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In vitro antioxidant and preliminary phytochemical screening of methanolic extract of *Macaranga denticulata* (Blume) Müll.Arg.

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Macaranga denticulata (Blume) Müll.Arg. (family Euphorbiaceae) is an evergreen tree and a common pioneer species in moist open and secondary forest. It is commonly known as Kharpa in Mizoram. Traditionally, the species of *Macaranga* are used in the treatment of swelling, cuts, sores, boils and bruises. Preliminary phytochemical screening and evaluation of *in vitro* antioxidant activity were carried out on the methanolic extract obtained from the bark of *M. denticulata*. The presence of alkaloids, tannins, flavonoids, saponins, steroids and triterpenoids was indicated by the tests conducted. The *in vitro* antioxidant activity was evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, reducing power and hydrogen peroxide radical scavenging activity. Ascorbic acid and butylated hydroxytoluene (BHT) were used as reference standards. The methanolic extract of the plant shows a strong antioxidant activity comparable to that of the reference standards.

Key words: Antioxidant activity, DPPH, hydrogen peroxide, *Macaranga denticulata*.

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Introduction

Free radicals are produced largely within the cells and may produce potentially damaging effect on the cells. There are a large number of physiological and pathological sources of oxygen free radicals and related oxidants. These oxygen free radicals are even known to be produced in small amounts during normal cellular processes.[1] It is becoming increasingly apparent that in addition to promoting direct toxicity, free radicals may also initiate and/or amplify inflammation via the up regulation of several different genes involved in the inflammatory response. This may occur by the activation of certain transcription factors, such as NF-κB. NF-κB is a ubiquitous transcription factor and pleiotropic regulator of numerous genes involved in the immune and inflammatory responses.[2] It has been reported that NF-κB plays major roles in leukemia, inflammatory bowel disease, arthritis, sepsis, asthma, multiple sclerosis, colitis, diabetic neuropathy and AIDS.[3]

Plants are endowed with many different free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinines, coumarins, alkaloids, amines, betalins and other metabolites, which are rich in antioxidant activity.[4, 5] *M. denticulata* is a deciduous tree, densely branched, up to 22 m tall, young stem brownish, pubescent, stem round

white-green, sometimes brown-green, spongy hollowed.[6] Traditionally, species belonging to *Macaranga* are used in the treatment of swelling, cuts, sores, boils and bruises.[7] The present study aimed at evaluating the antioxidant activity of the plant and to validate the traditional used of the plant for various inflammatory conditions.

Materials and Methods

Plant material

The plant material *M. denticulata* was collected from Lunglei, Mizoram, India in April 2017. The plant was authenticated (vide No. BSI/ERC/ Tech/2017/115) by Botanical Survey of India, Shillong.

Extraction of phytoconstituents

The dried powder material was extracted in a Soxhlet apparatus first with petroleum ether (60-80°C) to defat it and then the defatted material was extracted with methanol for 72 hours. The solvents were then evaporated using a simple distillation. The concentrated extracts were kept in refrigerator at 4°C for further use. The percentage yields of petroleum ether and methanol extract were found to be 0.863% and 7.044% respectively.

Phytochemical screening

The methanolic extracts of *M. denticulata* was screened for the presence of alkaloids, flavonoids, tannins, glycosides, steroids, triterpenoids, saponins, carbohydrates, saponins, fats and fixed oils.[8]

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging activity of methanolic extracts of *M. denticulata* and standard BHT were determined by using DPPH, which is a free radical. 3 ml of methanol extract at various concentrations (10, 20, 40, 60, 80, 100 µg/ml) were taken in a test tubes and 0.5 ml of 0.1 Mm DPPH solution was added to each. It was incubated in the dark for 30 minutes at 37 ºC. The absorbance was measured at 517 nm. The IC_{50} value was calculated. The percentage of DPPH scavenging effect was estimated by using equation:

$$I\ \% = [A_{control} - A_{sample} / A_{control}] \times 100$$

Where, $A_{control}$ is absorbance of control and A_{sample} is absorbance of the test sample.[9]

Reducing power assay

The reducing power was determined by using the methanolic extracts of *M. denticulata*. The extract was diluted at various concentrations. 1 ml of each dilution was mixed with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 30 min. After cooling, 2.5 ml of 10% trichloro acetic acid (TCA) was added and centrifuge for 10 min at 3000 rpm. 2.5 ml of the supernatant was diluted with 2.5 ml of distilled water; to it 0.5 ml of freshly prepared 0.1% ferric chloride solution was added and mixed. The absorbance of the mixture was measured at 700 nm. Ascorbic acid was used as a standard. The higher absorbance indicates increase in the reducing power.[10]

Hydrogen peroxide scavenging activity

The ability of the methanolic extracts of *M. denticulata* to scavenge hydrogen peroxide was determined. A solution of hydrogen peroxide (40 Mm) was prepared in phosphate buffer (pH 7.4) The extract (100 µg/ml) was diluted in distilled water at various concentrations and 1 ml of various concentrations were added to a hydrogen peroxide solution (0.6 ml, 4 Mm). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both *M. denticulata* and the standard compound ascorbic acid

was calculated:

$$\% \text{ Scavenged } [H_2O_2] = [(A_c - A_s)/A_c] \times 100$$

Where A_c is the absorbance of the control and A_s is the absorbance of the methanolic extracts of *M. denticulata* or ascorbic acid.[11]

Results

Phytochemical screening

Phytochemical screening of the extracts shows the presence of alkaloids, tannins, flavonoids, saponins, steroids and triterpenoids (Table 1).

Table 1 | Results of preliminary phytochemical test.

Sl. No.	Phytochemical tests	Petroleum ether extract	Methanol extract
1	Alkaloids	+	+
2	Tannins	-	+
3	Flavonoids	-	+
4	Steroids and triterpenoids	+	+
5	Saponins	-	+

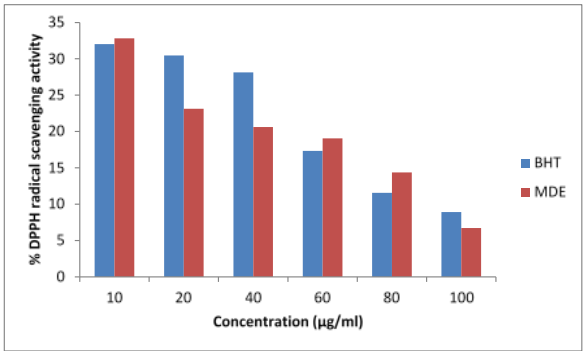

Figure 1 | DPPH radical scavenging activity of BHT and methanolic extract of *Macaranga denticulata* (MDE).

DPPH radical scavenging activity

DPPH is a free radical which is stable at room temperature and this method is often employed to determine the antioxidant activity of many plant extracts as it is a simple and reproducible method.[12] Fig. 1 shows that the methanolic extract of *M. denticulata* has a radical scavenging activity by inhibiting DPPH with the IC_{50} value of 2.97 µg/ml which is comparable to the standard BHT with an IC_{50} value of 2.009 µg/ml. The lower IC_{50} value indicates higher antiradical activity. The IC_{50} was calculated from the graph by plotting the % inhibition in Y-axis and concentration in X-axis.

Determination of reducing power

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. In this assay, the presence of antioxidant in the extract resulted in the reduction of ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}). Higher the absorbance of the reaction mixture higher would be the reducing power.[13] Figure 2 shows the reducing power of the standard ascorbic acid and methanolic extract of *M. denticulata*. The extract shows an increase in absorbance as the concentration increases which is comparable to the standard, which may suggest that the extract possesses a significant antioxidant activity capable of terminating a radical chain reaction.

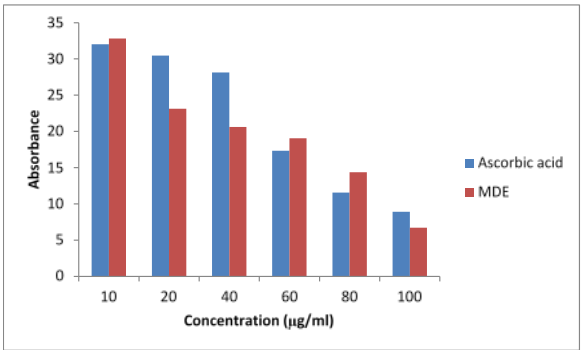

Figure 2 | Reducing power activity of ascorbic acid and methanolic extract of *Macaranga denticulata* (MDE).

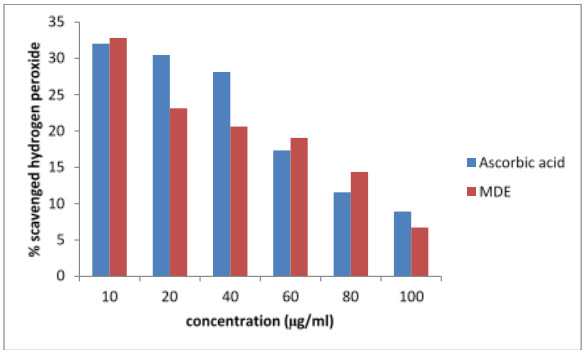

Figure 3 | Hydrogen peroxide activity of methanolic extract of *Macaranga denticulata* (MDE) against the standard ascorbic acid.

Hydrogen peroxide scavenging activity

Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate a biological membrane. However, it may be toxic if converted to hydroxyl radical in the cell.[12] The extract was capable of scavenging hydrogen peroxide in a concentration dependent manner. Figure 3 shows the hydrogen peroxide scavenging activity of the standard ascorbic acid and the methanolic extract of *M. denticulata*.

Discussion

A phytochemical review of the literatures indicates the genus *Macaranga* to be a rich source of the isoprenylated, geranylated and farnesylated flavonoids[14-17] and stilbenes.[17-19] Furthermore more classes of secondary metabolites like terpenes,[14,15,20] tannins,[21-23] coumarins [24,25] and other types of compounds [26-28] are known to be isolated from different species of the genus *Macaranga*. Flavonoids and stilbenes are regarded as the major constituents and are most likely responsible for most of the activities found in the plants of this genus.[7] The preliminary phytochemical investigation showed that methanolic extract of *M. denticulata* contains alkaloids, tannins, flavonoids, saponins, steroids and triterpenoids. The methanolic extract of the bark of *M. denticulata* was then evaluated for its antioxidant activities. The result of the tested activities showed a promising activities which may be attributed to the presence of active phytoconstituents such as flavonoids, steroids and triterpenoids, etc. The results of the preliminary phytochemical investigation showed that the methanolic extract of *M. denticulata* contained potential antioxidant compounds and the *in vitro* evaluation of methanolic extract of *M. denticulata* thus revealed that it does possesses a significant free radical scavenging activity when tested against known antioxidant compound such as Ascorbic acid and BHT.

Conclusion

From the results obtained, it can be concluded that the bark of *M. denticulata* contained active phytoconstituents which exhibited a significant potential in folk medicine. The plant also contained flavonoids which showed antioxidant activity. Flavonoids have been shown to exhibit their actions through their effect on membrane permeability, and by inhibition of membrane-bound enzymes such as ATPase and phospholipase A_2[29] and this may explain the antioxidant activity of *M. denticulata*. It is well established that there is crucial involvement of free radicals in many inflammatory conditions through different pathways like NF-κB, etc. Thus, this anti-oxidant studies support of the use of *M. denticulata* in folklore remedies in the treatment of cuts, sores, swelling, boils and bruises.

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