dhemaji is located at 27.48°N 94.58°E and it has an average elevation of 91 metres (298feet). The name is believed to be derived from two words "Dhe" which means fly and "Maji" which means creeping. Dhemaji become a fully fledged district on 14th October 1989 when it was split from Lakhimpur district.

### Results And Discussion

The leaves are mostly used for the medicines. They have also used roots, seeds, flowers; stems, fruits, tubers and sometimes the whole plant for preparing medicine. It is also found that some plants are used for curing more than one disease. 70% of the plants used are herbs 25% are shrubs and only around 5% medicinal plants are documented as tree. In the present study, it has been found that about 27 species of plants belonging to different families have been used traditionally by the Koch Rajbongshi people.

Total 27 Species belonging to 19 families are identified and recorded during the survey. Plants collected were identified comparing with Flora of Assam[7] and Weeds of North-East India [4]. The observed plants are recorded and enumerated in the Table 1

Table1: Observation of Data Analysis

Vernacular name	Scientific name	Family	Habit	Parts used	Methods of Preparation	Disease treated
Nephafu	<i>Clerodendrum glandulosum</i>	Verbenaceae	Shrub	Leaves	3 tea spoonful leaf extract is mixed with a small amount of salt and given it to patient to take thrice daily for four days.	Abdominal Pain
					Leaves are boiled with water and taken it orally.	High Blood Pressure
Bhatghila	<i>Oroxylum indicum</i>	Piperaceae	Tree	Seeds	About 50 gm of dried seeds were crushed and mixed with 200ml of water. The mixture was recommender twice daily for 10 days.	Malaria
TaruwaKadam	<i>Accasia farnesiana</i>	Mimosaceae	Shrub	Stem	The bark of stem is crushed into amorphous and paste on the head.	Headache
Chirata	<i>Andrographis paniculata</i>	Acanthaceae	Herb	Leaves and seeds	10 gm of powdered dry leaves and 25 gm of dry seeds were soaked in 250 ml of water for over night it is filtered. 2 spoon full of filtrate was recommender to take thrice a day for a week.	

Neem	<i>Azadiracta indica</i>	Acanthaceae	Tree	Leaves, Fruit, Seeds, Stem Bark	Leaves, fruits, seeds and stem bark were crushed together and small globules were prepared and dried in sun, 1 globule recommender thrice a day for 1 month to cure malaria.	Malaria
					The leaves are crushed and boiled with water. It is filtered.	Skin Disorder
Goonbhaduri	<i>Paederia foetida</i>	Rubiaceae	Climber	Leaves	The leaves of the plant is grinded and mixed with ricepowder and fried with oil to make pitha which is taken orally. Moreover it is also mixed with jira and used for treating various disorders.	Stomach Disorder
					The leaves are crushed and juice is squeezed out	
a) Amita	<i>Carica papaya</i>	Caricaceae	Tree	Root	Sidal is the traditional food that comes first which been used by the Koch rajbongshi people. For preparing the Sidal many items has been used that are root of the papaya plant, rice, black pepper, chillies, onion, garlic, mon gomari, kolor khar, kolful, "bettor xipa" which are mixed with dried fish and a paste is made that is converted to a solid round like structure and kept under the sun for drying. Then it is preserved for other purposes.	Cough, Malaria
b) Jaluk	<i>Piper nigrum</i>	Piperaceae	Climber	Seed		
c) Onion	<i>Allium sepa</i>	Alliaceae	Herb	Seed		
d) Ada	<i>Zingiberofficin ale</i>	Zingiberaceae		Root		
e) Jalakiy a	<i>Capsicum frutescens</i>	Solanaceae	Shrub	Seed		
f) Bet	<i>Calamus viminalis</i>	Arecaceae	Climber	Root		
Tezmui	<i>Zanthoxylum hamiltonianum</i>	Rutaceae	Climber	Root	The roots are crushed and juice istaken orally	Stop Bleeding during Menstrual Cycle
Dubaghas	<i>Cynodon dactylon</i>	Greaminae	Herb	Fresh Grass	Fresh grass is crushed and the juice is taken out	Menstrual bleeding stops, enhances fertility in male , quick healing of wounds
Keturi	<i>Curcuma aromatica</i>	Zingiberaceae	Herb	Rhizome	Juice extracted from equal amount of rhizomes of	

Antamul	<i>Tylophora asthamatica</i>	Asclepiadaceae	Herb	Leaves/ Stem	Leaves and roots are boiled and taken in empty stomach.	Blood purification
Bandor kekura	<i>Mucuna prurita</i>	Fabiaceae	Climber	Leaves	Root is mixed with honey and milk	Ovary Problems
Khar Gos	<i>Cassia alata</i>	Fabiaceae	Shrub	Leaves/ Flower/ Seed	Leaves juice are directly apply on the affected area	Ring Worm
					Flower and seeds are crushed and boiled with water for 15 minutes. Cool and recommender to take thrice a day .	Stomach disorder
Laijabori	<i>Drymaria cordata</i>	Caryophyllaceae	Herb	Whole plant	Leaves are crushed and the juice is extracted out and it is given dropwise into the eye	Bowl
Sagoonilota	<i>Tinospora cordifolia</i>	Menispermaceae	Climber	Leaves and stem	Leaves and stem are boiled and taken.	Diarrhea
Narashingho	<i>Murraya koeingii</i>	Rutaceae	Tree	Flower/L eaves	Flowers are boiled with water for 30-35minutes. Filtrate was recommended to take orally twice a day for 14 days.	Malaria
BonDhania	<i>Scoparia dulcis</i>	Scrophulariaceae	Herb	Leaves	Leaves are crushed and about 7 pieces of it. Mixed with water and are recommended to thrice a daily after food.	Malaria
Sookloti	<i>Perilla ocimoides</i>	Lamiaceae	Shrub	Leaves and stems	Leaves and stems are cooked along with pepper.	Helps fight Uterus infection, MC irregularity disappears.
a)Posotiya b)BogaBahak c)Ada d)Durun Bon	<i>Vitex negundo</i> <i>Adhatoda zeylanica</i> <i>Zingiberofficinale</i> <i>Leucas aspera</i>	Verbenaceae Acanthaceae Zingiberaceae Lamiaceae	Tree Herb Herb Herb	Leaves	Same amount of <i>V. negundo</i> , <i>A. zeylanica</i> , <i>Z. officinale</i> , <i>L. aspera</i> are crushed together. Juice was extracted and mixed with water and dry rhizome powder of <i>Z. officinale</i> and recommended to take orally twice a day for 10 days.	Malaria



A: *Zingiber officinale*



B: *Mucuna prurita*



C: *Calamus viminalis*



D: *Oroxylum indicum*



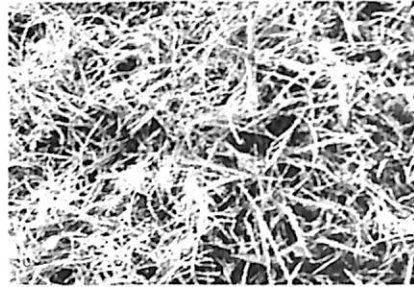
E: *Vitex negundo*



F: *Scoparia dulcis*



G: *Accasia farnesiana*



H: *Cynodon dactylon*



*Azadiracta indica*

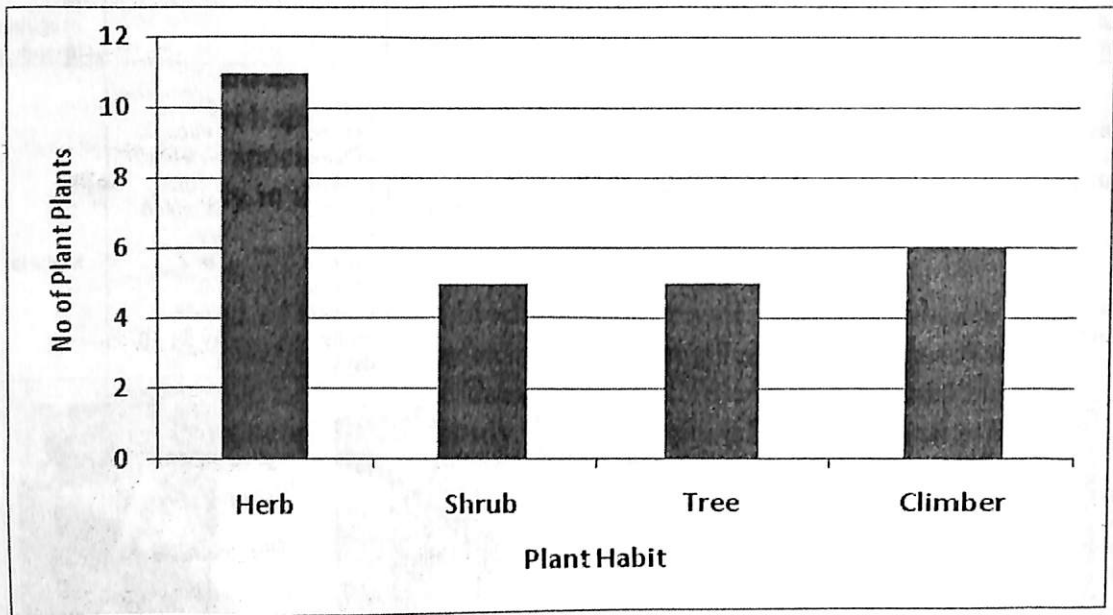


Fig1: Different types of plants used by the Koch Rajbongshi people of Dhemaji District

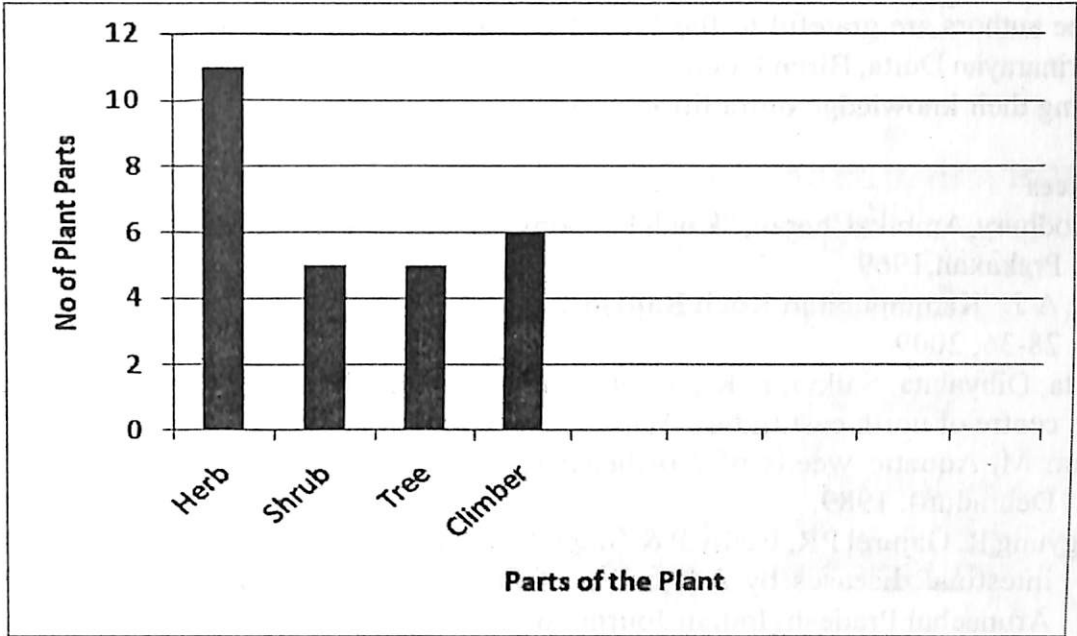


Fig 2: Different types of plant species used by the Koch Rajbongshi communitiy

The medicinal plant resources are mainly tree, shrub, herb, and climber in nature . The ethno-medico-botanical investigations reveals that the Koch Rajbongshi people of Dhemaji district are primarily dependant on the plant resources to cure various ailments and diseases. Herbs are found as the mostly used plant parts (11 species) followed by stem (1 species), fresh grass (1 species), rhizome (1 species), seed (3 species), leaves (7 species) and whole plant (1 species) . Most of the plants are taken orally in the form of extract or applied to the body in the form of paste.[5]

**Conclusion**

Dhemaji district is one of the richest biodiversity area of Assam. It harbours a good number of medicinal plants. There may exist a number of medicinal plant whose medicinal value is not known or cannot be documented, that are being traditionally used by the Koch Rajbanshi people in the study area. The people rely on such herbal system because the modern system is out of reach due to economy and less side effect and more curing capacity. The concept of disease is governed by their understanding of the environment . The results of the present study provide evidences that medicinal plants are still continuing to play an important role in traditional health care system.

## **Acknowledgement**

The authors are grateful to the local Koch rajbongshi people, Biswajit Narayan Ray, Harinarayan Dutta, Biren Koch, Dinesh Koch, Gobin Dutta, Ritumoni Rajbongshi for sharing their knowledge on traditional medicine system.

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# A study of pharmacognostic, antioxidant and antibacterial properties of *Dioscorea pubera*, Blume (Yam) tuber

Mir Bahar Uddin<sup>1</sup>, Farhana Sultana<sup>1</sup>, Bapan Banik<sup>1</sup>,  
Bhaskar Saikia<sup>2</sup> & Mousmi Saikia<sup>1</sup>

<sup>1</sup>Department of Herbal Science and Technology, ADP College, Nagaon

<sup>2</sup>Department of Botany, Cotton College State University, Guwahati

Corresponding author: baharmir22@gmail.com

Tel. No.: 8723008571(M)

## Abstract

The present study aims at evaluating the pharmacognostic, antioxidant and antibacterial studies of methanol, chloroform and aqueous extract of *Dioscorea pubera*, Blume tuber. Phytochemical screening, moisture content, ash value and vascular bundle studies were done in pharmacognostic studies. Total phenolic content was estimated by using the Folin Ciocalteu method and total flavonoid content was determined using 10% of aluminium chloride. The extracts were screened for its potential antioxidant activities using DPPH radical scavenging activity, superoxide radical scavenging activity. In vitro studies such as antimicrobial activities were determined by using agar well diffusion method, which revealed potential antibacterial activity. Thus the result gives a scientific base for use of *Dioscorea pubera* as nutritional food as well as therapeutic purposes.

## Introduction

Plants and herbs have played a significant role in maintaining the quality of food supplements and medicine. Nature is a big source is a big source of bioactive phytoconstituents or therapeutic agents which are being used by traditional method in traditional community from one generation to next generation. Phytoconstituents, especially phenolics compounds are the principal constituents present in plant body which have various types of biological functions. The species *Dioscorea* is one of the novel herbs which are being used in many pharmaceutical industries throughout the world. In India *Dioscorea* family is found commonly both tropical and subtropical region.

The tubers of *Dioscorea* species contains wide variety of secondary metabolites like starch, saponins, terpenoid, flavanoids, alkaloids, glycosides and different type of inorganic salts. *Dioscorea* species possesses different biological activities such as, anti-fungal, anti-bacterial and anti-cancer due to the presence of secondary metabolites (Sautour et al., 2004; Li et al., 2001). *Dioscorea* species have potential antioxidant activity due to the present of different bioactive metabolites (Sonibareet., 2012). Ethnolic leaf extract of *Dioscorea hispida* shows anti-inflammatory activity (Panduranga et al., (2011). The bulbils of *Dioscorea* species are also used to treat piles, syphilis and are applied to ulcers and inflammation (Mbiantcha et al., 2011; Kumer et al., 2013)

*Dioscorea pubera*, Blume belongs to the family Dioscoreaceae under the order of liliales which is commonly known as Haldiaalu (Assamese), Ruichelong (Karbi) and Bengukanda (Tea Garden Community) in Assam, India. It is known as Kosaaalu in Odissa (Kumer et al., 2013). Though it is widely distributed throughout the North-East region, India. It is mostly found in Tinsukia and Kamrup districts in Assam. Traditionally *Dioscorea pubera* has been used as an anti-rheumatic and to treat ophthalmic conditions. Ethnobotanically tubers of this species is boiled and mixed with rice & salts as eaten as famine food in both Assamese & tribal community in Assam. The tuberous rhizome and bulbil are cooked and given to relief colic pain (Sheikh et al., 2013 and Dutta, 2015) The tubers are eaten to remove weakness or as tonic also (Kumar et al., 2011).

## **Material and Methods**

### **Collection of plant sample preparation.**

The fresh tubers of *D. pubera*, Blume were collected from the Upper Dihing Patkai Sanctuary, Lakhipather range, Digboi in Tinsukia of Assam.

The tubers were washed under tap water and cut into small pieces. The fresh pieces were dried in hot air oven at 40°C. After drying samples were placed in a mixer grinder to the coarse powder and stored in air tight container in room temperature.

### **Physico-chemical parameters.**

The physico-chemical parameter of crude powder gives the purity and conformational identity of herbal drugs. The physicochemical constants such as total ash, water soluble ash values, acid insoluble ash values, extractive values and moisture content were calculated by following standard protocol (Indian pharmacopoeia., 1996).

### **Preparation of plant extract.**

10g powder of sample of *Dioscorea pubera* was subjected to Soxhlet extraction using solvents like methanol, chloroform and water according to their polarity. All the three different extracts of *D. pubera* were kept in air tight container and stored in refrigerator at 4°C.

### **Phytochemical investigation.**

The extracts of *D. pubera*, tuber were screened for the presence of alkaloids, flavanoid, glycosides, tannins, saponins, carbohydrate, resins following standard protocol.

### **Estimation of total phenolic content.**

The concentration of phenols in tuber extracts of *Dioscorea pubera*, Blume was determined by using Folin-Ciocalteu method (Waterman & Mole, 1994). The samples were dissolved in 3 mL distilled water and 0.5 mL Folin-Ciocalteu reagent. After 3 minutes 2 mL of 20% Na<sub>2</sub>CO<sub>3</sub> was added and shaken vigorously. The samples were thereafter paved in boiling water bath for 1 minute and then cooled. The absorbance was determined by using spectrophotometer at  $\lambda_{max}$  = 650 nm against a blank. Total phenolic content was expressed in terms of gallic acid equivalent (milli gram of GA/g of dry extract).

### **Estimation of flavanoids.**

The content of flavanoids was determined using method aluminium chloride method (Chang et al., 2002). An aliquot of 0.5 mL of each sample (1mg/mL) was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M potassium acetate and then 2.8 mL of distilled water. The reaction mixtures were then incubated for 30 minutes at room temperature and the absorbance was determined using UV spectrophotometer at  $\lambda_{max}$  = 415 nm. The content of flavanoids in extracts was expressed in terms of quercetin equivalent (mg/g extract).

### **In vitro antioxidant activity**

#### **DPPH radical scavenging activity**

The DPPH is a stable, sensitive and rapidly used method to assess the radical scavenging activity. Free radical is an unpaired number of electrons present in atom which lead the cellular damage.

The ability of all extracts to scavenge free radical was determined by DPPH method (Blois, 1957). 0.1 mL of each extract (1mg/mL) were mixed with methanol at different concentration (50, 100, 150, 200, 250 and 300  $\mu$ g/mL) and aliquot 2.5 mL of 75 $\mu$ M DPPH solution. The mixtures were then allowed to stand in darkness at room temperature for 90 minutes. Then the absorbance was recorded using UV-VIS spectrophotometer at wavelength of 517 nm. The ability of scavenging the DPPH radical activity was calculated as follows

$$\% \text{ of scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

### Hydrogen peroxide radical scavenging activity

The scavenging capability of extracts for hydrogen peroxide was measured according to Ruch et al, 1989 method. A solution of hydrogen peroxide was prepared in phosphate buffer at pH 7.4. The methanol solution of each extract (1 mg/mL) was added to 0.6 mL of hydrogen peroxide solution. The gallic acid mixtures were prepared by following same procedures without adding the plant extracts. Thereafter the absorbance of sample mixtures was determined at 230 nm by UV- VIS spectrophotometer. All the tests were performed in triplicate for each tuber extracts and results were evaluated in average. The hydrogen peroxide radical scavenging activity of the extract was reported as inhibition percentage and calculated by following equation

$$\text{Hydrogen ratical scavenging activity} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A<sub>0</sub> is the absorbance of control reaction

A<sub>1</sub> is the absorbance in presence of all of the extract sample and reference.

### Antibacterial studies

#### Test organisms

Four bacterial strains were used namely *Escherichia coli* (739), *Klebsiella pneumoniae* (432), *Bacillus subtilis* (441) and *Staphylococcus aureus* (96). The organisms were processed from MTCC, Chandigarh, Punjab, India and maintained in nutrient agar slants at 4°C.

The antibacterial evaluation of different bacterial strains was tested by Agar Well Diffusion method. The diluted bacterial culture was streaked over the nutrient agar plates by sterile bud. There are three wells of 5 mm diameter for each extract were made by using sterile cork borer in agar plate aseptically. The wells were then filled by each extract (20mg/1mL DMSO) at different concentration (50, 80 & 100 µl) and allowed to dry. The experiment was repeated in twice for each organism and the plates were incubated at 37°C for 18 hours. After incubation, the zone of inhibition was measured as diameter in millimeter.

### Results and discussion

#### Physicochemical parameters

The physiological parameters of powdered drug of *Dioscorea pubera* tuber like total ash value, acid insoluble ash value, water soluble ash value, alcohol soluble extractive value, water soluble extractive value and moisture content (%W/W) was evaluated and found some validate parameters. The results of physicochemical parameters prove the presents of different inorganic compound in the powdered drugs. The parameters are given in table no.1.

### **Phytochemical analysis**

Phenolic compounds are one of the major active components commonly present in plant body which act as free radical terminators and have different biological activities. The phytochemical investigation of extracts of methanol, chloroform and water of *Dioscorea pubera* revealed the presents of various phytoconstituents viz, flavanoid, Terpenoid Saponin, glycoside and carbohydrate etc. The investigated results are given in the table no. 2.

### **Estimation of total phenolic and flavanoid contents**

Total phenolic content was found to be highest in methanol extract 90 mg/g than water (65 mg/g) and chloroform (60 mg/g) extracts of *Dioscoreapuberain* gallic acid equivalent. Flavanoid content was found be highest in methanol extract 60 mg/g than water (53.33 mg/g) and chloroform extracts (43.33 mg/g) also. The results are shown in the fig.1.

### **Free radical scavenging activity**

Antioxidants are natural or man-made compound that have higher ability of donating a single electron or hydrogen atom for reduction that prevent or delay some types of cell damage by neutralizing the free radicals. Free radical are unpaired number of electrons present in atoms, molecules or ions which produced by breakdown of nucleotide sequence, amino acid sequence, certain medicines and other biological changes into human body. Free radicals may also exposed from external sources through X-ray, smoking, air pollutants and industrial chemicals like carcinogens. DPPH assay is one of the most widely used and reliable method to determine the free radical scavenging activity of plant extracts. The DPPH radical scavenging activity of *Dioscoreapubera* tuber extract increased with increasing concentration ranging from 50µg/mL upto 300µg/mL. In DPPH assay the free radical scavenging activity of methanol extract of *Dioscoreapubera* was found to be increased from 46.14 to 76.34% at a concentration of 50-300 µg/mL. Free radical scavenging activity of aqueous & chloroform extracts of *Dioscoreapubera* tuber was found to be respectively 33.47-64.11% & 29-57% at a concentration of 50-300 µg/mL. The inhibition percentage of ascorbic acid found to be 61.31 to 89%. These results indicated that the methanol extract of *Dioscorea pubera* tuber exhibit the highest ability to scavenge DPPH free radical than the water and chloroform extracts which is remarkable to standard used. The results are shown in the figure 2.

Hydrogen peroxide radical is one of the major reactive oxygen species in the biological system which causes various cellular damage to leads to various pathological conditions like cancer, AIDS, asthma, arthritis, diabetes, autoimmune diseases, cardiovascular disorder and neurodegenerative disorder etc. The ability of the tuber

extracts of *Dioscorea pubera* to scavenge the hydrogen peroxid radicals was found to be slightly increased with increasing amount of extract concentration. In this assay the inhibition percentage of the methanol extract of *Dioscorea pubera* tuber reached 64 % a concentration of 50-300 µg/mL while at the same concentration that water & chloroform extracts of *Dioscorea pubera* tuber was found to be 55-58 % respectively. The parameters are graphically represented in figure 3.

The present antioxidant studies of *Dioscorea pubera* tuber extract reveals that methanol extract have the strong free radical scavenging activity in various in-vitro methods than water and chloroform extract which is comparable to standard used and the DPPH method has the strong ability to scavenge the free radicals among the other two in-vitro methods.

### Antibacterial evaluation

The antibacterial activity of *Dioscorea pubera* tuber extracts of different solvents in different working concentrations were evaluated by disc diffusion method and zone of inhibition was measured in mm diameter. The methanol extract of *Dioscorea pubera* was potent effective results in variable zone of inhibition (16-22mm) against for all the bacterial strains in the concentration of 100µl/mg. The Methanolic tuber extracts of *Dioscorea pubera* showed potent antibacterial activity towards *E.coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* at a concentration of 100µl/mL and the zone of inhibition was found to be 22 mm, 16 mm and 20mm in diameter respectively. The methanol and chloroform extract was showed more effective against *Bacillus subtilis* with zone of inhibition was 18-22 mm, respectively followed by water extract with 18 mm in diameter at a concentration of 100µl/mL. The different bacterial strains were subjected to the standard antibiotic chloramphenicol and the results were found to be variable inhibitory zones of 31-52 mm in diameter at a concentration of 1 mg/mL. The zone of inhibition showed by the standard (1mg/mL) reveals that methanol extract showed the remarkable effect against the microorganisms tested in vitro than the other two chloroform and water extracts. The zone of inhibition is given in the table no. 3.

Table 1: Physiochemical parameters of *Dioscorea pubera* tuber powder

S.I. No.	Parameter	Average % W/W
1	Ash values	
	Total ash value	6
	Acid insoluble ash value	4.6
	Water soluble ash value	5.3
2	Extractive values	
	Alcohol soluble	18
	Water soluble	32
3	Moisture content	9

Table 2: Preliminary qualitative phytochemical observation of tuber extracts of *Dioscorea pubera*, Blume

Name of the Phytoconstituents	Test Performed		Extract		
			Methanol	Chloroform	Water
Alkaloid	Dragendroff's test	Mayer 's test	-	-	-
Flavanoid	Lead acetate test		+	+	+
Terpenoid	Standard test		+	+	+
Glycoside	Keller-Killiani test		+	+	-
Saponin	Froth test	Foam test	++	+	+
Carbohydrate	Fehling's test	Benedict test	+	+	+

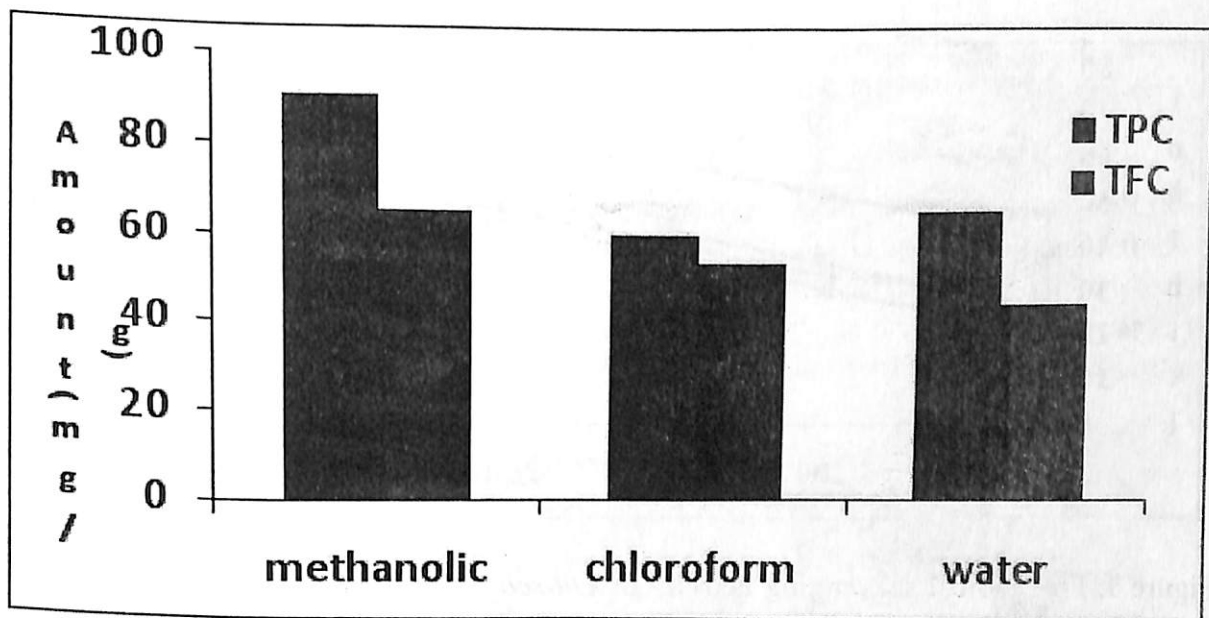


Figure1: Concentration of total phenolic and flavonoid content in *Dioscorea pubera* tuber

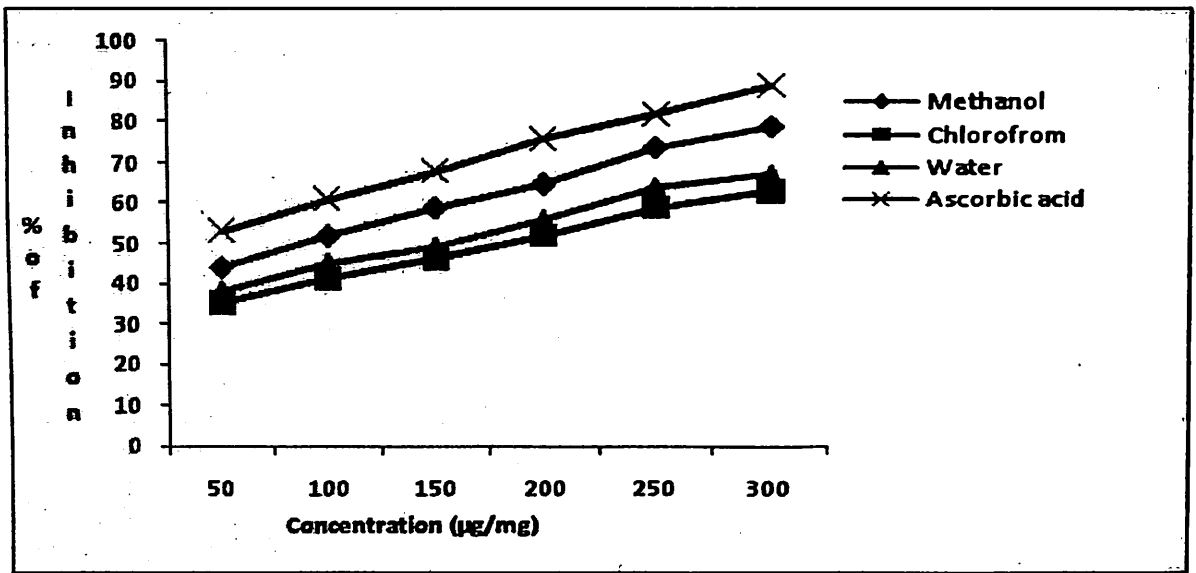


Figure 2: DPPH free radical scavenging activity *Dioscorea pubera* tuber extract

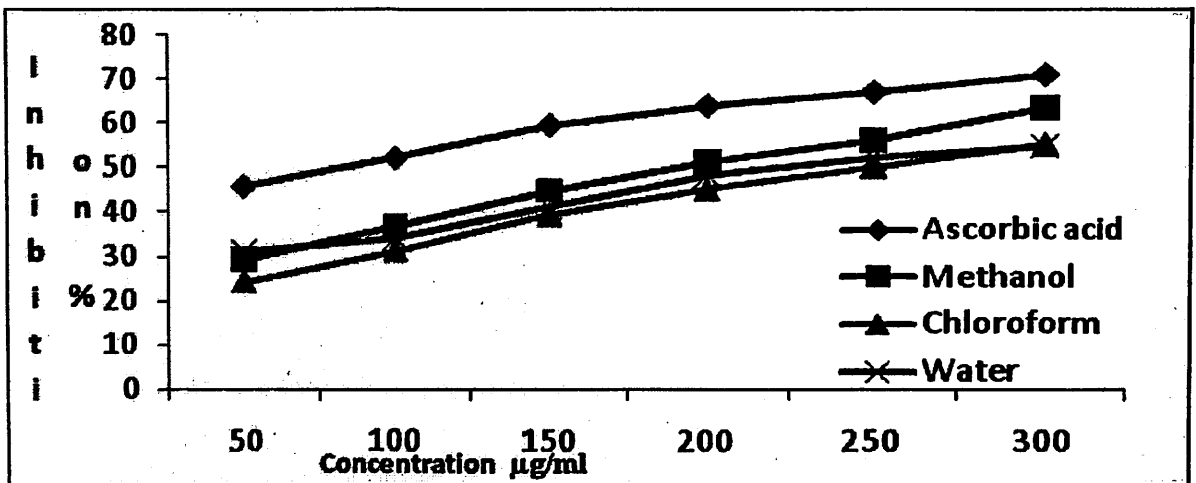


Figure.3: Free radical scavenging activity of *Dioscorea pubera* extracts by hydrogen peroxide method

Table : 3. Antibacterial activity of *Dioscorea pubera* showing zone of inhibition

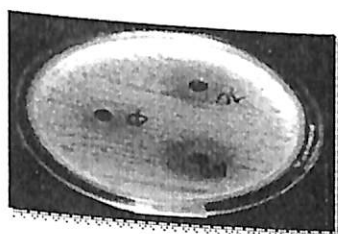
Tested organism	Zone of inhibition (mm)			
	Methanol	Chloroform	Water	Chloramphenical
<i>Escherichia coli</i>	19	16	14	42
<i>Klebsiella pneumoniae</i>	22	14	16	52
<i>Staphylococcus aureus</i>	24	20	20	42
<i>Bacillus subtilis</i>	22	22	18	31

### Conclusion

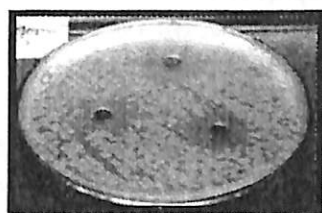
The present study provides useful information about the different phytoconstituents present in the tubers of *Dioscorea pubera* and many other in vitro activities which may lead to identifying and isolating the pure compounds from the plant body.

### Acknowledgement

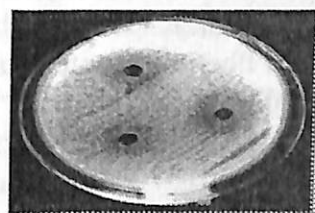
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Methanol

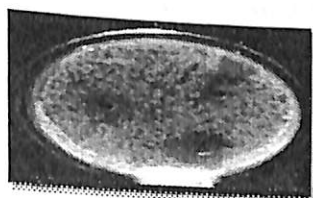


Chloroform

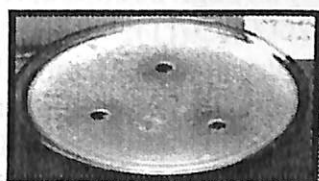


Water

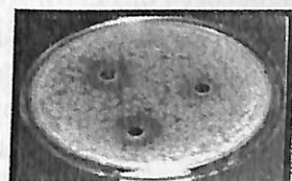
Figure 4: Zone of Inhibition against *Klebsiella pneumonia* by *Dioscorea puber* tuber extracts



Chloroform



Methanol



Water

Figure 5: Zone of inhibition against *Bacillus subtilis* by *Dioscorea pubera* tuber extracts.

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# Ethnomedicinal System Practised by the Deori Community of Dhemaji District, Assam

Pranab Borah<sup>1</sup>, Pabitra Gogoi<sup>1</sup>, Rajib Kagyung<sup>2</sup>

<sup>1</sup>Herbal Science and Technology, A.D.P. College, Nagaon, Assam

<sup>2</sup>Department Botany, A.D.P College, Nagaon, Assam

Email: borahpranab2@gmail.com

## Abstract

Treatment of diseases with medicinal plants in different ethnic groups of Assam is widespread, because of effectiveness, easy availability and inaccessibility to modern healthcare system, cultural preferences and century old association with plant resources. Due to rapid fragmentation of natural habitat and improper knowledge about harvesting management the natural vegetation of plants has been decreased in alarming way. The present study is conducted among Deori communities in Siripani area of Dhemaji District. This paper recorded 26 plant species of medicinal plants used by the tribe in the treatment of Hysteria, Diptheria, Gynecological problems, Dysentery, Diarrhoea, Fever, Headache etc. the most commonly used plant species are *Lagenaria cineraria* (Mol) Stand., *Mimosa pudica* L., *Thelypteris agustifolia* (Wild.) Proctor., *Amaranthus spinosus* L., *Elusine indica*, *Mirabilis jalapa* L. Moreover, most of these plants are used both as medicinal as well as nutraceuticals.

**Keywords:** Deori community, harvesting management, medicinal plant, nutraceuticals

## Introduction

The primitive societies in India have been dependent on herbal medicines from the time immemorial. In fact all traditional systems of medicine had their roots and origin in folk medicines or ethno medicines [2].

Selection of Deori tribes of Dhemajidistrict for the present study is based on the fact that they are large secluded from urban cultures. This is due to poor communication and transport, and lack of development of resources, which hinder the growth of urban center. The Deori prefer to inhabit in areas in and around forest several isolated habitats are even scattered in deep interior in to the forest.

Deori tribe is one of the ethnic tribes of Assam which is belongs to the great Tibeto-

Mongolian race belonging to the Tibeto-Burman linguistic family. The tribe is divided into four khel (territorial group) namely, *Dibogongyas*, *Tengapaniya*, *Borgongya*, and *Patorgongya*. The name of each khel is derived from a particular river of their original homeland [1].

The main aim of the studies is to evaluate the plants used by Deori of Sripani area of Dhemajidistrict of Assam for various purposes to record the new and the less known uses of the plants by them.

## Methodology

### 1. Study area

Geographically, the Dhemaji district is situated between the 94°12'18"E and 95°41'32"E longitudes and 27°05'27"N and 27°57'16"N latitudes. The district covers an area of 3237 sq.km and is a basically plain area lying at an altitude of 104 meter above the sea level.

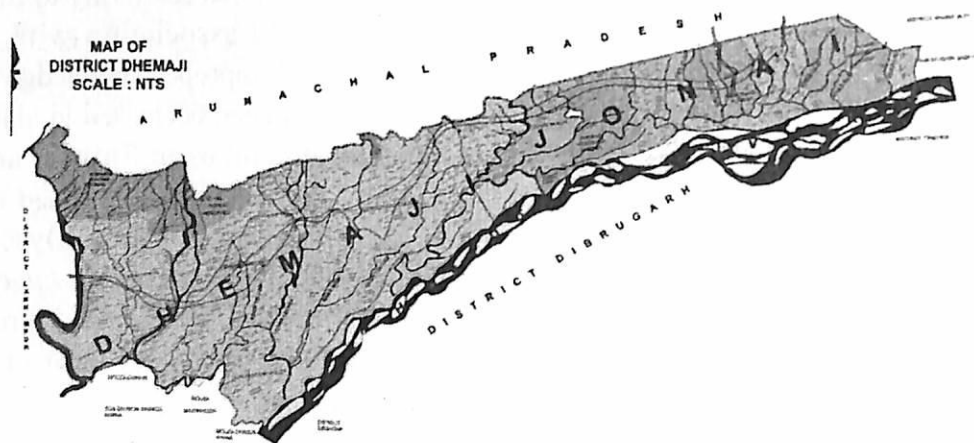


Fig 1: Map of Dhemaji District

### 2. Methods

Intensive field work has been carried out among the Deori community belonging to two villages of Sripani area of the district Dhemaji particularly in remote area lying near to the border region of Arunachal Pradesh and collected the information in respect to medicinal uses of different plants consulting with the age old people and experienced herbal practitioners[6].

## Results and Discussion

A total of 26 species belonging to 25 genus and 22 families were identified. For each species the scientific name, local name, family name, habit, part used and medicinal uses were Table 1: Observation of data analysis

Sl no	Botanical name	Family	Local name	Parts used	Habit	Mode of preparation	Disease treated
1	<i>Rubus mollecanus</i> L.	Rosaceae	Jetulipoka	Yong shoot	climber	All the ingredients are boiled in water and make a concentrated extract. The extract is then taken through mouth.	Diphtheria & Tonsil
2	<i>Abrus fruticosus</i> L. Medic	Fabaceae	Bogalatumoni	Yong shoot	climber		
3	<i>Chrysophyllum lanceolatum</i> (Bl.) D.C	sapotaceae	Bonpitha	Seed	tree		
4	<i>Croton caudatus</i> Geisel	Euphorbiaceae	Lotamahudi	Yong shoot	climber		
5	<i>Livistonjenkinsiana</i> Griff	Arecaceae	Tokow	Leaf & stem	Shrub		
6	<i>Psidium guajava</i> L.	Myrtaceae	Madhuri	Yong shoot	tree		
7	<i>Lagenaria siceraria</i> Mol Stand.	Cucurbitaceae	Jatilao	Root	climber		All roots are grinded well and boiled with water in a bamboo cylinder. The extract is then taken through orally in the morning
8	<i>Dendrocnide sinuate</i> Bl Chew	Urticaceae	Surat	Root	tree		Used in premature birth
9	<i>Callicarpa arborea</i> Roxb	Verbenaceae	Tong loti	Root	shrub		
10	<i>Mimusapudica</i> L.	Mimosaceae	Nilaji bon	Root	herb		The extracted juice from roots is used through orally for 3 days
11	<i>Mirabilis jalapa</i> L.	Nyctaginaceae	Godhuligopal	Rhizome	shrub		The root juice is mix with milk & molasses and given orally
12	<i>Amaranthus spinosus</i> L.	Amaranthaceae	Hatikhutura	Root	herb		The root extract is taken through orally
13	<i>Elysieneindica</i> L.	Poaceae	Bobosa bon	Leaf & stem	herb		The leaf & stem is crushed and rub on the forehead
14	<i>Cocciniagrandsis</i> L. Voigt.	Cucurbitaceae	Belipoka	Rhizome	climber		The rhizome extract is directly applied to the sinus gland of the nose through nasal route

15	<i>Plumbagozeylanical.</i>	Plumbaginaceae	Agiachita	Root	herb				Any kind of Liver disorder
16	<i>Entadascandens</i> Benth.	Fabaceae	Ghila	Seed	tree			The root of agiachita&ghila seed is fried with a chicken egg in mustard oil & given to eat	
17	<i>Urenalobata</i> L.	Malvaceae	Sonborial	Root	herb			The fresh root made into a garland and wore around the neck.	Used in fever
18	<i>Solanumindical.</i>	Solanaceae	Titabhekuri	Root	shrub			All the ingredients are grind and mix with water and boil.The liquid extract is then given to the patient to eat in the morning	Intestinal disorder
19	<i>Solanumtorvum</i> Swartz.	Solanaceae	Hatibhekuri	Root	shrub				
20	<i>Aristolochiatagala</i> Cham	Aristolochiaceae	Nilokontho	Root	climber				
21	<i>Phlogamihustyriformis</i>	Acanthaceae	Titaful	Root	shrub				
22	<i>Syzygiumaromatium</i>	Myrtaceae	Long	Seed	shrub				
23	<i>Elettariacardamom</i>	Zingiberaceae	elachi	seed	herb				
24	<i>Cinnamomumzeylanicum</i>	Leuraceae	Dalseni	Bark	tree				
25	<i>Garciniamorella</i> Gaertn. Desr	Clusiaceae	kujithekera	Fruit	tree			Dry fruit is soaked in water overnight and the extract is given to the patient in empty stomach	hypertension
26	<i>Eclipta alba</i> L.Hassk	Asteraceae	kehraj	Stem and leaf	herb			Liquid extract is prepared by crushing the plant and given to eat	Nosebleed or epistaxis

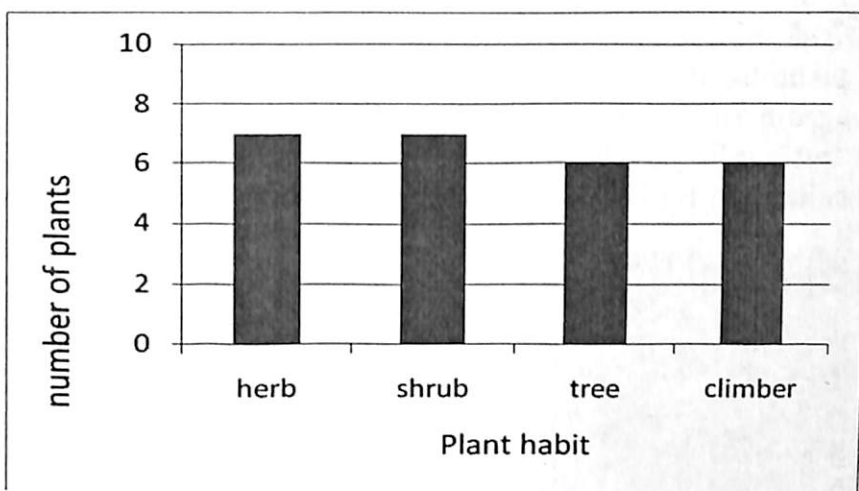


Fig 2: Different types of plants used by deori community

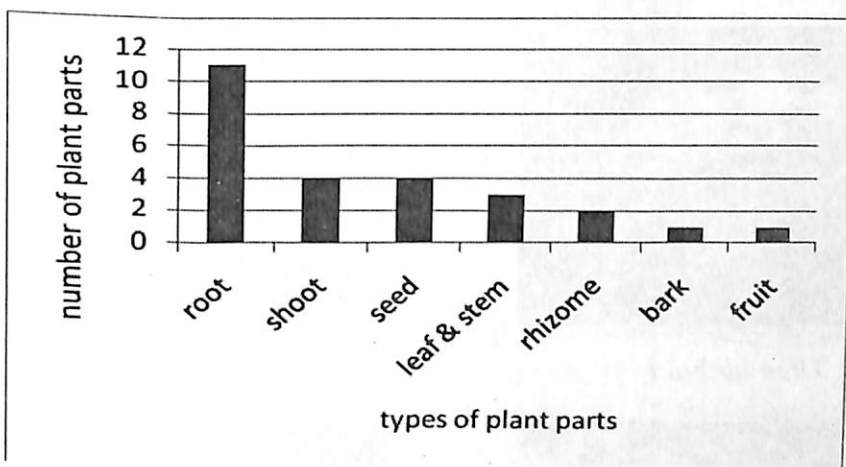


Fig 3: Different kinds of plant parts used for preparing herbal formulation by deori community

The above ethno-medicinal studies reveals that the Deori community of Sripani subdivision, Dhemaji district are primarily dependent on the plant resources to treat their different diseases like Diphtheria, Infertility, Liver disease, Hysteria, Kidney stone, Sinusitis, Hypertension, Nosebleed or epistaxis etc [4]. The medicinal plant resources are mainly tree, shrub, herb, and climber in nature. Roots are most widely used plant parts (11species) followed by shoots and seed (4 species each), leaf and stem (3 species), rhizome(2 species), bark (1 species), and fruit (1 species). Most of

preparations are polyherbal formulation which are taken orally in the form of decoction, infusion, or extract for curing the disease like diphtheria, premature birth,infertility, kidney stone, hypertension, nosebleed. On the other hand, some formulation are taken locally or topically[4].

In fig:2 bar diagram indicates the different types of plants used by the deori community and fig:3 indicates the different types of plant parts used for preparation of polyherbal formulation by Deori community.



*Eclipta alba L*



*Plumbagozeylanica L*



*Uren alobata*



*Phloganthus thyrsiformimis*

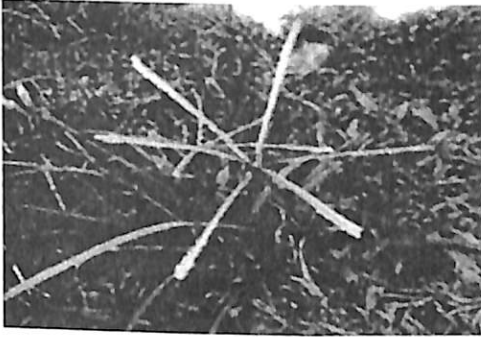


*Amaranthus spinosus L*



*Coccinia grandis*

**Fig 4: Different types of plant species used by the Deoricommuntiy**



*Eleusine indica* L



*Mirabilis jalapa* L



*Mimosa pudica* L

### **Conclusion:**

Herbal medicine and traditional herbal practitioners play an important role in the healthcare system in those villages. The recovery of knowledge and practices associated with these plant resources may be an important strategy linked to the conservation of biodiversity. Some of the medicinal plant species has lost their habitats due to the inadequate knowledge about the collection procedures of the medicinal plant. So proper harvesting management is required for conservation of these medicinal plants. There is an urgent need for inventory and documentation of traditional knowledge of this which may lead to development of a new drug.

### **Acknowledgement:**

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# Investigation into the Antioxidant, Biochemical and Antimicrobial properties of *Rhus chinensis* an important traditional medicinal plant of North East India

Esther Jamir<sup>1</sup>, Yutika Nath<sup>2</sup>, Alak Kumar Buragohain<sup>3</sup>  
and Ranjan Dutta Kalita<sup>1\*</sup>

<sup>1</sup>Department of Applied Biology, University of Science and Technology, Meghalaya, Techno City Campus Ri-Bhoi District, Kling Road, Meghalaya- 793101

<sup>2</sup>Department of Molecular Biology and Biotechnology, Tezpur University, Napaam, Tezpur-784028

<sup>3</sup>Dibrugarh University, Dibrugarh, 786004

\* Corresponding Author: Email: dkranjan@gmail.com, Phone No: 09864455067

## Abstract

*Rhus chinensis* is a traditional medicinal plant being used by the people of different tribes and communities in the North Eastern part of India. In the present work the seeds of the plant were investigated for their antimicrobial, antioxidant and biochemical properties. The seeds were dried and crushed and then extracted using ethanol as a solvent. The crude extract was found to exhibit antimicrobial activity against both Gram positive and Gram negative bacteria using *Klebsiella pneumonia* and *Pseudomonas aeruginosa* as the representative microorganisms. The antioxidant properties of the extract was assessed using DPPH radical scavenging activity. It was found that the ethanol extract possessed more than 90% radical scavenging properties. The extract was also found to contain significant amount of phenolics and flavonoids. The presence of different pharmacological properties present in the ethanol extract of the plant justifies its use as a traditional medicinal plant.

**Keywords:** *Rhus chinensis*, phenolics, flavonoids, antioxidant, antimicrobial

## Introduction

Traditional system of medicine is one of the most affordable, inexpensive and readily available medicine in many parts of the world where modern medical healthcare is still a distant dream. Medical professionals are scarce in these parts and

therefore the people are dependent on traditional healers. Such healers depend on medicinal plants for their therapy. Medicinal plants due to their rich antioxidant properties and antimicrobial efficacies neutralize many of the most common diseases. The North-Eastern part of India lies in one of the most ecologically hotspot region of the world- the Indo Burma region, and is home to a wide diversity of medicinal plant species which are endemic to the region (Myers et al, 2000).

*Rhuschinensis*, the plant investigated in the present study is native to North Eastern India, China and Japan (Wang et al, 2008). The plant is used in to treat a variety of diseases like cold, cough, fever and malaria (Rayne and Mazza, 2007). The aqueous extract of the plant has also been found to be inhibiting the alpha-glucosidase activity (Wang et al, 2006, Shim et al, 2003) thus making it a promising anti-diabetic medicinal plant. Significant works on the plant has led to the identification of anti-HIV molecules from the stem (Wang et al, 2006, 2008). Decoction of the roots and the leaves of the plant is used as a remedy for treatment of different ailments like snakebites, stomachache, laryngitis, inflammations etc (Djakpo and Yao, 2010). The water extract of the fruit is reported for its use against diarrhea and dysentery (Pradhan and Badola, 2008). The seeds of the plant are also reported to be used against cough, malaria, rheumatism, Jaundice etc (Abbasi et al., 2009). The present work reports the phytochemical composition of the fruit extract of the plant, its antimicrobial activity and antioxidant activity.

## Materials and Methods

**Plant Collection and Extract Preparation:** The fruits of the plant were collected from Kohima, Nagaland, India. The fruits were washed, dried and then made into fine powder using a traditional grinder available in the villages which is made up of wood and carried out manually. The powdered fruits were then stored in refrigerator. Extraction of the fruits was then carried out using 90% ethanol. 100 grams of the powder was taken in a beaker and 300ml of 90% ethanol was added in it. The beaker was constantly stirred. The extract solvent was filtered in Whatman Filter Paper No 1. The crude extract was obtained by concentrating the filtrate. The extract was further stored at 4°C in the refrigerator.

**Antimicrobial Activity:** The antibacterial assay was done by using the agar well diffusion method. Bacterial culture was adjusted to McFarland standard No. 0.5 before the tests. The media used for the assay was Mueller Hinton Agar. 1% DMSO was taken as the negative control and streptomycin sulfate (10 mg/ml) (Sigma) was taken as the positive control. A stock concentration of 20 mg/ml was made from which 50% was added into each well. The plates were incubated at 37°C for 16 hours. The test was performed in triplicates. The zone of inhibition around the wells indicated the

antimicrobial potential of the plant extracts. The organisms tested included *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

**Antioxidant Activity:** The antioxidant activity of the latex methanol extract was determined by the DPPH assay according to Sharififar et al, (2007). The assay uses DPPH radical as a reagent. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep—violet to light—yellow) is then measured (Miliauskas et al., 2004). Fifty microliters of various concentrations of the samples in methanol were added to 5ml of 0.004% methanol solutions of DPPH. The absorbance was read at 517nm after 30 minutes incubation in dark at room temperature. Inhibition of DPPH free radical in percentage (I %) was calculated using the following formula:

$$I\% = (A \text{ blank} - A \text{ sample} / A \text{ blank}) \times 100,$$

where, A blank is the absorbance of the control reaction containing all the reagents except the extract. A sample is the absorbance of the extract i.e. the test compound. The concentration of the extract that provided 50% inhibition was calculated from the graph plotting inhibition percentage against the extract concentration. All the tests were carried out in triplicates. Ascorbic acid (BDH Chemicals) was taken as the standard.

**Total Flavonoid Assay:** The total flavonoid assay of the methanol extract was done to determine the total flavonoid constituents. The assay was done according to the protocol taken from Siddique et al. (2010). 0.5 ml solution of the extract at various concentrations was prepared in methanol. To this 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of milli Q water was added. The mixture was then incubated at room temperature for about 40 minutes. The absorbance of the reaction mixture was measured at 415nm in a spectrophotometer (Thermo Fischer). Quercitin (Sigma) was taken as the standard.

**Saponin Assay:** The assay was carried out according to the protocol of Mir et al, (2013). 2 gm of the powdered sample of the fruits was boiled in 20ml of distilled water by using a water bath. The sample was filtered and the filtrate was mixed with 5ml of distilled water in a test tube. The tube was then shaken vigorously to see the formation of froth. Formation of froth indicates the presence of saponins.

**Tannin Assay:** The assay was carried out according to Mir et al, (2013). For the assay 0.5gm of the powdered fruits of *Rhuschinensis* was taken and boiled in 20ml of distilled water in a beaker. The solution was then filtered and the filtrate was taken in a test tube. 0.1% of  $FeCl_3$  was added to the test tube containing the filtrate. The formation of a brownish green or a blue black ring on the surface of the solution indicated the presence of tannins.

## Results and Discussions

The ethanol extract was found to have potential antimicrobial activity as was observed from the agar well diffusion assay. Presence of zone of inhibition around the wells indicated antimicrobial efficacy of the ethanol extract. It was found that the ethanol extract has a zone of inhibition of 17 mm against both *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

The ethanol extract was also found to possess promising radical scavenging activity. It was observed that at 1mg/ml concentration the extract inhibited 96.1% of the total DPPH radicals indicating its very high antioxidant properties. At 250µg/ml the extract was found to have 95.4% radical scavenging activity. The flavonoid content of the extract was found to be high. 1mg of the crude ethanol extract contained flavonoid concentration equivalent to that of 31.25µg of the standard quercitin.

The extract was also found to contain high amount of tannins. The presence of a blue black colouration on the surface of the test tube indicated the presence of tannins. The ethanol extract was also found to contain high content of saponins as was observed by its ability to form froth on addition of water.

## Conclusion

Thus from the present study it was concluded that the ethanol extract obtained from the fruits of *Rhuschinensis* possessed antimicrobial activity as well as high antioxidant activity. The pharmacological importance of the fruits can be observed by its composition which included flavonoids, tannins and saponins. The fruits are rich in their contents of different phytochemical components which supports its use as a traditional medicinal plant. More research works into its contents needs to be carried out to ascertain its importance and further works on its molecular components will highlight its chemical composition.

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# Effect of pH on the synthesis of silver nanoparticles using leaf extract of *Ficus hirta* and its antibacterial activity

**Kritika Bhattacharyya<sup>1</sup> and Probin Phanjom<sup>2</sup>**

Department of Applied Biology  
University of Science and Technology, Meghalaya  
Email: 2phanjom@gmail.com

## **Abstract**

In nanotechnology, particles sized between 100 and 1 nanometres are considered to be nanoparticles. Nanoparticles have a wide variety of potential applications in biomedical, optical and electrical fields. Due to these applications research in nanoparticles is an area of intense scientific interest. There are several methods for synthesizing nanoparticles like wet chemical method where harmful and toxic chemicals are used. In the present study, effect of pH (3-10) on the synthesis of silver nanoparticles from aqueous solution of silver nitrate ( $\text{AgNO}_3$ ) using the leaf extract of *Ficus hirta* in an environment friendly method was carried out. The nanoparticles size decreased with increase in pH. For characterisation of the nanoparticles UV-Vis absorption spectroscopy, XRD and TEM were done. Size dependent anti-bacterial activities were also investigated by disc diffusion method.

## **1. Introduction**

Nanotechnology is the engineering of functional systems at the molecular scale. Generally, nanotechnology deals with developing materials, devices, or other structures possessing at least one dimension sized from 1 to 100 nanometres. Nanotechnology entails the application of fields of science as diverse as surface science, organic chemistry, molecular biology, semiconductor physics, microfabrication, etc. Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields. There are several methods for creating nanoparticles, including both attrition and pyrolysis. Synthesis and characterization of nanoparticles is an important area of research as selection of size and shape of nanoparticles provide an efficient control over many

of the physical and chemical properties (Alivisatos, 1996; Steven et al., 1998). Biological materials like plant leaf extract (Parashar et al., 2009), bacteria (Saifuddin et al., 2009), fungi (Bhainsa and D'Souza, 2006) and enzymes (Willner et al., 2007) are used for the green synthesis of silver nanoparticles. Green synthesis process offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Leaf extract of various plants such as geranium (*Pelargonium graveolens*) (Shanker et al., 2003), *Helianthus annuus* (Asteraceae), *Basella alba* (Basellaceae), *Oryza sativa*, *Saccharum officinarum*, *Sorghum bicolor* and *Zea mays* (Poaceae) were used for the bioreduction of aqueous solution of silver nitrate solution to form stable silver nanoparticles (Arangasamy and Munusamy, 2008). Reports on the effect of pH on the synthesis of silver nanoparticles using leaf extract of Olive and *Cinnamomum zeylanicum* are available where increase in pH effect the rate of synthesis and morphology of the nanoparticles (Satishkumar et al., 2009; Mostofa et al., 2014). In the present study, the effect of pH on the synthesis of silver nanoparticles using leaf extract of *Ficus hirta* and its antibacterial has been reported.

## **2. Materials and Methods**

### **2.1 Preparation of leaf extract**

25gm of the *Ficus hirta* leaf was washed thoroughly with sterilised distilled H<sub>2</sub>O and then air dried. The leaves were then cut into fine pieces and then boiled at 30°C with 100 ml distilled H<sub>2</sub>O for 20 minutes. The leaf extract was allowed to cool at room temperature and then filtered using Watmann filter paper No.1 (25 µm pore size).

### **2.2 Synthesis of silver nanoparticles under different pH conditions**

For the synthesis of silver nanoparticles 1mM aqueous solution of silver nitrate solution (AgNO<sub>3</sub>) was prepared. To 1ml of *Ficus hirta* leaf extract adjusted to different pH (4-10), 20ml of the prepared AgNO<sub>3</sub> solution was mixed and kept at room temperature to react.

### **2.3 UV-Vis Spectra analysis**

Monitoring of the reduced silver particles was done by measuring the UV-Vis spectrum of the reaction medium after 3 hours. UV-Vis spectral analysis was done by using PC Based Double Beam Spectrophotometer 2202 (Systronic).

### **2.4 XRD measurement**

The biosynthesized silver nanoparticles thus obtained were purified by repeated centrifugation at 8000 rpm for 15 minutes followed by redispersion of the pellet of

silver nanoparticles into 10 ml of deionised water. After freeze drying of the purified silver particles, the structure and composition were analyzed by XRD. The dried mixture of silver nanoparticles was collected for the determination of the formation of Ag nanoparticles by XRD (D8 ADVANCE, BRUKER).

### 2.5 TEM analysis of silver nanoparticles

Samples for transmission electron microscopy (TEM) analysis were prepared by drop coating biologically synthesized silver nanoparticles solution (24 hours reaction of the AgNO<sub>3</sub> solution with the *Ficus hirta* (leaf broth) on to carbon-coated copper TEM grids. The films on the TEM grid were allowed to stand for 2 minutes, following which extra solution was removed using a blotting paper and grid allowed to dry prior to measurement. TEM measurements were performed on a JEM 2100, 200 kV, Jeol.

### 2.6 FTIR analysis

The AgNO<sub>3</sub> reduced by *Ficus hirta* extract was centrifuged at 9,000 rpm for 10 min. The deposited residue was freeze dried and grinded with KBr to obtain pellet for the purpose of FTIR analysis.

### 2.7 Antimicrobial activity

Disk diffusion test was done to determine the size specific antibacterial activity of silver nanoparticles, the procedure specified by Ruparelia et al.(2008).

## 3. Result and discussion

To investigate the effect of pH on the morphology and rate of synthesis of silver nanoparticles, to the leaf extract of *Ficus hirta*, 1mM AgNO<sub>3</sub> was added and the reaction was carried out in dark at 27°C at different pH ranging from 4-10. The reaction mixture exhibited a gradual change in colour towards brown within 12 h of reactions which further darkened with increased incubation period time as shown in Fig.1 due to reduction of AgNO<sub>3</sub> and generation of surface plasmon resonance. When pH was increased from 4 to 10, maximum synthesis was observed at pH10 and the time taken was reduced to 30 min only. The absorbance peak shifted towards the UV region with increase in pH as shown in the Fig. 2, which reflect the size of the silver nanoparticles as revealed by TEM. The SPR peak shifts to longer wavelengths with increase in particle size (Brause et al., 2002). TEM also revealed that the size of the particles decreases with increase in pH and were found to be more uniform in sizes almost spherical in shape as seen in Fig.3. The average particles size obtained at different pH is shown in the Table 1. These observations indicate that alkaline condition is necessary for the reduction of metal ions. The presence of hydroxide ions

increased the reduction capacity of the active molecules involved in the reduction process, which might act as a reducing agent to carry out the reduction reaction of metal ions. Similar observation has been reported earlier by Satishkumar et al., 2009. Silver nanoparticles were synthesized using *Cinnamomum zeylanicum* at different pH range of 1 to 11. At low pH, large-sized particles ellipsoidal in shape were observed, whereas at higher pH, a large number of spherical-shaped nanoparticles with smaller diameter were observed.

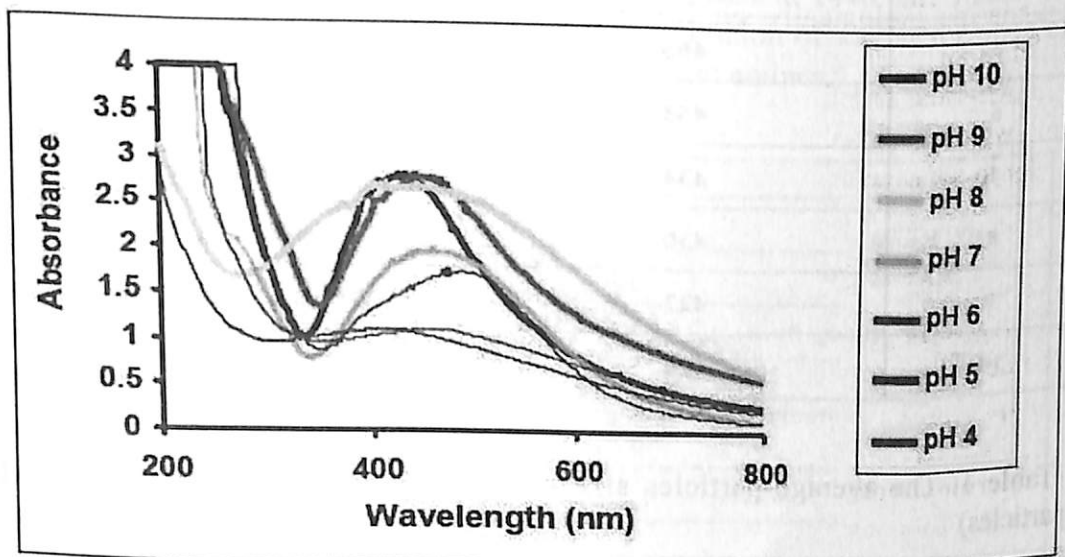


Fig.2. UV-vis spectrum of biosynthesized silver nanoparticles under different pH conditions.

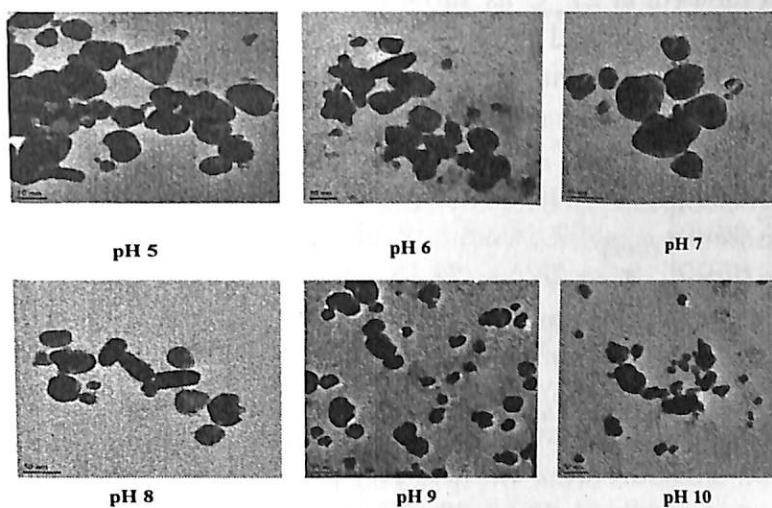


Fig. 3. TEM micrograph of biosynthesized silver nanoparticles

pH ranges (4-10)	<i>Ficus hirta</i>	
	Absorbance peak (nm)	Average SNPs size (nm)
4	-	-
5	465	71.1 ± 6.51
6	453	58.21 ± 6.51
7	434	48 ± 7.19
8	430	41.88 ± 5.68
9	427	36.16 ± 2.73
10	424	29.14 ± 2.41

Table 1. The average particles size obtained at different pH (SNP: silver nanoparticles)

The XRD pattern showed four intense representative XRD patterns of silver nanoparticles formed after reaction of plant extract of *Ficus hirta* with 1mM AgNO<sub>3</sub> aqueous solution for 24 h at 27 °C as shown in Fig. 5. The 2 theta peak values of around 38.06°, 44.64°, 64.58° and 77.62° observed confirmed the face centered cubic (fcc) structure when compared with the standard.

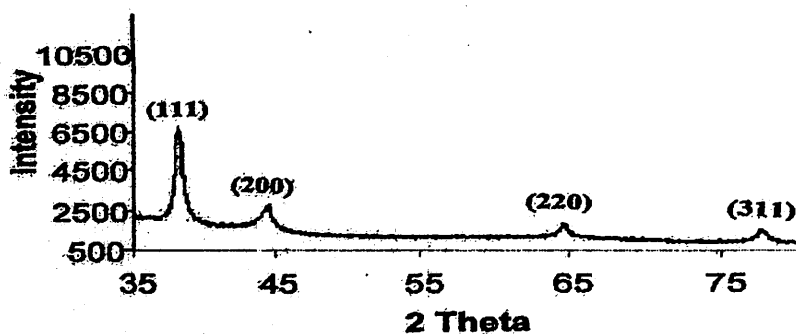


Fig. 4 XRD spectrum of silver nanoparticles synthesized by leaf extract of *Ficus hirta*

The nanoparticles synthesized by the leaf extract of *Ficus hirta*, were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent which was confirmed by FTIR analysis Fig.5. The FTIR spectrum of biosynthesized showed five distinct peaks, 3463cm<sup>-1</sup>, 1639 cm<sup>-1</sup>, 1440 cm<sup>-1</sup>, 1367cm<sup>-1</sup> and 1020 cm<sup>-1</sup>. The peak at 3463cm<sup>-1</sup> refers to NH stretch vibration of primary and secondary amides of protein. The peak at 1639 cm<sup>-1</sup> refers to carbonyl stretch, which is assigned to the amide bond. The peaks at 1440 cm<sup>-1</sup> refer to the C=N stretches, 1367cm<sup>-1</sup> refers to C-H bending vibration of saturated carbon chain and 1020 cm<sup>-1</sup> refers to C-N stretch for non-aromatic amines.

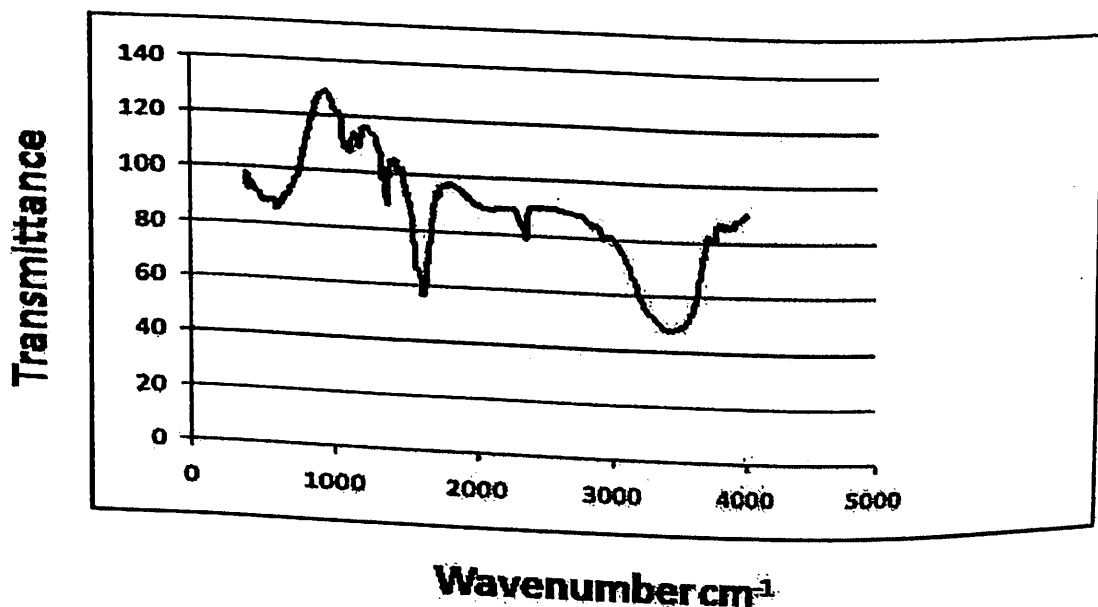


Fig.5. FTIR spectrum of biosynthesized silver nanoparticles.

Size dependent antibacterial activities of silver nanoparticles were tested against two bacterial strains *E. coli*, and *S. aureus*. Silver nanoparticles (20 µg/ml) with different average size ( $29.14 \pm 2.41$ ,  $41.88 \pm 5.68$  and  $71.1 \pm 6.51$ ), impregnated on filter paper disks (~5 mm diameter), were placed on an Muller-Hinton agar plate having uniform bacterial suspension (*E. coli* and *S. aureus*)  $10^4$  to  $10^5$  CFU/ ml for 24 h and incubated at 37 °C. The zone of inhibition (ZOI) obtained in the petri discs was measured. Silver nanoparticles having an average size of size  $29.14 \pm 2.41$ , showed maximum antibacterial activity against the tested bacterial strains, followed by nanoparticles having an average size of  $41.88 \pm 5.68$  and the minimum was showed by the nanoparticles with an average size of  $71.1 \pm 6.51$  as shown in the Fig.6.

Nanoparticle with small size was found to be more effective as antibacterial as large size against the test organisms as shown in the Table 2. Antibacterial activity was maximum in the Gram negative bacterial strain *E. coli* than in the Gram positive bacterial strain *S. aureus* which may be due to the difference in the cell wall compositions. The possible reason for maximum antibacterial activity shown by particles having average size of  $29.14 \pm 2.41$ , may be due to the presence of much smaller nanoparticles ranges, which can bind more efficiently because of large surface area and could penetrate the cell wall due to its small size. Similar observation on size dependent antibacterial activity of silver nanoparticles was reported earlier (Agnihotri et al., 2014; Kethirabalan and Gurusamy, 2014).

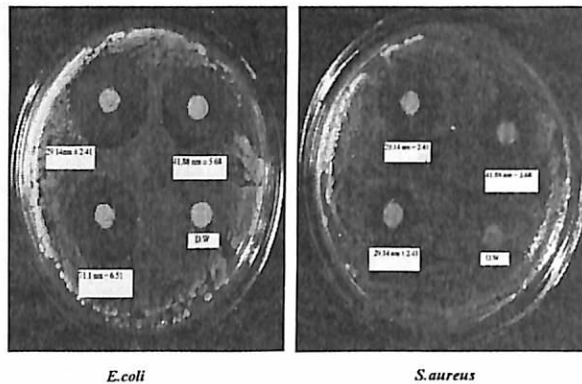


Fig.6. Size dependent antibacterial activity of silver nanoparticles against *E. coli* and *S. aureus*

Bacterial strains	Zone of inhibition (mm)			
	D.W	29.14 ± 2.41 (SNP 20µg/ml)	41.88 ± 5.68 (SNP 20µg/ml)	71.1 ± 6.51 (SNP 20µg/ml)
<i>E. coli</i>	-	16 ± 1.15	11 ± 1	8 ± 1
<i>S. aureus</i>	-	8 ± 1	6 ± 1	4 ± 0.57

Table 2. Size dependent antibacterial activity of silver nanoparticles against *E.coli* and *S.aureus*

#### 4. Conclusion

Effect of pH on synthesis of silver nanoparticles by using leaf extract of *Ficus hirta* was studied. Synthesis of smaller particles with increased in pH (4-10) was observed, indicating the importance of alkaline condition for the reduction process. The bioactive compounds present in the leaf extract might play an important role in reduction process and as capping agents. Size dependent antibacterial activity of silver nanoparticle was demonstrated against two test organisms viz., *E. coli* and *S. aureus*. Maximum antibacterial activity was demonstrated by nanoparticle with smaller average size against the tested bacterial strains.

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# A study on the pharmacological and antioxidant properties of four different medicinal plants used in the traditional system of medicine of North East India

Sahanaj Begum<sup>1</sup>, Rinku Kr. Barman<sup>1</sup>, Pranjal Rajbangshi<sup>1</sup> and Ranjan Dutta Kalita<sup>1\*</sup>

<sup>1</sup>Department of Applied Biology, University of Science and Technology, Meghalaya, Techno City Campus Ri-Bhoi District, Kling Road, Meghalaya- 793101

\*Corresponding Author: Email: dkranjan@gmail.com, Phone No: 09864455067

## Abstract

Four different plant species used in the traditional system of medicine were investigated for their pharmacological constituents. Different parts of the plants *Costus speciosus*, *Moringa oleifera*, *Alstonia scholaris* and *Cassia tora* were studied. The plant parts were extracted using various solvent systems based on their polarity. Investigations were carried out to assess the alkaloid, tannins, saponins, phenolics, cardiac glycosides and flavonoid composition of the crude extracts. The extracts prepared from the diverse parts of the plants were found to be rich in most of these parameters. Some of the extracts were found to possess antioxidant activities when assayed using the DPPH free radical scavenging assay. The richness of the different biochemical composition of the plant parts reflects their use in the traditional system of medicine.

**Keywords:** *Costus speciosus*, *Moringa oleifera*, *Alstonia scholaris*, *Cassia tora*, phenolics, cardiac glycosides, tannins, antioxidants

## Introduction

Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies. Plant based medicines were initially dispensed in the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations and now serve as the

basis of novel drug discovery (Jachak et al., 2007). Plants have been utilized as medicines for thousands of years (Balunas et al., 2005). The plant based drug discovery resulted mainly in the development of anti-infectious agents and continues to contribute to the new leads in clinical trials (Saklani et al., 2008). Assam with her vast and unique plant resources has huge potential and possibilities in the field of drug discovery. Assam lies in the North East India which falls in the IndoBurma region and has been identified as one of the eight hotspots for biodiversity in the world depending on five factors that include Endemic plants (Myers et al., 2000). Of the three thousand odd plant species found in the state, some 950 species are known to possess medicinal properties. Some of these species have been used in the ayurvedic, unani, and other traditional alternative systems since time immemorial. This huge body of traditional knowledge is an invaluable asset in the process of drug discovery. Many of the native plant species of Assam are traditionally known to have antibacterial activity. These plants need to be investigated for validating their antibacterial properties.

In the present work four different plants- *Moringa oleifera*, *Alstonia scholaris*, *Cassia tora* and *Costus speciosus* were investigated for their different phytochemical constituents.

## Materials and Methods

**Plant Collection and Extract Preparation :** The different parts of the plant were collected, dried and powdered using a mixer grinder. The powdered samples were then stored in refrigerator. Extraction of the different samples was then carried out using 90% ethanol, methanol and hexane. 100 grams of the powder of each plant was taken in a beaker and 300ml of the solvent was added in it. The beaker was constantly stirred. The extract solvent was filtered in Whatman Filter Paper No 1. The crude extract was obtained by concentrating the filtrate. The extract was further stored at 4°C in the refrigerator. *Moringa oleifera* and *Costus speciosus* stems were extracted using methanol, *Alstonia scholaris* flowers were extracted using hexane and *Cassia tora* seeds by using ethanol.

**Antioxidant Activity :** The antioxidant activity of the latex methanol extract was determined by the DPPH assay according to Sharififar et al, (2007). The assay uses DPPH radical as a reagent. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep-violet to light-yellow) is then measured. Fifty microliters of various concentrations of the samples in methanol were added to 5ml of 0.004% methanol solutions of DPPH. The absorbance was read at 517nm after 30 minutes incubation in dark at room temperature. Inhibition of DPPH free radical in percentage (I %) was calculated using the following formula:

$$I\% = (A \text{ blank} - A \text{ sample} / A \text{ blank}) * 100,$$

where A blank is the absorbance of the control reaction containing all the reagents except the extract. A sample is the absorbance of the extract i.e. the test compound. The concentration of the extract that provided 50% inhibition was calculated from the graph plotting inhibition percentage against the extract concentration. All the tests were carried out in triplicates. Ascorbic acid (BDH Chemicals) was taken as the standard.

**Saponin Assay :** The assay was carried out according to the protocol of Mir et al, (2013). 2 gm of the powdered sample of the fruits was boiled in 20ml of distilled water by using a water bath. The sample was filtered and the filtrate was mixed with 5ml of distilled water in a test tube. The tube was then shaken vigorously to see the formation of froth. Formation of froth indicates the presence of saponins.

**Tannin Assay :** The assay was carried out according to Mir et al, (2013). For the assay 0.5gm of the powdered fruits of *Rhus chinensis* was taken and boiled in 20ml of distilled water in a beaker. The solution was then filtered and the filtrate was taken in a test tube. 0.1% of  $FeCl_3$  was added to the test tube containing the filtrate. The formation of a brownish green or a blue black ring on the surface of the solution indicated the presence of tannins.

**Alkaloid Assay :** The alkaloid assay was carried out using Wagner's Reagent and Mayer's Reagent. 50mg/ml concentration of the samples were prepared in methanol. To the solution, the reagents were added dropwise. The presence of alkaloids is represented by precipitation of the solution on addition of the reagents.

**Total Flavonoid Assay :** The total flavonoid assay of the methanol extract was done to determine the total flavonoid constituents. The assay was done according to the protocol taken from Siddique et al. (2010). 0.5 ml solution of the extract at various concentrations was prepared in methanol. To this 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of milli Q water was added. The mixture was then incubated at room temperature for about 40 minutes. The absorbance of the reaction mixture was measured at 415nm in a spectrophotometer (Thermo Fischer). Quercetin (Sigma) was taken as the standard.

## Results and Discussions

None of the extracts demonstrated any antioxidant activities. The percentage of free radical scavenging was found to be very low and therefore it was not accounted as antioxidant property. In terms of flavonoid composition also none of the extracts demonstrated any potential flavonoid content. Tannins were found to be present in all the plant extracts except for hexane extract of the flowers of *Alstonia scholaris*. The alkaloids were found to be present in the methanol extracts of *Moringa oleifera* and *Costus speciosus* but absent in the other plant extracts. Except for the hexane extract

of the flowers of *Alstonia scholaris*, all other plant extracts were found to contain saponins.

### Conclusion

It has been observed that out of all the four plants, only the methanol extracts of *Moringa oleifera* and *Costus speciosus* are rich in major phytochemicals and can be justified of their traditional uses. However much more investigations has to be carried out to justify the usage of the plants.

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# Enumeration of wild plants used as medicines found around dhing area of nagaon district, assam

**Uddhab Talukdar<sup>1</sup> and Sanjeeb Kumar Nath<sup>2</sup>**

<sup>1</sup>Research Scholar, Biotech Hub, Dhing College, Dhing, Nagaon, Assam

<sup>2</sup>Associate Professor, Department of Botany, Dhing College, Dhing, Nagaon, Assam

Email: sanjeebkumarnath@gmail.com

## Abstract

Plants have always been the prime source of man for medicine. The Indian System of Medicine directly deals with the plants, and according to different classics of Indian system of Medicine they believe that all plants have medicinal values. The medicinal plants are used since ancient times i.e. from the Vedic period for the treatment of different diseases, by which the society has been benefited utmost since ancient times. An ethno medicinal study of plants carried out by the traditional healers, rural dwellers residing around Dhing area was conducted in Nagaon district. This study revealed 10 plant species which are used for medicinal value.

**Keywords:** Medicinal plants, Traditional medicine, Dhing

## Introduction

Plants have been playing significant role in curing diseases. The importance of plant based medicine which indeed waxed and waned earlier has now found its resurgence by the end of the last century. The demand for medicinal plants is ever increasing, as people are more and more fascinated towards herbals. Even in the modern age too Ethno-Botany, specially Ethno medicine play a major role in treating diseases. India with her 45,000 plant species and 550 tribal communities belonging to 160 linguistic groups inhabited in varied geographic and climatic zones with diversified plant species, which harbours Ethno-botanical world. The tribal communities are rich in traditional knowledge of medicinal plants that flow with their blood generation after generation.

## Materials and Method

**Description of the Study area :** Dhing is situated at the North-West part of Nagaon District in the state of Assam, India. It is located at 26°28'N 92°28' E, which

has an average elevation of 190 feet. Dhing is located at a distance of around 25 kilometres from Nagaon District. The road from Nagaon town is the main road through which Dhing is connected. Dhing is connected with Guwahati by a road-gauge railway tract. The climate of this area is in general monsoon type of climate. It is a rainy area having rainfall of 1200-1800 mm. Most of the people of this region in the locality of Dhing are cultivators.

In the present study information was collected during ethnomedicinal studies, conducted in study site. Data about the uses of medicinal plant were collected on the basis of oral interviews, discussion with the local people. The works of Chopra et.al (1969) and Sarma (2002) were referred for taxonomic identification.

### Results and Discussion

The present study revealed that 10 species of flowering plants are known to be an ethno botanical significance for treating diseases. These plants are enumerated below which are arranged family wise as per Bentham and Hooker's system of classification. Botanical names are arranged alphabetically under each family followed by local name.

1. *Mimosa pudica* L.

Family: Mimosaceae

Local name: Nilagibon

Part used: Roots

Uses: Roots are used to treat diarrhea, amoebic dysentery, against wounds, itching. Roots are also used to treat stones, diabetes, heavy menstrual bleeding.

2. *Commelina benghalensis* L

Family: Commelinaceae

Local name: Kona simolu

Part used; Stem

Uses: It can be used for treating skin diseases, fever, wounds, boils and prickly heat.

3. *Eclipta prostrata* (L) L.

Family Asteraceae

Local name: Keheraj

Part used: Leaves

Uses: Juice from leaves is used to treat urinary infections. Fresh leaves can cure constipation.

4. *Ipomoea aquatica* Forsk

Family: Convolvulaceae

Local name: Kolmou

Part used: Twigs & leaf

Uses: The twigs are used to treat skin diseases such as ringworm. Leaves are used to treat diabetes in pregnant women.

5. *Adhatora vasica* (L) Nees.

Family: Acanthaceae

Local name: Vasaktita

Part used: Leaves

Uses: The leaves are used as an herbal treatment of cold, cough, asthma treatment. Extract from the leaves cure diarrhea, dysentery, vomiting, gonorrhoea.

6. *Ocimum basilicum* (L)

Family: Lamiaceae

Local name: Tulshi

Part used: Leaves, Seed

Uses: Leaves are as antimicrobial, antioxidant. Seeds can be used for treating urinary infections. Seeds are also useful in controlling of Blood sugar. Weight reduction may also be observed if one takes regular doses.

7. *Catharanthus roseus* (L)

Family: Apocynaceae

Local name: Nayantora

Part used: Leaves, Whole plant, Roots

Uses: It is used to control high blood pressure, Dysentery, Diarrhea. It effectively cures skin diseases. It works against insect bites, urinary problems.

8. *Amaranthus spinosus* L.

Family: Amaranthaceae

Local name: Khutura

Part used: Juice of roots

Uses: A paste of the root is used in the treatment of gonorrhoea, eczema and colic. The juice of the root is used to treat fever, urinary troubles, diarrhea and dysentery.

9. *Asperagus racemosus* Willd,

Family: Liliaceae

Local name: Shatamul

Part used: Roots

Uses: Roots are useful in nervous disorders, tumors, cough, and restless sleep. It is also use as antiseptic, antidiarrheal and other purposes.

**10. *Holarrhena antidysenterica* Wall.**

**Family:** Apocynaceae

**Local name:** Dudhkhari

**Part used:** seed, bark

**Uses:** It is used for the treatment of dysentery. The plant is used for the treatment of skin diseases such as scabies, ringworm and other diseases. It is also used for the treatment of urinary tract infection. The seeds are used for curing fever and cold.

**Observation and Discussion:**

The data collected for the ethnomedicinal plant have been arranged according to botanical name, family, local name, plant part used.

The collected information's are based on oral interviews with the local people during this study so, it is not recommended for use. This report needs proper chemical and pharmacological experiments for use as effective drug.

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