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Research Article

IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY OF AERIAL PART OF *MAERUA APETALA*. ROTH (JACOBS) (CAPPARACEAE)

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ABSTRACT

Objective: To determine the total phenolics, flavonoids and *in vitro* antioxidant activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of whole plant of *Maerua apetala* using various antioxidant model system viz, DPPH, hydroxyl, superoxide, ABTS and reducing power.

Methods: Total phenolic content was estimated by folin-ciocalteau method. Flavonoids were determined by Aluminium chloride method. *In vitro* antioxidant activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts was evaluated by studying 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity, ABTS radical cation scavenging activity and reducing power using standard procedure.

Result: The total phenolics and flavonoids in methanol extract were found to be 1.25g100g^{-1} and 2.44g100g^{-1} respectively. Among the solvent tested, methanol and ethanol extracts of whole plant of *M. apetala* showed potent *in vitro* antioxidant activities.

Conclusion: This investigation explored immense free radical scavenging potential of whole plant of *M. apetala* which can be used for the treatment of various free radical mediated ailments.

Keywords: anti oxidant, *Maerua apetala,* ethanol, DPPH, ABTS, reducing power.

INTRODUCTION

Antioxidants help the organisms in dealing with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Reactive oxygen species (ROS) readily combine and oxidize biomolecules such as carbohydrates, proteins and lipids and thus making them inactive with subsequent damage to cells, tissues and organs [1,2].

It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defences or by supplementing with proven dietary antioxidants [3]. This is one of the reasons why discovery and synthesis of novel antioxidants is a major active area. In recent years, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value [4]. Antioxidants derived from fruits, vegetables, spices and cereals are very effective and have reduced interference with the body's ability to use free radicals constructively [5,6]. Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols) ascorbic acid and carotenoids. The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the last two decades. Efforts to gain extensive knowledge regarding the power of antioxidants from plants and to tap their potential are therefore on the increase. Many medicinal plants have been investigated for their beneficial use as antioxidants or source of antioxidants using presently available experimental techniques.

Maerua is an important ayurvedic drug used as one of the ingredients in many Ayurvedic preparations. Ethnomedical survey reveals that *Maerua* is used to cure various diseases such as fever, stomach ache, skin infections, urinary calculii, diabetes mellitus, epilepsy, pruritis, rigidity in lower limbs, and abdominal colic [7].

Maerua is an important controversial drug used in diseases like anaemia, fever, diabetes, stomach disorders, typhoid, urinary infection and cough [8]. *Maerua apetala* (Capparaceae) is one of the botanical sources of the Ayurvedic drug. So far, no attempts have been made to evaluate the antioxidant properties of *Maerua apetala.* Hence the present study was performed to investigate the *in vitro* antioxidant activity of different extracts of *Maerua apetala* aerial part using different models viz: DPPH, hydroxyl, superoxide and ABTS radical cation scavenging activity.

MATERIALS AND METHODS

The aerial part of *Maerua apetala* Roth (Jacobs) were collected from Vattakottai, Kanyakumari District, Tamil Nadu. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

Preparation of plant extract

The coarse powder (100g) of aerial part of *Maerua apetala* was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250mL in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No.41 filter paper. All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for *in vitro* antioxidant activity. The methanol extract was used for the estimation of total phenolics and flavonoids.

Estimation of total phenolic content

Total phenolic contents were estimated using Folin-Ciocalteau reagent based assay as previously described [9] with little modification. To 1mL of each extract (100µg/mL) in methanol, 5mL of Folin-Ciocalteau reagent (diluted ten-fold) and 4mL (75g/L) of Na_2CO_3 were added. The mixture was allowed to stand at 20ºC for 30min and the absorbance of the developed colour was recorded at 765nm using UV-VIS spectrophotometer. 1mL aliquots of 20, 40, 60, 80 and 100µg/mL methanolic gallic acid solutions were used as standard for calibration curve. The absorbance of solution was compared with gallic acid calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE g/100g dry weight of extract).

Estimation of flavonoids

The flavonoids content was determined according to Eom *et al* [10]. An aliquot of 0.5ml of sample (1mg/mL) was mixed with 0.1mL of 10% aluminium chloride and 0.1mL of potassium acetate (1M). In this mixture, 4.3mL of 80% methanol was added to make 5mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H [11].

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method [11]. Briefly, an 0.1mM solution of DPPH in methanol was prepared and 1mL of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (50,100,200,400 & 800µg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell [12]. Stock solutions of EDTA (1mM), $FeCl_3$ (10mM), Ascorbic Acid (1mM), H_2O_2 (10mM) and Deoxyribose (10mM) were prepared in distilled deionized water.

The assay was performed by adding 0.1mL EDTA, 0.01mL of $FeCl_3$, 0.1mL H_2O_2, 0.36mL of deoxyribose, 1.0mL of the extract of different concentration (50,100,200,400&800µg/mL) dissolved in distilled water, 0.33mL of phosphate buffer (50mM, pH 7.9), 0.1mL of ascorbic acid in sequence. The mixture was then incubated at 37^{0}C for 1 hour. 1.0mL portion of the incubated mixture was mixed with 1.0mL of 10% TCA and 1.0mL of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Srinivasan *et al* [13]. The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16mM, pH 8.0), containing 0.5mL of NBT (0.3mM), 0.5mL NADH (0.936mM) solution, 1.0mL extract of different concentration (50,100,200,400 & 800µg/mL), and 0.5mL Tris – HCl buffer (16mM, pH 8.0). The reaction was started by adding 0.5mL PMS solution (0.12mM) to the mixture, incubated at 25^{o}C for 5 min and the absorbance was measured at 560nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula

Antioxidant activity by radical cation (ABTS +)

ABTS assay was based on the slightly modified method of Huang *et al* [14]. ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS solution with 2.45mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS+ Solution were diluted with ethanol to an absorbance of 0.70+0.02 at 734nm. After addition of sample or trolox standard to 3.9mL of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC). The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Reducing power

The reducing power of the extract was determined by the method of Kumar and Hemalatha[15]. 1.0mL of solution containing 50,100,200,400&800µg/mL of extract was mixed with sodium phosphate buffer (5.0mL, 0.2M, pH 6.6) and potassium ferricyanide (5.0mL, 1.0%): The mixture was incubated at 50^{o}C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980g (10 minutes at 5^{o}C) in a refrigerator centrifuge. The upper layer of the solution (5.0mL) was diluted with 5.0mL of distilled water and ferric chloride and absorbance read at 700nm. The experiment was performed thrice and results were averaged.

Statistical analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

RESULTS

Total phenolic and total flavonoid content

The total phenolic content and total flavonoid content of *M. apetala* were found to be 1.25g100g^{-1} and 2.44g100g^{-1} respectively.

DPPH radical scavenging activity

Petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of aerial part of *M. apetala* and standard ascorbic acid on DPPH radical scavenging activity were compared and shown in Figure-1. The scavenging effect increases with the concentration of standard and samples. At 800µg/mL concentration of methanol, petroleum ether and ethanol extracts of *M. apetala* aerial part possessed 108.56%, 98.18% and 96.54% scavenging activity on DPPH respectively. All the concentration of *M. apetala* extracts showed higher activity except benzene and ethyl acetate extracts than standard ascorbic acid. The scavenging ability decreased in the order of methanol>petroleum ether>ethanol>ethyl acetate> benzene.

Hydroxyl radical scavenging activity

The effect of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *M. apetala* aerial part and standard ascorbic acid on hydroxyl radical scavenging activity were compared and shown in Figure-2. At 800µg/mL concentration of ethanol, methanol and petroleum ether extracts of *M. apetala* aerial part showed 114.46%, 103.84% and 93.94% scavenging activity on hydroxyl radical respectively. All the concentration of *M. apetala* aerial part extracts showed higher activity except ethyl acetate and benzene extracts

than the standard ascorbic acid. Hydroxyl radical scavenging activity of extracts were in following order ethanol> methanol>petroleum ether> ethyl acetate> benzene.

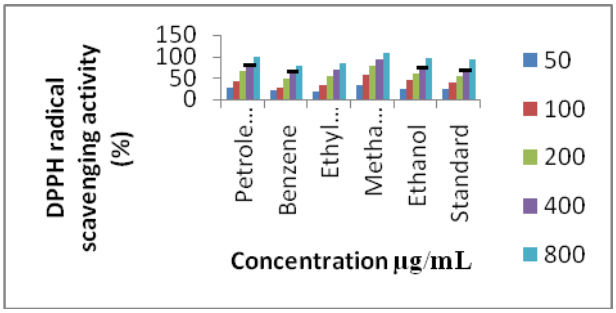

Fig 1: DPPH radical scavenging activity of different extracts of *M. apetala* aerial part

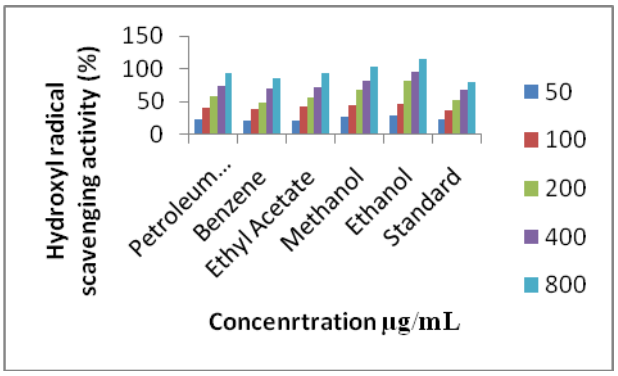

Fig 2: Hydroxyl radical activity of different extracts of *M.apetala* aerial part

Superoxide radical scavenging activity

The *M. apetala* aerial part extracts were subjected to be superoxide radical scavenging assay and the results were shown in Figure-3. It indicates that ethanol, methanol and petroleum ether extracts of *M. apetala* aerial part (800µg/mL) exhibited the superoxide radical scavenging activity of 112.66%, 109.16% and 103.56% respectively, which is higher than the standard ascorbic acid whose scavenging effect is 93.51%. Superoxide radical scavenging activity of extracts were in following order ethanol> methanol> petroleum ether> benzene> ethyl acetate

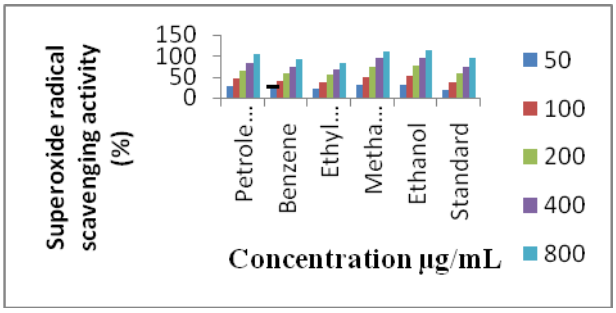

Fig 3: Superoxide radical scavenging activity of different extracts of *M. apetla* aerial part

ABTS radical scavenging activity

The effect of *M. apetala* aerial part extracts and standard trolox on ABTS radical cation were compared and shown in Figure-4. The scavenging effect increases with the concentration of standard and samples. At 800µg/mL concentration of ethanol, methanol and petroleum ether extracts of *M. apetala* aerial part possessed 101.22%, 98.91% and 91.34% scavenging activity on ABTS respectively. All the concentration of *M. apetala* extracts showed higher activity except ethyl acetate extract than the standard trolox. ABTS radical cation scavenging activity were in following order ethanol>methanol>petroleum ether> benzene>ethyl acetate.

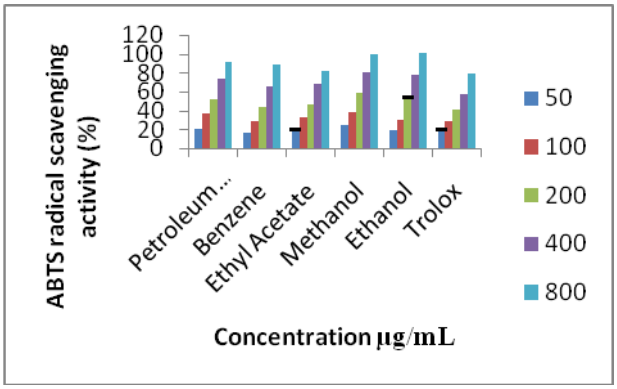

Fig 4: ABTS radical cation scavenging activity of different extracts of *M. apetla* aerial part

Reducing power

The reducing power of *M. apetala* extracts was compared with the standard ascorbic acid. The reducing power increases with an increasing concentration. The reducing power of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *M. apetala* aerial part was shown in Figure -5. At 800µg/mL concentration of ethanol and methanol extracts of *M. apetala* aerial part showed higher reducing power than the ascorbic acid.

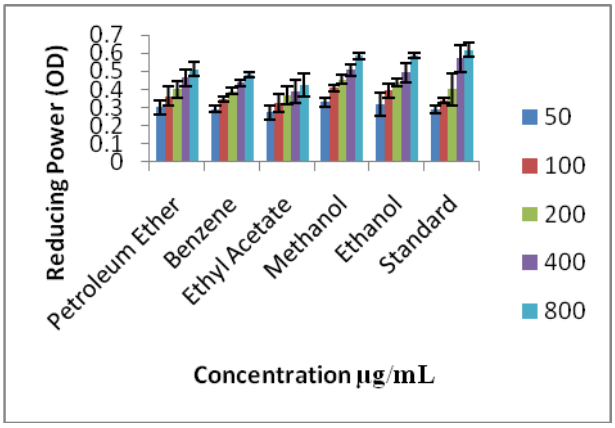

Fig 5: Reducing power ability of different extracts of *M.apetla* aerial part.

IC_{50} values

IC_{50} values of petroleum ether extracts of *M. apetala* aerial part and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 21.65µg/mL and 20.11 µg/mL; 22.18 µg/mL and 19.46 µg/mL; 23.97µg/mL and 22.18µg/mL; 21.97µg/mL and 20.67µg/mL respectively.

IC_{50} values of benzene extracts of *M. apetala* aerial part and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 18.23µg/mL and 20.11µg/mL; 20.54 µg/mL and 19.46µg/mL; 22.04 µg/mL and 22.18µg/mL; 20.83µg/mL and 20.67µg/mL respectively.

IC_{50} values of ethyl acetate extracts of *M. apetala* aerial part and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 19.18µg/mL and 20.11µg/mL; 22.51µg/mL and 19.46µg/mL; 21.64µg/mL and 22.18µg/mL; 20.11µg/mL and 20.67µg/mL respectively.

IC_{50} values of methanol extracts of *M. apetala* aerial part and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were

found to be 22.67µg/mL and 20.11µg/mL; 25.28µg/mL and 19.46 µg/mL; 24.11µg/mL and 22.18µg/mL; 23.65µg/mL and 20.67µg/mL respectively.

IC_{50} values of ethanol extracts of *M. apetala* aerial part and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 20.93µg/mL and 20.11µg/mL; 26.11µg/mL and 19.46µg/mL; 25.63 µg/mL and 22.18µg/mL; 24.59µg/mL and 20.67µg/mL respectively (Table-1).

Table 1: IC_{50} values of different solvent extracts of *M. apetala**

	IC_{50} (µg/mL)			
Solvent	**DPPH**	**Hydroxyl**	**Superoxide**	**ABTS**
Petroleum ether	21.65	22.18	23.97	21.97
Benzene	18.23	20.54	22.04	20.83
Ethyl acetate	19.18	22.51	21.64	20.11
Methanol	22.67	25.28	24.11	23.65
Ethanol	20.93	26.11	25.63	24.59
Ascorbic acid	20.11	19.46	22.18	-
Trolox	-	-	-	20.67

***All the values are mean by triplicate determines**

DISCUSSION

Phenolics are ever-present secondary metabolites in plants and possess a wide range of therapeutic uses. The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups. Total phenolic assay by using Folin-Ciocalteu reagent is a simple, convenient and reproducible method. It is employed usually in studying phenolic antioxidants [16]. Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as antiinflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral anticancer activities. They also inhibit enzymes such as aldose reducatse and xanthine oxidase. They are able of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups and are potent antioxidants [17]. The presence of high phenolic and flavonoid contents in the extracts has contributed directly to the antioxidant activity by neutralizing the free radicals [18].

The free radicals are chemical species which contains one or more unpaired electrons. They are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems.

In vitro antioxidant activity of the petroleum ether, benzene, ethyl acetate, ethanol and methanol extract of *M. apetala* were investigated in the present study by DPPH, hydroxyl, superoxide radical scavenging and ABTS radical cation scavenging activity. It is probably due to the presence of respective phytochemicals like flavonoids, phenolics etc in these species [19, 20]. The main function of the antioxidants is to neutralize the free radicals, which routinely produced in the biological system.

Free radicals are known to play a vital role in a wide variety of pathological manifestations. Antioxidant fights with free radicals and protect us from various disease. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanism.

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts [21]. DPPH is a stable, nitrogen-centered free radical which produces violet colour in ethanol solution. It was reduced to a diphenylpicryl hydrazine, with the adding of the fractions in a concentration-dependent manner. The lessening in the number of DPPH molecules can be associated with the number of available hydroxyl groups. All the fractions showed significantly higher inhibition percentage (stronger hydrogen – donating ability) and positively correlated with total phenolic content. In the present study, the extracts had significant scavenging effect on the DPPH radical which was increasing with the increase in the concentration of the sample from 50-800µg/mL.

The present study shows the abilities of the extracts to inhibit hydroxyl radical deoxyribose degradation in a concentration dependent manner. The extract had significant scavenging effects on the hydroxyl radical, which was increasing with the increase in concentration from 50-800 µg/mL. Aerial part of *M. apetala* possessed higher hydroxyl radical scavenging activity than that of the standard ascorbic acid. The radical scavenging capacity may be attributed to phenolic compounds in plant extracts with the ability to accept electrons, which can combine with free radical competitively to decrease hydroxyl radical [22].

Superoxide anion plays an important role in the formation of more reactive species such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA [23].Therefore, studying the scavenging activity of plant extracts on superoxide radical is one of the most important ways of clarifying the mechanism of antioxidant activity. In the present study, ethanol extract of *M. apetala* showed 112.66% superoxide inhibition at the concentration of 800µg/mL followed by the methanol extract (109.16%). All the tested extracts exhibited higher ability in scavenging superoxide radical, when compared to the standard ascorbic acid (93.51% at 800µg/mL). The results revealed that the *M. apetala* aerial part extracts have superoxide radical scavenging activity which can be of significant interest in health point of view in reducing the level of superoxide radical which is elevated during oxidative stress in the body.

ABTS is used as a substrate with hydrogen peroxide for a peroxidase enzyme. The formal reduction potentials for ABTS are high enough for it to act as an electron donor for the reduction of molecular oxygen and hydrogen peroxide. It is thus useful for testing food extracts and most food extracts are highly-coloured. It is also viable for both aqueous and lipophilic systems. The ABTS radical cation is reactive towards most antioxidants including phenolics, thiols and Vitamin C. The concentration of the antioxidant in the sample is inversely proportional to the absorbance of the radical cation produced by 2,2'-azino-bis-(3-ethylbenzothiozoline-6-sul-fonate) (ABTS). Trolox standard is used as standard in this indicator of potential antioxidant activity. Although, different mechanism was proposed for the antioxidant activity such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [24]. In the present study, ethanol extract is highly potent in neutralizing ABTS cation radicals (101.22% at 800µg/mL). Phenolics of this plant extracts are probably involved in the ABTS radical scavenging activity. This study indicated that the extracts have the hydrogen donating ability and could serve as free radical scavengers by activity as primary antioxidants.

In reducing power assay, the presence of antioxidants in the sample would result in reducing Fe^{3+} to Fe^{2+} by donating an electron by the extract. The extract with reducing power reveal that they are electron donors, reduce the oxidized intermediates and act as primary antioxidant substances [25]. In the present study, the reducing power of ethanol extract of aerial part of *M. apetala* was very potent and the reducing power of the extract was increased with increasing concentration.

CONCLUSION

The findings of this study support the view, that all extracts particularly ethanol extract shows promising sources of potential antioxidant and may be efficient as preventive agents in some diseases and can be considered as a natural herbal source in pharmaceutical industry. Further detailed studies on isolation phytoconstituents of the plant extract are essential to characterize them as biological antioxidants.

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