

## Total Phenol Content, Antioxidant and Antimicrobial Capability of Traditional Medicinal Plants of Mizoram, Eastern Himalayas, Northeast India

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### Abstract

Mizoram inhabits a wide variety of medicinal plants which are traditionally used by the local tribes for the treatment of a range of ailments. The present study was initiated to screen the antimicrobial and antioxidant properties of *Albizia chinensis*, *Callicarpa arborea*, *Mussaenda macrophylla* and *Stereospermum tetragonum*. Among the selected plants, *A. chinensis* possessed highest total Phenolic content, superoxide dismutase (SOD) (9.235 units/mg protein), catalase (93.212  $\mu\text{M H}_2\text{O}_2$  Decomposed/min/ mg protein), ascorbic acid oxidase (1.66 units/mg protein) and antibacterial activity against *Staphylococcus aureus* (MTCC-96) and *Escherichia coli* (MTCC 739). Methanol extract of *S. tetragonum* showed highest antioxidant activity (41.53 mg GAE/g DW). A positive correlation was seen between total Phenolic content and total antioxidant activity of all four plants with having correlation coefficient ( $R^2$ ) ranges from 0.969-0.995. Further studies on isolation and structural elucidation of biologically active compounds present in the plants will be helpful for pharmaceutical and industrial applications.

**Keywords:** Medicinal plants; Phenolic content; antibacterial activity; antioxidant activity; Superoxide dismutase

### Introduction

Mizoram, a state of north-east India, is known as a biodiversity hotspot abounds with dense forest favouring diversified species with a wide variety of medicinal plants. Several rural tribal people in Mizoram are still dependent on plant-derived herbal drugs for their primary health care requirements and treatment of ailments such as scorpion sting, maniacal cases, skin diseases and those related with oxidative damage. World Health organization estimated that in developing countries about three quarters of the population is still dependent on plant based treatment used in their traditional system as the basic need of primary health care. In that admiration, it appears practical to achieve a scientific basis for the possible use of the medicinal plants in second-hand conventional treatments to cure range of diseases for thousands of years. Hence, a proper scientific study is required to evaluate these folk medicines [1, 2].

Medicinal herbs contain diverse classes of compounds such as polyphenols, tocopherols, alkaloids, tannins, carotenoids, etc. Among them, flavonoids and Phenolic compounds are particularly attractive as they are known to exhibit various beneficial pharmacological properties such as vaso protective, antiviral, anti-inflammatory, ant-allergic activities as well as anti-proliferative activity on tumour cells [3]. Some of these properties have been related to the action of these compounds as antioxidants and triple oxygen and inhibitors of peroxidation. Earlier study on phyto-pharmacological properties of *Albizia* species reveals different group of natural product (triterpenoids, saponins, pyridine glycosides and diterpenoids lignans) [4].

Antioxidant compounds protect the cells against the damaging effects of reactive oxygen species (ROS) such as singlet oxygen, hydroxyl radicals and peroxy nitrite. An antioxidant will slower down or prevent the oxidation of other molecules, which produces free radicals, and thereby inhibit the reaction. Phenolic compounds are important because of their free radical scavenging ability and directly

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contribute to the antioxidant properties. Hence it is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in human beings [5-7].

The present study has been carried out to find out the in vitro antioxidant and antimicrobial activity of 4 ethno-medicinally important plants viz. *A. chinensis*, *S. tetragonum*, *M. macrophylla* and *C. arborea* of Mizoram, North East India.

## **Materials and Methods**

### **Materials**

Fresh and disease free leaves were collected from the selected medicinal plants: *Albizzia chinensis*, *Stereospermum tetragonum*, *Mussaenda macrophylla*, *Callicarpa arborea* from different parts of Mizoram based on their ethano-botanical knowledge, availability and medicinal properties (Table 1). The plant materials were bought to the laboratory in sterile polythene bag and stored in refrigerator until processed. Voucher specimens were identified in the institutional herbarium. All chemicals and reagents used for biochemical assays were of analytical grade and were obtained from Himedia, India and Sigma-Aldrich, USA.

### **Preparation of Plant Extracts**

Fresh leaf samples were washed thoroughly for 2-3 times with Millipore water and cut into thin slices of 2-3cms using sterile blade and dried in hot air oven at 37°C for 72-96h. The dried plant materials were ground into powder and stored in air-tight bottles. 10 g of each plant material was extracted using distilled water (100 ml), 60% methanol (100 ml) and 60% ethanol (100 ml) for 30 min at 40°C. Extracts were filtered by using Whatman No.1 filter paper and the filtrate was collected. Aqueous extracts were freeze-dried and solvent (ethanol and methanol) extracts were concentrated to dryness in a Soxhlet apparatus at 60°C for 30 min. For the enzymatic antioxidant activity, 1 g of each fresh leaf tissue was weighed, ground in mortar and pestle with 10 ml buffer solution (0.05M Tris HCl, pH 7 containing 3 mM MgCl<sub>2</sub> and 1mM EDTA). The extracts were centrifuged for 10 min at 5000 rpm and stored at 4°C until analysis [8, 9].

### **Determination of Total Phenolic Content**

Total Phenolic content was determined using the Folin-Ciocalteu reagent following the method. 100 µl of the extracted solution (0.03 mg/ml) was mixed with 900 µl of Millipore water. 500 µl Folin-Ciocalteu reagents was added and incubated for 10 min. followed by addition of 3 ml of 2% Na<sub>2</sub>CO<sub>3</sub> solution. The mixture was made up to 10 ml by the addition of Millipore water and incubated for 2h in room temperature. The absorbance was measured at 760 nm using a spectrophotometer [10].

### **Ferric Reducing Antioxidant Power (FRAP) Assay**

Ferric reducing activity of plant extracts was performed according to Oyaizu, [11]. One millilitre of the extract solution was mixed with 2.5 ml of potassium buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min and the reaction was stopped by adding 2.5 ml trichloro acetic acid. To the solution, 2.5 ml of distilled water and 0.5 ml of FeCl<sub>3</sub> (0.1%) were added and allowed to stand for 30 min before measuring the absorbance at 593 nm. The absorbance obtained was converted to Gallic acid equivalent in mg per gm of dry weight (GAE/g) using Gallic acid standard curve.

### **Estimation of Enzymatic Antioxidants**

#### **Superoxide Dismutase Assay**

0.5 ml of leaf extract was taken in test tube and mixed with 1 ml of sodium carbonate (125 mM), 0.4 ml of nitro blue tetrazolium (NBT 25 µM) and 0.2 ml of EDTA (0.1 mM). The reaction was started after the addition of 0.4 ml of Hydroxylamine hydrochloride (1 mM) in the mixture. The absorbance was measured at 560 nm at every 5 minute interval. Units of SOD were expressed as amount of enzyme required for inhibiting the reduction of NBT by 50% and the activity was expressed in terms of units per mg of protein [12].

#### **Catalase Assay**

One ml of plant extract was added to 5 ml of phosphate buffer (300 µM, pH 6.8) containing hydrogen peroxide (100 µM) and incubated at 25°C for 1 minute. The reaction was terminated by the addition of 10 ml of sulphuric acid (2%), and the residual H<sub>2</sub>O<sub>2</sub> was

titrated with potassium permanganate until pink colour appeared. Enzymatic activity was calculated by the decomposition of  $H_2O_2$  per minute per mg protein [13].

#### **Ascorbic acid oxidase Assay**

0.1 ml of leaf extract was added to 3 ml of substrate solution (8.8 mg of ascorbic acid in 300 ml phosphate buffer, pH 5.6) and the change in absorbance at 265 nm was measured at 30 sec intervals for 5 min. One enzyme unit was expressed as to 0.01 OD change per minute per mg protein [14].

#### **Antimicrobial Screening Test**

##### **Strains and Media**

Antimicrobial screening of the plant extracts was carried out using *Staphylococcus aureus* (MTCC-96), *Escherichia coli* (MTCC 739) and plant pathogenic fungus, *Fusarium oxysporum* f. sp. *ciceri* (MTCC-2791) and *Fusarium graminearum* (MTCC 1893). Bacterial strains were maintained at 37°C in nutrient agar and fungal isolates at 25 + 2°C in potato dextrose agar.

##### **Antimicrobial Assay of Standard Antibiotics**

The test microorganisms were spread on solidified Nutrient agar and potato dextrose agar for bacteria and fungi, respectively. The agar surface was dried and antibiotic discs of Gentamycin, Chloramphenicol, Nitrofurantcin, Ofloxacin, Furazolidine and Miconazole (Hi-Media) were placed on the top of agar surface. The plates were incubated at 37°C for 24h for antibacterial and 25 + 2°C for 48h for antifungal assay. After incubation, antibiotic sensitivity was measured by calculating the inhibition zone (in mm) around each antibiotic disc.

##### **Antimicrobial Assay of Plant Extract**

Antibacterial activity of plant extracts was determined by disc diffusion method [15]. 100 µl from each standard bacterial stock suspension was spread on the surface of the solidified agar using sterile spreader. Sterile Whatman filter discs (6 mm dia.) was made and soaked with different plant extracts and placed on the inoculated plate. Each plate also contained one filter disc, as control, soaked in the respective solvent. Plates were then incubated at 37°C for 24h and the inhibition zones (in mm.) measured. The cup-plate agar diffusion method was adopted with some minor modifications to assess the antifungal activity of prepared extracts. From each of the fungal stock suspension, 100 µl was thoroughly mixed with 20 ml of sterile molten potato dextrose agar (45°C - 50°C), poured into sterile Petri-dishes and allowed to solidify. Five cup-shape wells (10 mm dia.) were made and filled with four plant extracts using sterile p5 pettes. In each petridish, one well was filled with the respective solvent as a control. Plates were incubated at 25 + 2°C for 72h and the inhibition zone (in mm dia.) was measured.

#### **Statistical Analysis**

All the assays were carried out in triplicate. The results were expressed as mean ± standard deviation. The experiments were repeated at least twice. The percentage data represented in the tables were arcsine transformed before being analyzed for significance using ANOVA (analysis of variance,  $P < 0.05$ ). Further, the differences in means were contrasted using Duncan's new multiple range test following ANOVA. All statistical analysis was carried out using SPSS statistical software package version 16.0.

#### **Results and discussion**

##### **Total Phenolic Content**

Plants with high phenol content are important for food industry as it can inhibit oxidative degradation of lipid and improve the quality and nutritional value of food. Phenolic compounds can stabilize and delocalize the unpaired electron, chelate metal ions, protecting against different microbial infection and thereby acts a good source of antioxidant. Thus, it is needed to estimate total Phenolic content in plant species. In the present study, the total Phenolic content of *A. chinensis*, *S. tetragonum*, *M. macrophylla* and *C. arborea* ranged from 42.714 - 13.282 mg GAE /g dry weight basis. The highest concentration of phenol was found in ethanolic extract of *A. chinensis* (42.714 mg GAE /g DW) followed by ethanolic extract of *S. tetragonum* (40.28 mg GAE /g DW). The results were in agreement with the previous

studies on the genus *Albizia*. The concentration of the Phenolic compounds is controlled by different factors like environmental, development stage, type of solvent used, and the degree of polymerization of phenolic as well as by the formation of insoluble compounds. Most medicinal plants possess higher antioxidant activities because of relatively more abundance of phenolic compounds in these plants. Phenolic compounds have directly linked with antioxidant activities of medicinal plants as these compounds are regarded as the main contributor to antioxidant activities [16-20].

Some studies also claim that the Phenolic compounds have a role to play in antimicrobial activities of plant extracts. Previous study showed a positive linear relationship existing between antioxidant activity and total Phenolic content in some spices and herbs [21]. Shan., *et al.* [22] had mentioned the positive correlation occurring between antioxidant capacity and the antibacterial activity of some plant extracts. Thus, it can be stated that total phenol content of plant extract is having contribution in both antioxidant and antimicrobial activities of plant which found true in our studies also.

### Ferric Reducing Antioxidant Power (Frap) Assay

At low pH, the reduction of a ferric complex to the ferrous form can be measured by monitoring the absorbance at 593 nm. Absorbance change is directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture. *In vitro* antioxidant activity of the plant extract is shown in Table 2.

Methanolic extract of *S. tetragonum* has highest reducing activity of 41.53 mg equivalent to Gallic acid (GAE)/g. These finding prove that tested plant extracts might act as an electron donor and can react with free radicals to stop the radical chain reaction. Khatoun., *et al.* [17] studied the antioxidant activity of *Albizia procera* leaves through DPPH, reducing power and total antioxidant capacity. Their leaf extract exhibited an IC<sub>50</sub> value of about 90% among that of DPPH radicals.

This high up free radical scavenging may be correlated to the high Phenolic content or due to synergistic activity of various chemical entities present in the organic extracts. A positive correlation was observed between total Phenolic content and total antioxidant activity of all the plants having correlation coefficient (R<sup>2</sup>) values ranges from 0.969-0.995 (Figure 1). The highest correlation was found in *C. arborea* with R<sup>2</sup> value of 0.995 followed by *A. chinensis*, *S. tetragonum* and *M. macrophylla* with R<sup>2</sup> values 0.982, 0.973 and 0.969 respectively. The findings are in agreement with the earlier report showed a positive correlation among the total phenolic content and antioxidant properties of *Mussaenda macrophylla* [23].

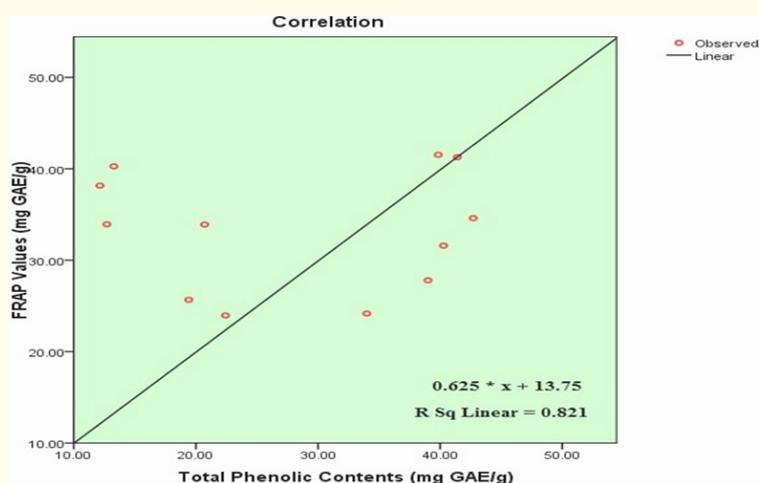
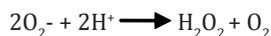


Figure 1: Correlation between total Phenolic content and total antioxidant capacity of all plants extractives.

### Estimation of Enzymatic Antioxidants

Reactive oxygen species (ROS) like Superoxide anion, hydroxyl radical and hydrogen peroxide produced during cellular metabolism are highly toxic to cellular macromolecules. Superoxide anion is considered as a weak oxidant, as it can produce hydroxyl radical and singlet oxygen, generated during oxidative stress. Superoxide dismutase's (SODs) can remove superoxide anion ( $O_2^-$ ) which carries out the following reaction:



Catalase is another antioxidant enzyme widely distributed in animal tissue. They protect the cell from hydroxyl radicals by hydrogen peroxide decomposition. Depletion of this enzyme may enhance oxidative stresses. The antioxidant activity of the medicinal plants is shown in Table 3. High superoxide dismutase (9.235 SOD Units/mg protein), catalase (93.212  $\mu$ M  $H_2O_2$  Decomposed/min/mg protein) and ascorbic acid oxidase (1.663 Units/mg protein) activities were more in *A. chinensis* as compared to other tested plants. Ascorbic acid is responsible for the first line of defence against various oxidative stresses. Ascorbic acid oxidase contains eight atoms of copper per molecule; it is widely distributed in plants and microorganisms catalyze the oxidation of ascorbic acid to dihydro ascorbic acid. The definitive biological function of ascorbate oxidase is not known, although report says that it may participate in a redox system involving ascorbic acid [24, 25].

### Antimicrobial Screening Test

The findings presented in this paper indicate that the chosen plants serve as a very effective source of new antimicrobial agents for use in pharmaceuticals industries and medicine. Ethanolic and Methanolic extracts of all the four tested plants show good antibacterial capability against tested bacteria. Antimicrobial activity of aqueous, ethanol and methanol extracts are exhibited by all the plant samples except *S. tetragonum* which shows no antibacterial effect with the aqueous extract. Ethanol extract of *A. chinensis* shows highest antibacterial activity over *S. aureus* (Table 4). Plant extracts were more effective against Gram positive bacteria than Gram negative bacteria. This can be due to the difference in cell wall composition between them that effect permeability and susceptibility of these bacteria to different compounds. The antimicrobial effect is associated with some compounds which can penetrate the outer membrane and reach its site of action, partial by their size and shape. None of the plant extracts shows any effect over fungal plant pathogens (Table 4). Phenolic compounds are not only provide antioxidant activity but also act as an antimicrobial agent in the plant extracts. Several mechanisms can be involved in antimicrobial activity of the plant extracts such as disruption in protein and cell wall, inactivation of enzyme and interfering in DNA replication [26, 27]. All the standard antibiotics used as positive control showed antibacterial activity against the tested microorganisms.

### Conclusion

From the present study, it can be concluded that Methanolic extract of *S. tetragonum* have significant reducing / in vitro antioxidant activity and ethanolic extract of *A. chinensis* has good antibacterial activity. Based on our findings, it can be concluded that medicinal plant extracts have great capability as antimicrobial compounds against pathogenic microorganisms and can be used in the treatment of infectious diseases caused by resistant microorganisms. The result obtained clearly signifies that these plants are rich source of antioxidants (both enzymatic and non-enzymatic). Since antioxidants play crucial role in prevention of many degenerative diseases, these plants are the capability targets to make acquainted for future investigations towards the finding of new, potent and safe antioxidant and antimicrobial compounds.

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**Conflict of Interest**

There is no conflict of interest.

Scientific name	Traditional / Local name	Family	Date and Place of collection	Geographical location	Traditional uses
<i>Albizzia chinensis</i>	Vang	Mimosaceae	June 2012 Dampa Tiger Reserve Forest	23°25''00N and 92°00''01E	lotion in scabies and other skin diseases
<i>Callicarpa arborea</i>	Hnahkiah	Verbenaceae			abdominal colic
<i>Mussaenda macrophylla</i>	Vakep	Rubiaceae	July 2012 Tanhril	23°44.269/N and 92°039.778/E	treatment of cough
<i>Stereospermum tetragonum</i>	Zihngghal	Bignoniaceae	June 2012 Mamit	23°92.934/N and 92°49.055/E	treatment of scorpion sting(a cutaneous condition usually resulting in pain, paresthesia, and variable swelling.) and maniacal cases

**Table 1:** Details of plant leaves used in the present investigation.

Medicinal plant	Solvent used for extraction	In vitro antioxidant activity- FRAP method (mg GAE/g DW)	Total phenol content (mg GAE/g DW)
<i>Albizzia chinensis</i>	water	24.17 ± 0.30 <sup>g</sup>	34.00 ± 0.03 <sup>d</sup>
	ethanol	34.60 ± 0.12 <sup>c</sup>	42.71 ± 0.01 <sup>a</sup>
	methanol	41.27 ± 0.06 <sup>a</sup>	41.40 ± 0.28 <sup>ab</sup>
<i>Stereospermum tetragonum</i>	water	27.80 ± 0.14 <sup>e</sup>	39.00 ± 0.04 <sup>c</sup>
	ethanol	31.60 ± 0.12 <sup>d</sup>	40.28 ± 0.14 <sup>bc</sup>
	methanol	41.53 ± 0.16 <sup>a</sup>	39.85 ± 0.03 <sup>c</sup>
<i>Mussaenda macrophylla</i>	water	23.96 ± 0.12 <sup>g</sup>	22.42 ± 0.17 <sup>e</sup>
	ethanol	25.67 ± 0.01 <sup>f</sup>	19.42 ± 0.01 <sup>f</sup>
	methanol	33.91 ± 0.02 <sup>c</sup>	20.71 ± 0.1 <sup>4f</sup>
<i>Callicarpa arborea</i>	water	38.16 ± 0.09 <sup>b</sup>	12.14 ± 0.17 <sup>h</sup>
	ethanol	33.93 ± 0.24 <sup>c</sup>	12.71 ± 0.30 <sup>h</sup>
	methanol	40.27 ± 0.05 <sup>a</sup>	13.28 ± 0.40 <sup>g</sup>

The values are the average of three replicates and are expressed as mean ± S.D. Mean (± SD) followed by the same letter(s) in each column are not significantly different at P < 0.05 using Duncan's new multiple range test.

**Table 2:** In vitro antioxidant activity and total Phenolic content of plant extracts.

Medicinal plant	SOD Units/ mg protein	Catalase $\mu\text{M H}_2\text{O}_2$ Decomposed/min/ mg protein	Ascorbic acid oxidase Units/ mg protein
<i>A. chinensis</i>	9.235 $\pm$ 0.58 <sup>a</sup>	93.212 $\pm$ 1.05 <sup>a</sup>	1.663 $\pm$ 0.24 <sup>a</sup>
<i>S. tetragonum</i>	7.341 $\pm$ 0.72 <sup>bc</sup>	89.428 $\pm$ 1.26 <sup>bc</sup>	0.992 $\pm$ 0.05 <sup>bc</sup>
<i>M. macrophylla</i>	8.292 $\pm$ 0.65 <sup>ade</sup>	90.210 $\pm$ 1.11 <sup>ace</sup>	1.264 $\pm$ 0.092 <sup>ace</sup>
<i>C. arborea</i>	6.230 $\pm$ 0.67 <sup>bcd</sup>	74.860 $\pm$ 1.52 <sup>bdf</sup>	0.364 $\pm$ 0.01 <sup>bdf</sup>

The values are the average of three replicates and are expressed as mean  $\pm$  S.D. Mean ( $\pm$  SD).

**Table 3:** Enzymatic antioxidant activity of the medicinal plants.

Tested Microorganisms	A. <i>chinensis</i>			S. <i>tetragonum</i>			M. <i>macrophylla</i>			C. <i>arborea</i>			Standard antibiotics					
	A	E	M	A	E	M	A	E	M	A	E	M	Chl	Nit	Ofl	Gen	Fur	Mic
<i>S. aureus</i>	-	14	7	-	3	-	-	5	3	-	6	7	8	8	11	10	8	-
<i>E. coli</i>	-	12	5	-	-	-	-	3	-	-	4	6	7	8	7	7	8	-
<i>F. graminearum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16
<i>F. oxysporum cicer</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18

A - Aqueous extract, E - Ethanol extract, M - Methanol extract, Gen - Gentamycin, Chl - Chloramphenical, Nit - Nitrofurantcin, Ofl - Ofloxacin, Fur - Furazolidine, Mic - Miconazole.

**Table 4:** Antimicrobial properties of plant extracts.

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