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The assessment of the free radical scavenging activity and flavonoid contents of selected medicinal plants of Mizoram

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Abstract

Herbal medicine has proven to be one of the most well-known fields of traditional medicine worldwide. Extracts from different traditional medicinal plants have been screened to discover the source of therapeutic effects and nature has been a source of medicinal agent for thousands of years. Antioxidants protect the body from the harmful damage produced by free radical induced oxidative stress. The present study is the assessment of the total flavonoid contents and antioxidant potential of medicinal plants. Different parts of the plant were used namely the stem bark of *Schima wallichi* (DC) Korth., *Milletia pachycarpa* Benth., the leaves of *Eleagnus caudata* Schlecht, *Dysoxylum gobara* Buch.-Ham and the fruit of *Castanopsis indica* (Roxb.)A.DC. The chloroform and ethanol extracts of all these plants showed free radical scavenging activity in a concentration dependent manner. *S. wallichi* showed the maximum scavenging activity followed by *E. caudata*, *M. pachycarpa*, *C. indica* and *D. gobara* accordingly. The chloroform and ethanol extracts both showed an increase in the flavonoid content in a concentration dependent manner. The amount of total flavonoids was higher in the ethanol extracts than that of chloroform extracts. The ethanol extracts also has greater flavonoid content and possess higher antioxidant activity when compared to the chloroform extracts. Our study concluded that *S. wallichi* showed highest ABTS scavenging activity among all the five plants. The antioxidant activity was not directly proportional to the total flavonoid contents of a plant species. The amount of total flavonoids was found to be lower in *S. wallichi*, which shows that other secondary compound like alkaloids, phenols, etc may have contributed to this effect.

Key words: ABTS; Total flavonoids; Medicinal plants.

Introduction

In spite of the recent domination of the synthetic chemistry as a method to discover and produce drugs, the potential of bioactive plants or their extracts to provide new and novel products for disease treatment and prevention is still enormous (1). For thousands of years nature has been the major source of medicine (2). Bioactive plants and their extracts have the potential to provide novel products for prevention and treatment of diseases. Due to its effectiveness in treating various diseases, minor side effects and cheaper cost, medicinal plants are highly popular among developing countries (3). Medicinal plants are indispensible to the global economy. Many secondary metabolites are commercially sold and are crucial to many pharmaceutical companies (4).

In India, the traditional knowledge of medicinal plants used by indigenous people are well documented and has been passed on the next generation for many years (5). Phyto-chemicals are natural chemical compounds found in plants. Plants produce these chemicals as a mode of defense mechanism for their protection, but these phytochemicals also provide protection

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for humans from diseases according to recent studies (6). Flavonoids are ubiquitous in nature and occurs as glycones, glycosides and methylated derivatives in vascular plants. Flavonoids are polyphenolic compounds (7). Due to their extensive biological and pharmacological properties, flavonoids have been widely studied. Flavonoids are potent antioxidants that can protect human beings from free radicals and reactive oxygen species. Their antioxidant capacity and scavenging activities depends upon their molecular structure, mainly on the position of hydroxyl groups in its chemical structure (8). Flavonoids contain many substances that protect biological systems from the toxic effects of oxidative processes (9). Free radicals have harmful effects on human beings and is related to toxicity and causing diseases like diabetes, chronic renal failure, cancer, mellitus, atherosclerosis, immune dysfunction and aging (10). Food sources like fruits and vegetables contain many free radical scavenging antioxidants (11). Free radicals have a damaging effect on cells and cause various degenerative disorders when they are produced excessively (12).

Antioxidants can intervene the production of free radicals during the main steps of the free radical mediated oxidative processes, viz., initiation, propagation and termination (13). Most living organisms have an effective defense system to protect themselves against the oxidative stress induced by Reactive Oxygen Species (ROS) (14). Due to the certain health benefits, less toxicity, cheap price and accessibility, antioxidants from plant sources are getting popular (15). Therefore, this study targets to investigate antioxidant potential of different medicinal plants including *Schima wallichi*, *Milletia pachycarpa*, *Eleagnus caudata*, *Castanopsis indica* and *Dysoxylum gobara in vitro* by evaluating ABTS scavenging activity.

Medicinal uses:

***Schima wallichii* (DC) Korth:** The leaves and the stem bark are traditionally used for its medicinal properties. The bark is used as an antiseptic for wounds. It is also used as a vermicide, and treating gonorrhea (16), decoction of bark is effective against head lice and reduces fever. The bark juice of Chilauni is used in animals as a liver fluke disinfesting agent (17). People of Western Mizoram use the fruit juice of Chilauni for treating snakebite (18). *Schima wallichii* has anti-cancer activities, and have the ability to induce apoptotic mechanisms (19).

***Elaeagnus caudata* Schlecht:** The fruit is taken as a health tonic (20). The extract of the fruit or stem bark is mixed with *Piper longum* and is taken for 2-3 weeks on a daily basis to cure jaundice and other liver troubles (21). The root decoction is taken to expel the retained placenta, ease labor and as a treatment after child birth. The leaf infusion is used for strengthening the function of uterus after child birth (22).

***Milletia pachycarpa* Benth:** *M.pachycarpa* is used in Chinese traditional medicine for the preparation of 'Jixueteng' that induce the growth of red blood cells (23). The compounds isolated from *M. pachycarpa* has been reported to be cytotoxic and induce apoptosis in HeLa cells (24) and also show cytotoxic effect in Brine shrimp assay (25) with anti-inflammatory activity (26). In India and China, it is used traditionally in treating cancer and infertility. It is also used as a pesticide and as a blood tonic (27). The bark paste is also used in treating diseases like skin infections and itches (28).

***Castanopsis indica* (Roxb.) A. DC:** *C. indica* is traditionally used for treating stomach disorders, chest pain, skin diseases, headache and diarrhea (29). The leaf decoction is used to treat stomach disorder and skin diseases (30). The seeds are consumed raw in Nepal (31) and Mizoram (32). The resin is given to treat diarrhea and the leaf paste is applied for headache. The bark paste is also applied on the chest to control chestpain (33).

***Dysoxylum gobara* Buch.-Ham:** The leaf and bud decoction is used to treat diarrhea and dysentery (34-36). The tender leaves and flowers are cooked

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and eaten as a vegetable. The decoction of leaves is used as a remedy for food poisoning, diarrhea and dysentery (37).

Materials and Methods

Chemicals: Ascorbic acid, trolox and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), aluminium chloride, chloroform, ethanol, potassium acetate, quercetin, potassium persulfate.

Collection of Plant Materials: The healthy stem bark of *Milletia pachycarpa* Benth., *Schima wallichi* (DC) Korth., the leaves of *Eleagnus caudata* Schlecht., *Dysoxylum gobara* Buch.-Ham. and the fruit of *Castanopsis indica* (Roxb.) A.DC. were collected during the dry season from different parts of Mizoram. The plant specimens were identified by Prof. Lalramnghinglova, Department of HAMP, Mizoram University, Aizawl. The herbarium specimens are deposited at the Department of Zoology, Mizoram University. The stem bark, leaves or fruits were examined visually for infection, washed thoroughly with clean water and allowed to shade dry at RT in the dark, clean and hygienic conditions. The dried plant material was powdered using an electrical grinder at room temperature and sequentially extracted with chloroform and ethanol using a Soxhlet apparatus. The liquid extracts were filtered and dried using rotary vacuum evaporator and stored at-70^{o} C until further use.

Total flavonoid content: The total flavonoid content was determined by $AlCl_3$ method (38). The ethanol and chloroform extracts of different concentrations were mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. Then it was incubated at RT for 30min. The absorbance was measured at 415 nm with double beam UV spectrophotometer). The calibration curve was prepared by preparing Quercetin solution at different concentrations.

Antioxidant capacity using ABTS scavenging assay: The ABTS cation scavenging activity was determined for the different extracts using a minor modification of Re R et al (39). 37.5 mg of potassium persulfate was taken and dissolved in 1 ml of distilled water. 44 ml was taken from this solution and dissolved in 2.5 ml of distilled water with 9.7 mg of ABTS. The absorbance was measured at 734 nm after the solution was kept in dark condition at RT for 16 hours. The results have been represented as ascorbic acid equivalent. The scavenging activity was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{(A control OD-Atest OD)}}{\text{(A control OD)}} \times 100$$

Results and Discussion

Phytochemicals found in plants possesses significant benefits to human health such as ascorbic acid, carotenoids and phenolic compounds (40). Phytochemical compounds that naturally possess anticarcinogenic (41-42) and other beneficial properties are referred to as chemo-preventers and this protective action is due to their antioxidant activity and their capacity to scavenge free radicals (43).

The total flavonoid contents of chloroform extracts of *Milletia pachycarpa*, *Schima wallichi*, *Eleagnus caudata*, *Castanopsis indica* and *Dysoxylum gobara* showed a concentration dependent rise up to 2500 μg/ml (Figure.1). The highest total flavonoid was present in *E. caudata* (130.36±2.15 mg/g) followed by *D. gobara* (94.62±1.58 mg/g), *M. pachycarpa* (51.27±2.14 mg/g), *C. indica* (48.61±3.40 mg/g) and *S. wallichi* (26.82±1.25 mg/g). The least total flavonoid content was detected in *S. wallichi.*

The presence of total flavonoid contents in ethanol extracts of all five plants also showed an increase in flavonoid content with an increase in concentration manner. The highest flavonoid content were also found at 2500 μg/ml for all the extracts with the highest value present in *Dysoxylum gobara* (75.73±0.98 mg/g), followed by *E. caudata* (53.43±3.27 mg/g), *C. indica* (38.04±23 mg/g), *S. wallichi* (32.06±2.29 mg/g). The lowest content was found in *M. pachycarpa* (19.43±0.71 mg/g). The order of the flavonoid

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content observed was as follows: ECC (130.36±2.15) > DGC (94.62±1.58) > DGE (75.73±0.98) > ECE (53.43±3.27) > MPC (51.27±2.14) > CIC (48.61±3.40) > CIE (38.04±23) > SWE (32.06±2.29) > SWC (26.82±1.25) > MPE (19.43±0.71) at the concentration 2500 µg/ml. The present study revealed high content of both phenols and flavonoids (Figure 1), both flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties (44).

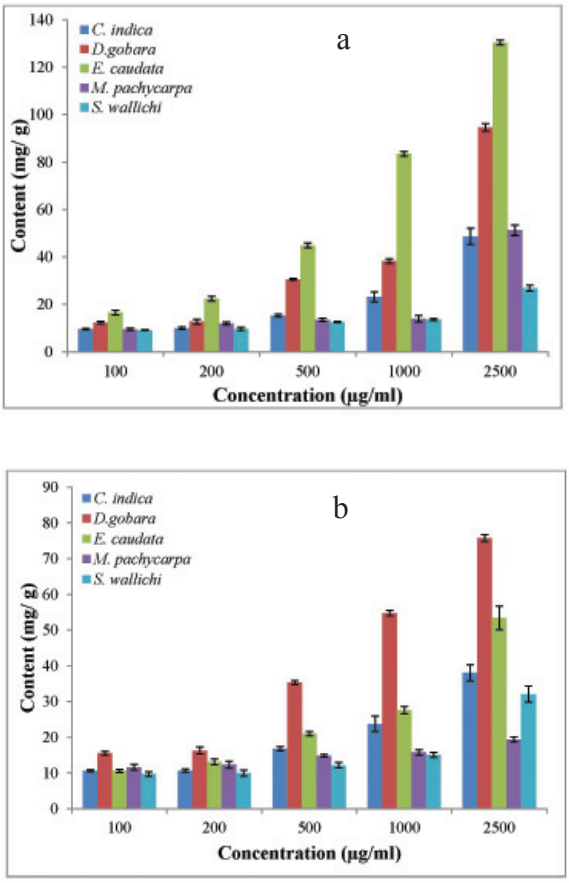

Fig. 1: The total flavonoid content of various medicinal plant of Mizoram extract with chloroform (a) and ethyl alcohol (b) as Quercetin equivalent. The values represented as Mean+ SEM, n=3.

The chloroform extracts of the five plants showed a concentration dependent rise up to 5000 µg/ml in the ABTS scavenging activity. The maximum ABTS inhibition for chloroform extract was found in *S. wallichi* (78.45±0.95 µg/ml) followed by *C. indica* (65.78±0.76 µg/ml), *M. pachycarpa* (63.44±2.58 µg/ml), *E. caudata* (63.28±2.44 µg/ml) and *D. gobra* (22.93±0.53 µg/ml). The ethanol extract of all the plants also showed the highest ABTS scavenging at 5000 µg/ml. The ethyl alcohol extract of *M. pachycarpa* showed the highest activity (89.02±1.79 µg/ml), followed by, *S. wallichi* (88.84±2.46 µg/ml), *E. caudata* (88.60±0.94 µg/ml), and *C. indica* (79.90±1.65 µg/ml) and *D. gobara* (78.67±2.21 µg/ml).

The order of scavenging activity was observed as follows: MPE (89.02±1.79%) > SWE (88.84±2.46%) > ECE (88.60±0.94%) > CIE (79.90±1.65%) > DGE (78.67±2.21) > SWC (78.45±0.95%) > CIC (65.78±0.76%) > MPC (63.44±2.58%) >ECC (63.28±2.44%) > DGC (22.93±0.53%) at the concentration 5000 µg/ml.

The analysis of ABTS scavenging in respect of TROLOX equivalent was similar as maximum scavenging activity was observed at concentration of 5000 ìg/ml for chloroform extracts and ethyl alcohol extracts of all the five plants.

Correlation analysis of total flavonoid content and scavenging activity of certain medicinal plants of Mizoram, extracted with chloroform and ethyl alcohol shows that an increase in the flavonoid content causes an increase in the scavenging activity. The chloroform extracts of *M. pachycarpa*, *S. wallichi*, *E. caudata*, *C. indica* and *D. gobara* did inhibit the generation of ABTS free radicals in concentration dependent manner and this activity was highest at the concentration of 5000 µg/ml with EC50 values of 2140 µg/ml, 475.9 µg/ml, 74.18 µg/ml, 2295 µg/ml respectively, however *D. gobra* did not show insignificant effective concentration compared to the others, the maximum effect was observed for 5000 ìg/ml with 22.93±0.53% of scavenging activity.

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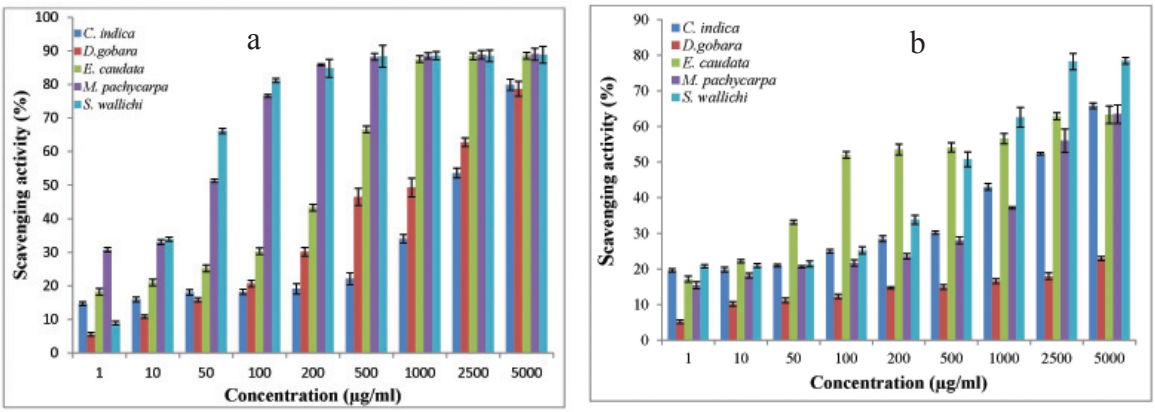

Fig. 2: Scavenging activity of various medicinal plant of Mizoram extracted with chloroform (a) ethyl alcohol (b). The values represented as Mean+ SEM, n=3.

Table 1(a) : Correlation of total flavonoid and scavenging activity certain medicinal plants of Mizoram, extracted with chloroform.

Conc.	*C. indica*		*D.gobara*		*E. caudata*		*M. pachycarpa*		*S. wallichi*	
(µg/ml)	TF	SA	TF	SA	TF	SA	TF	SA	TF	SA
100	9.51	24.94	12.35	12.26	16.6	51.97	9.47	21.65	9.14	25.22
200	9.97	28.49	12.68	14.68	22.35	53.51	11.9	23.55	9.67	33.81
500	15.26	30.19	30.51	14.95	44.83	54.14	13.49	28.04	12.55	50.76
1000	23.18	43.02	38.2	16.58	83.5	56.6	13.97	37.15	13.61	62.57
2500	48.61	52.36	94.62	17.99	130.37	62.89	51.28	56.02	26.83	78.24
Correlation (R- value)			**0.95**	**0.85**	**0.98**	**0.94**	**0.88**			

Table 1(b) : Correlation of total flavonoid and scavenging activity certain medicinal plants of Mizoram, extracted with ethyl alcohol.

Conc (µg/ml)	*C. indica*		*D.gobara*		*E. caudata*		*M. pachycarpa*		*S. wallichi*	
	TF	SA	TF	SA	TF	SA	TF	SA	TF	SA
100	10.62	18.17	15.57	20.68	10.6	30.29	11.64	76.6	9.76	81.21
200	10.68	19.12	16.31	30.11	13.15	43.34	12.33	85.87	9.99	84.84
500	16.79	22.12	35.36	46.5	21.05	66.55	14.85	88.23	12.21	88.41
1000	23.76	34.05	54.76	49.29	27.58	87.55	15.77	88.53	15.05	88.55
2500	38.04	53.6	75.73	62.77	53.43	88.38	19.44	88.84	32.06	88.56
Correlation (R- value)		**0.99**		**0.95**		**0.82**		**0.71**		**0.55**

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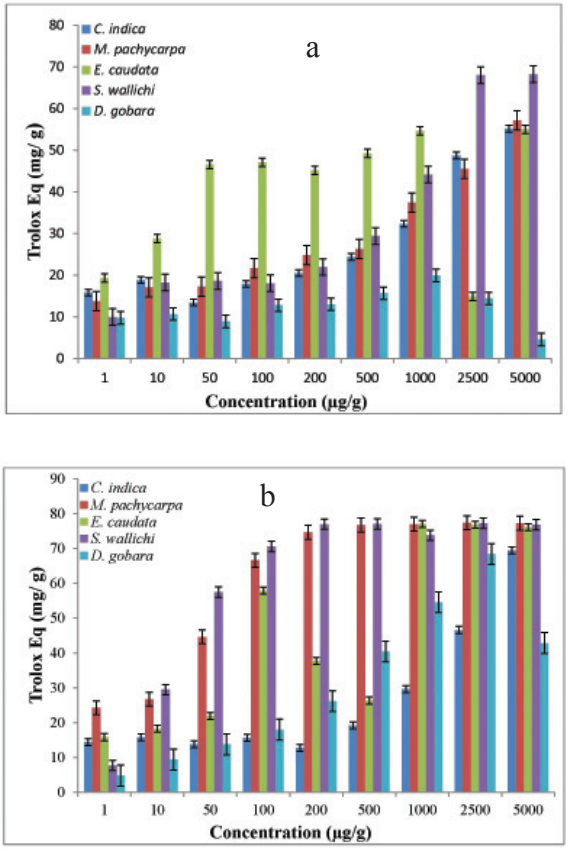

Fig. 3: The trolox equivalent of various medicinal plant of Mizoram extracted with chloroform (a) ethyl alcohol (b). The values represented as Mean+ SEM, n=3.

The ethyl alcohol extracts of *C. indica* and *D. gobra* also showed maximum inhibition at the concentration of 5000 µg/ml, with EC50 value of 2524 µg/ml and 908 µg/ml, respectively.

The maximum ABTS inhibition for ethyl alcohol extract of *E caudata* was at 200 µg/ml, which remained almost same at 1000 µg/ml, that remained almost similar up to a concentration of 5000 µg/ml with an EC50 value of 239 µg/ml. The maximum scavenging was observed at 200 µg/ml for *M. pachycarpa* with an EC50 value of 1.27 µg/ml, whereas this concentration was 100 µg/ml for *S. wallichi* with EC50 value of 0.060 µg/ml, both remained almost similar up to a concentration of 5000 µg/ml. The comparison between activity and content show positive correlation and the order of positive correlation were as follows: CIE (r = 0.99) > ECC (r = 0.975) > CIC (r = 0.951) > DGC (r = 0.950) > MPC (r = 0.93) > SWC (r = 0.88) > DGC (r = 0.85) > ECE(r = 0.82) > MPE (r = 0.71) > SWE (r = 0.55).

The free radical scavenging activity of plants extract against ABTS cations revealed significant reduction with less concentration (Figure 2). The active metabolites present in the plants are responsible for the antioxidant activity and the natural phenol and flavonoid contents are present in fruit, leaves, flower and the seeds of plants (45). The TROLOX equivalent activity showed the plant extract possesses high efficacy of scavenging properties (Figure 3). The correlation analysis of both concentration and activity revealed that the activity increased with increase in concentration (Table. 1). The natural antioxidants maybe useful as they may have fewer side effects or no side effects, because of their biologic origin. *E. caudata* scavenged the ABTS free radicals in a concentration dependent manner indicating its antioxidant potential. The other species of *Eleagnus* including *E. angustifolia* have been reported to scavenge ABTS radical (46).

S. wallichi inhibit the generation of ABTS free radicals in a concentration dependent manner and this activity was highest for this plant (Figure. 2). The present study correspond with the previous reported by (47, 17, 48). The resulting antioxidant effect observed was almost akin to the present study. The chloroform and ethanol extracts of *M. pachycarpa* did inhibit the generation of DPPH free radicals in concentration dependent manner. The ethyl alcohol extract of *M. pachycarpa* showed the highest activity.

M. pachycarpa has been known to scavenge free radicals (49-50) and the results are also in accordance with the findings in this study.

C. indica scavenged the ABTS free radicals in a concentration dependent manner indicating

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its antioxidant potential. The *C. indica* has been reported to scavenge superoxide, hydroxyl and ferric free radicals earlier and this activity was attributed to the presence of phenolic compunds (51).

The other species of Castanopsis including *Castanopsis cuspidate* have been reported to have antioxidant property (52).

The antioxidant activity of *D. gobara* was lesser than the other plants and has the least antioxidant activity among all the five plants. *D. gobara* has been reported to possess antioxidant activity in DPPH assays earlier (50) and the results are also in accordance with the findings in the present study. The other species of *Dysoxylum* namely *Dysoxylum cauliflorum* have been reported to possesses antioxidant activity in both the DPPH scavenging and FRAP assay.

Conclusion

The present study of medicinal plants including *M. pachycarpa, S. wallichi, E. caudata, C. indica* and *D. gobara* revealed high content of bioactive compound and potential antioxidant efficiency, which might be useful resources for future ethno-medicine. However, the mechanism of action of secondary metabolites required further investigation in future.

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