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A Less known folklore medicinal plant *Maytenus emarginata* of "Nallamala forest in Southern INDIA" is evaluated for its Free Radical Scavenging and α-glucosidase inhibitory activities

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Abstract
Background: Medicinal plants are source of a wide variety of natural antioxidants. The dietary antioxidant consumption may be a worthy approach for inhibiting or delaying the oxidation of susceptible cellular substrates and thus is relevant to disease objection in many paradigms. *Maytenus emarginata* is a locally available plant and it is less known folk medicinal plant. This plant used by the tribes of Nallamala forest of Kurnool district for the inflammation, ulcers, and even for the treatment of gastritis and tonsils.
Purpose of this study: In our present study we demonstrated the reducing power, total antioxidant potential, radical scavenging capabilities and α-glycosidase inhibitory property of different crude extracts of Maytenus emarginata leaf.
Methodology: In this study The leaf powder was subjected to sequential extraction in a soxhlet apparatus using different solvents such as Hexane, Ethyl acetate, Ethanol and Water. The capability of deactivation of free radicals scavenging activities were evaluated with *in vitro* bioanalytical methods like •OH, DPPH•, SO2- NO, and then α-glucosidase inhibitory property is also evaluated.
Results: The extracts of *Maytenus emarginata* scavenged radicals effectively in varied degree. Similarly, the total reducing power of alcohol extract was found higher in both phospho molybdenum and FRAP methods. *In vitro* assay of α-glucosidase activity of MEA and MEW showed an IC50 of 173.47 and 286.66 µg/ml respectively, while other two extracts did not show any significant effect. In other hand, total phenolic and total flavonoid contents of extracts were studied, where values of Aqueous extract were found to be higher than that of other extracts.
Statistical Analysis: Graph pad prism 5software was used for statistical analysis and to prepare the graphical representation of results.
Conclusion: we found that Ethanol and Aqueous extracts of *Maytenus emarginata* contain significantly effective antioxidant and radical scavenging activities as compared to other extracts. Addition to this *Maytenus emarginata* has shown a remarkable α-glycosidase inhibition also.
Potential implication: Our experimental study provides an evidence for the ethno-botanical claims and reported ethno pharmacological activities that *Maytenus emarginata* leaves have a significant therapeutic potential.

Keywords: *Maytenus emarginata*, Radica Scavenging, South India, α-Glucosidase, Folk medicine.

Introduction

Background

Reactive oxygen species (ROS) which includes singlet oxygen (1O2), hydrogen peroxide (H2O2), superoxide anion (O2−) and hydroxyl (OH) radical are often generated as byproducts of many biological and metabolic reactions or may be from exogenous. Thus These ROS creates homeostatic imbalance which generates an oxidative stress cause cell death and in turn leads to tissue injury [1]. Free transition metal ions and ROS are well known inducers of cellular and pathological processes including extensive oxidative damage to cellular biomolecules, cell proliferation, inflammatory conditions and many neu-rodegenerative disorders [2]. Hence, there is great interest in the use of naturally occurring antioxidants for treatment or prophylaxis of various oxidative stress-related diseases [3]. So the Dietary antioxidant intake may be an important approach for inhibiting or delaying the oxidation of susceptible cellular substrates and is thus relevant to disease prevention in many models. Phenolic compounds such as flavonoids, phenolic acids, diterpenes etc. have received attention for their high antioxidative activity [4]. Therefore the *in vitro* bioassay systems have been largely used to monitoring the biological activities of medicinal plant extracts used in an

ethno and traditional medicines. Thus the exploration of natural antioxidants and their preparations of plant to attain this purposeful objective has been gained a great importance over the years *Maytenus emarginata* (Willd.) belongs to the family Celastraceae, popularly known as "kankero", locally it is known as "danthi" [5]. It has great therapeutic value like the root is used in gastro-intestinal troubles, especially to cure dysentery. Pulverized leaves are given in milk to children as a vermifuge. A decoction of the leafy twigs is used as a mouth wash to relieve toothache. The extract of plant shows cytotoxic effect on some cancers. The plant is reported to possess Antispasmodic properties. The bark is ground to a paste and applied with mustard oil to kill lice in the hair [6].

In this present study, we have evaluated the antioxidant efficiency of different extracts of *Maytenus emarginata* using series of *in vitro* biological assays. In addition to this, we have also demonstrated its α-glycosidase enzyme inhibitory activity.

Methodology

Materials

All solvents used in this study were of analytical grade. Methanol, ethyl acetate, hexane, and Folin-Ciocalteau reagent obtained from Sd-Fine Chemicals, Maharashtra, India while other chemicals were procured from Sigma chemicals, India.

Plant Material

ME leaves (*M. emarginata* L.) were obtained from the Nallamala forest of Kurnool district (Figure1), India in the month of September, 2014. The identity of the plant was confirmed by Dr.G.Meera Bai, Head, Dept. of Botany, Rayalaseema University, Kurnool, AP, India. The voucher specimen of the plant was kept for future reference (Figure 2). Two hundred gms of leaves were dried at ambient temperature for 10–15 days. After drying the leaves were grinded to a coarse powder using an electric grinder.

Preparation of Extracts

The coarse powder was subjected to successive extraction in a soxhlet apparatus using different solvents such as Hexane, Ethyl acetate, Ethanol and Water. Each time before extracting with the next solvent, the plant material was dried in a hot air oven. Furthermore, extracts were filtered through Whatman No.1filter paper and concentrated to the dry mass with the aid of rotary evaporator. The yield of each extract was measured and residues were stored in dark for further assay. Different extracts were designated as MEH (for Hexane extract), MEE (for Ethyl acetate extract), MEA (for Ethanol extract) and MEW (for Water extract). Dried extracts of 20 mg/ml stock solution were prepared and different concentrations were used in various experiments. The extract sample is further tested for its IR and HPLC analysis for the separation and identification of the compounds. The FT-IR spectroscopy [7] and HPLC was done for the aqueous extracts of *Maytenus emarginata* in dried form [Figure 8, 9].

Determination of Phytochemical Constituents Total Phenolic Content

Total phenolic content was determined as per the method of Folin–Ciocalteu [8] with slight modifications, In brief, 50 µl of the extracts were taken to which 500 µl of double distilled water was added, followed by 100 µl of Folin Ciocalteu's reagent. After incubating the mixture for 10 min at room temperature, 300 µl of 20% Na2CO3 was added, thoroughly vortexed and the volume of the reaction mixture was adjusted to one ml with double distilled water. The mixture was then incubated for 2 hrs in dark and the absorbance was measured at 730 nm against blank. The total phenolics content was expressed as gallic acid equivalents (GAE) in mg per gram of dry sample.

Total Flavanoid Content

Total flavonoid content was quantified by following the method of Barreira [9] with little modifications.10 µl of each extract were mixed with 500 µl double distilled water and then the 30 µl of 5% sodium nitrite NaNO2 solution. After few min of incubation at room temperature, 60 µl of 10% Aluminum Chloride AlCl3 solution was added. Subsequently, 350 µl of 1 M NaOH and 50 µl of double distilled water were added to make the final volume to 1ml. Samples were further incubated for 10 min at room temperature and the absorbance of samples was measured at 510 nm. The total flavonoids were determined as quercetin equivalents (mg QE)/g of dry weight and the values were expressed as means of triplicate analysis.

Determination of Total Antioxidant Activity

The Total antioxidant activity of all our extracts were evaluated by phospho molybdenum method [10]. The assay is based on the reduction of Mo+6–Mo+5 by the antioxidant com- pounds and subsequent formation of a green phosphate/ Mo+5 complex at acidic pH. The reagent solution contains ammonium molybdate (4mM), disodium hydrogen phosphate (28 mM) and sulfuric acid (0.6 M) mixed with the extracts. Samples were incubated for 60 min at 90°C and the absorbance of the green phosphomolybdenum complex was recorded at 695 nm. Ascorbic acid was used as reference and reducing capacity of the extracts was expressed as the mg ascorbic acid equivalents per gram dry weight.

Determination of Reducing Antioxidant Power (FRAP)

The reducing antioxidant ability of the ME extracts were determined according to the method described by Oyaizu [11] with minor modifications. Briefly, 20 µl of each extract was taken and the volumes were made to 200 µl with double distilled water. Further, 300 µl of potassium ferricyanide (1%) was added to the tubes and incubated for 20 min at 50°C. Then 250 µl of trichloroacetic acid (10%) was added to the incubated mixture. Upper part of the mixtures (500 µl) were taken and mixed with 400 µl of double distilled water and 100 µl of ferric chloride (0.1%). The absorbance of mixture was measured at 700 nm and reducing power of extracts was expressed as mg ascorbic acid equivalents (AAE) per gram (g) of dry weight.

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) Radical Scavenging Activity

The DPPH radical scavenging activity of the plant ex-tracts were determined by the method described by Braca [12]. The activity was assessed using stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH). DPPH solution (0.004% w/v) was prepared in 95% methanol, mixed with different dilutions of

plant extracts and absolutely vortexed. The reaction mixture was then incubated in dark at room temperature for 30 min and absorbance was measured at 517 nm against the blank. Methanol (95%) and ascorbic acid were used as blank and reference compound respectively.

Nitric Oxide Radical Scavenging Activity

The activity was measured according to the method of Sreejayan and Rao [13]. To the 100 µl of the extract possessing different concentrations (50–500 µg/ml), 20 µl of sodium nitroprusside solution (10 mM) was added and then incubated for 20 min. After incubation, the mixture was diluted with 300 µl of Griess reagent (1% sulfanilamide in 2% H3PO4). The reaction mixture was again incubated for 40 min at 30°C followed by addition of 10 µl of 0.1% naphthyl ethylene diamine dihydrochloride in 2.5% H3PO4. The final volume was made up to 1 ml with double distilled water. The chromophore absorbance was taken immediately at 545 nm and compared to the ascorbic acid as standard.

Superoxide Radical Scavenging Activity

Super oxide Radical Scavenging activity was evaluated by using nitro blue tetrazolium (NBT) reduction method [15]. The reaction mixture consists of 0.5 ml of NBT solution (156 µM, 0.5 ml nicotinamide adenine dinucleotide (468 µM, NADH), and extracts of different concentrations (50–500 µg/ml). The reaction was induced by adding 50 µl of phenazine methosulfate solution (60 µM, PMS) in phosphate buffer (pH 7.4). Then it was incubated at 25°C for 20 min and then the absorbance was measured at 560 nm. Ascorbic acid was used as the standard reference.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was calculated according to the method of Fenton's reaction [14]. Hydroxyl radicals were generated by mixing ferrous ion (Fe2+) with hydrogen peroxide and 1, 10-phenanthroline. Phenanthroline-Fe2+ is an indicator of redox reaction. The H2O2/Fe2+ system generate hydroxyl radicals through Fenton reaction and Phenanthroline-Fe2+ complex oxidises to Fe3+. Reaction mixture contained 60 µl of 1.0 mM FeCl2, 90 µl of 1 mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µl of 0.17 M H2O2 and 1.5 ml of extract at various concentrations. Adding H2O2 started the reaction.

After incubation at room temperature for 10 min, the absorbance of the mixture at 560 nm was measured with a double beam spectrophotometer. The radicals scavenging activity calculated as per the following formulae.

$$\% \textit{Inhibition} = [(A0 - A1)/ A0 \times 100]$$

Where A0 was the absorbance of the control/blank (without extract) and A1 was the absorbance of the extract or standard.

Evaluation of α-glucosidase Activity

The α-glucosidase enzyme inhibition activity was evaluated as per the method of Matsui [16] with the little modifications Briefly, the enzyme reaction was performed by using PNP-glycoside as a substrate in 0.1 M phosphate buffer (pH 6.8). PNP-glycoside (10 mM) and 10 µl of GSH (3 mM) was pre-mixed with samples at different concentrations. Each mixture was added to an enzyme solution (0.01 units) to make 1 ml of final volume. The reaction was terminated by adding 10 µl of 100 mM Na2CO3 solution. Enzymatic activity was quantified by measuring the Para Nitrophenol released from PNP-glycoside at 400 nm. All reactions are carried out at 36°C for 30 min with 3 replications. The IC50 values are calculated by the graphic method.

Results and Discussion:

Determination of Phytochemical Constituents

In this, the results of total phenol content of different leaf extracts of *M. emargingata* were effective and has found in the range of 2.23 to 28.26 mg GAE/g dw (Table 1). Out of the tested extract, the higher one of total phenolics was observed in MEW (2.23 mg GAE/g dw), where as MEH has shown the lowest (28.26 mg GAE/g dw). The flavonoid contents of our extracts in terms of quercetin equivalent were in the range of 8.48 to 38.46 mg QE/g dw (Table 1), highest being in MEW (38.46 mg QE/g dw) and lowest in MEH (8.48 mg QE/g dw). It is always interesting to know that both the polyphenol and flavonoid contents of MEW are higher than other extracts. This is may be due to the higher solubility of the polyphenol and flavonoid type of constituents in the aqueous solvent than that of other medium. As per our present study, the high contents of these phytochemicals in *M. emargingata* can explain its high radical scavenging capacity.

Determination of Total Antioxidant Activity

In this study, the antioxidant activity was in the range of 5.65 to 24.54 mg AAE/g dw in the leaf extracts. Here, MEW has shown the higher value of 24.54 mg AAE/g dw, as same was in the case of both total phenols and flavonoids, where as the lower value is of 5.65 mg AAE/g dw was found in MEH. Hence in this study, total poly phenol, flavonoid content and antioxidant activity of our plant leaf extracts are correlated significantly and would also contributing to the whole antioxidant potential.

Determination of Reducing Antioxidant Power (FRAP):

The reducing property to convert Fe3+ to Fe2+ is also an indirect evidence for the antioxidant activity of an extract or a compound [18]. In our present assay system, the anti oxidants present in the extract can causes the reduction of the Fe3+/ ferri cyanide comples to form Fe2+ ions, which is can be monitored spectrophotometrically by recording the absorbance of the reaction at 700nm. All the tested extracts has shown the degree of electron donation capacity (Table 1). The reducing power of ethanol extract MEA was the higher amongst all the other extracts with 16.77±0.82 mg AAE/gm dw, where as the others were much lower with a versatile range from 2.68, 2.44, 8.96 mg AAE/gm dw. So the presented data is an indicative for the marked reducing power of *M. emarginata.*

Table 1: Quantitative Evaluation of Phytochemicals and Antioxidant activities of various extracts of *Maytenus emarginata*

Extract	Total polyphenol content a	Total flavonoid content b	Total antioxidant activity c	Ferric reducing power c
MEH	2.23±0.10	8.48±0.25	5.65±0.60	2.68±0.85[a]
MEE	12.29±0.20	13.97±0.34	20.61±0.83	8.96±0.84
MEA	21.03±0.34	21.68±0.36	22.57±0.85	16.77±0.82
MEW	28.26±0.39	38.46±1.006	24.54±0.89	2.44±0.51[a]

Results represented in means ± standard deviation (n = 3)

DPPH Radical Scavenging Activity

Compounds that are able to donate hydrogen or an electron to DPPH, nitrogen centered free radical are considered as antioxidants and therefore, radical scavengers. The degree of discoloration of violet color of DPPH, as it gets reduced, it indicates that the radical scavenging potential of an antioxidant [19]. In our present analytical study, all extract has shown the effective dose dependant DPPH radical scavenging activity (Figure 3). The IC50 values are ranged from 21.52±0.37 to 30.75±0.52. Among this MEE was the most efficient, with lowest IC50 value of 21.52. Hence our experimental studies suggest that the Plant extracts could contain more bioactive compounds that may be attributes the antioxidant properties of *M. emarginata.*

Nitric Oxide Radical Scavenging Activity

Abnormal level of NO has been linked with the chronic inflammation and may be associated with the etiology and pathophysiology of number of chronic diseases [20]. In our present study, we have tested M. emarginata extracts for their inhibitory effect on nitric oxide production and nitric oxide radicals. The extracts at varied concentrations (50-500 µg/ml) showed significant inhibitory effect of NO scavenging activity. The percentage scavenging activity increased with the increasing concentration. Among all the extracts, the lower IC50 value was observed for MEE 162.03±0.29 and the highest value is for MEA 267.13±0.48 (Table 2; Figure 4).

Superoxide Radical Scavenging Activity

Superoxide anion (O2) is one of the much important representative of free radicals. It acts as a initiator of more reactive oxidative species such as single O2, H+ radicals that have the potential of reacting with biological macromolecules and thereby inducing tissue damage,
and palys a vital and key role in peroxidation of lipids [21-23]. It was observed that superoxide scavenging activities of different extracts of *M. emarginata* were increased remarkably with increasing concentrations (Figure 5). The IC50 values of extracts were found to be in an order of MEW > MEA > MEE > MEH (Table 2). Hence These results imply that the aqueous extract is best superoxide scavenger and its ability to scavenge super oxide may contribute to its antioxidant activity.

Table 2: The Half maximal inhibitory concentration (IC_{50}) values procured in the Antioxidant activity assays

Extract	DPPH	Nitric oxide	Superoxide	Hydroxyl radical	α-glucosidase inhibition
MEH	30.75±0.52	188.04±0.34	89.94±0.35	380.91±0.37	NA
MEE	21.52±0.37	162.03±0.29	128.95±0.34	534.71±0.35	NA
MEA	27.91±0.33	267.13±0.48	140.63±0.39	176.48±0.35	286.66±0.35
MEW	27.35±0.30	162.91±0.38	202.65±0.33	81.96±0.38	173.47±0.35

Values expressed in µg/ml; Results represented in means ± standard deviation (n = 3); NA: No activity

Hydroxyl Radical Scavenging Activity

Hydroxyl radicals are the one of the major reactive oxygen species and that are responsible even for oxidation of macro biomolecules and massive biological damage [24, 25]. The evaluation of radical scavenging activity was based on the generation of OH- by means of Fenton reaction. The percentage of inhibition against the hydroxyl radical of different extracts of M. emarginata was shown in Figure 6. In our experimental study we found that MEW has shown the effective scavenging activity of hydroxyl radicals with least IC50 value (81.96±0.38 ug/ml), while other extracts were found to be less efficient scavengers. By observing the IC50 values of all extract, we can be able to say that the aqueous extract (MEW) was more efficient hydroxyl radical scavengers than its other counterparts

Determination of α-glucosidase Activity

As we all know that the natural products are still the most readily available source of α- glucosidase inhibitors [26]. To strengthen this, the recent reports from other traditional plants, poly phenols were observed to contribute to strong α-glucosidase inhibition [27]. Therefore we are enthusiastic to investigate the activity of our different extracts. It was found that among the four extracts tested for α-glucosidase inhibition activity, only MEA MEW has shown a significant inhibition property (Figure 7), where as the other extracts (MEH and MEE) did not inhibit α-glucosidase at all. The percentage inhibition of α-glucosidase by MEA and MEW exhibited significant inhibitory activity at dose-dependent acceleration suggesting a competitive type of inhibition. In this, MEW (IC50=173.47µg/ml) exerted the most significant inhibitory activity, where as The IC50 value for MEA was found to be 286.66µg/ml. By taking the consideration our results presented here, we can say that *M. emarginata* exert inhibitory effect on α-glucosidases, with MEW being the most effective.
With these results, we can further assure and support that the traditional use of the medicinal plants for its broad therapeutic applications.
The extracts of *Maytenus emarginata* scavenged radicals effectively in varied degree. Similarly, the total reducing power of alcohol extract was found higher in both phospho molybdenum and FRAP methods. *In vitro* assay of α-glucosidase activity of MEA and MEW showed an IC50 of 173.47 and 286.66 µg/ml respectively, while other two

extracts did not show any significant effect. In other hand, total phenolic and total flavonoid contents of extracts were studied, where values of Aqueous extract were found to be higher than that of other extracts.

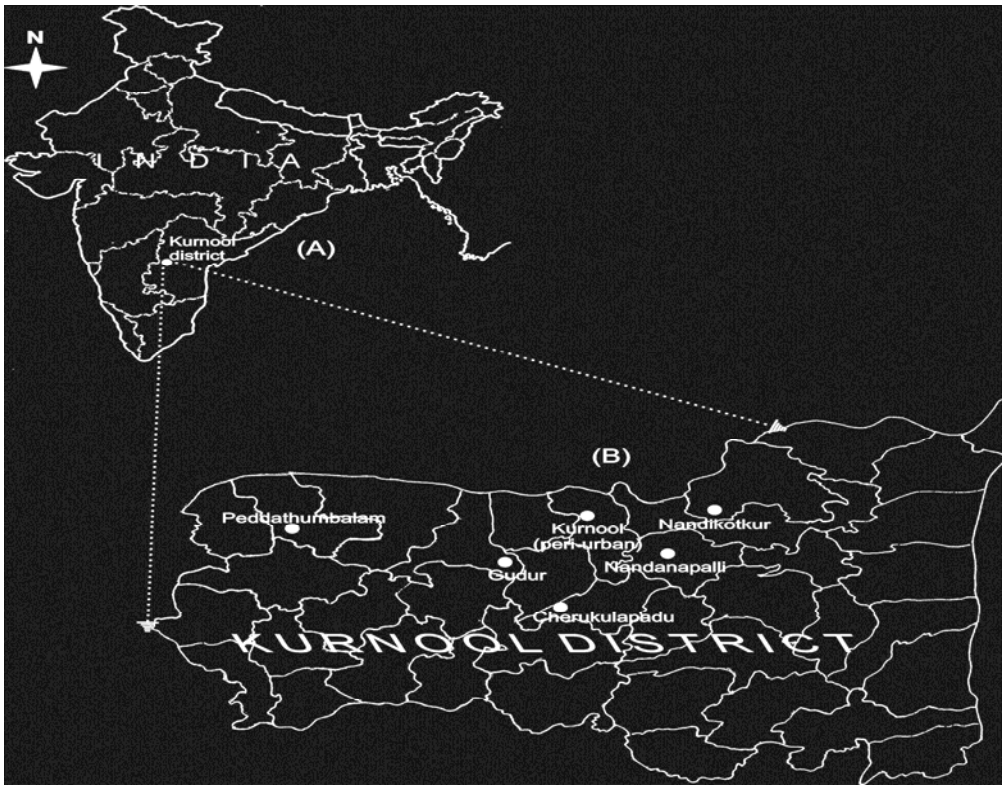

Fig 1: Map of India showing location of the Kurnool district, AP, India [17]. (B) Map of Kurnool district Showing the location and availability of the plant *Maytenus emerginata*

Fig 2: The leaves of *Maytenus emerginata*

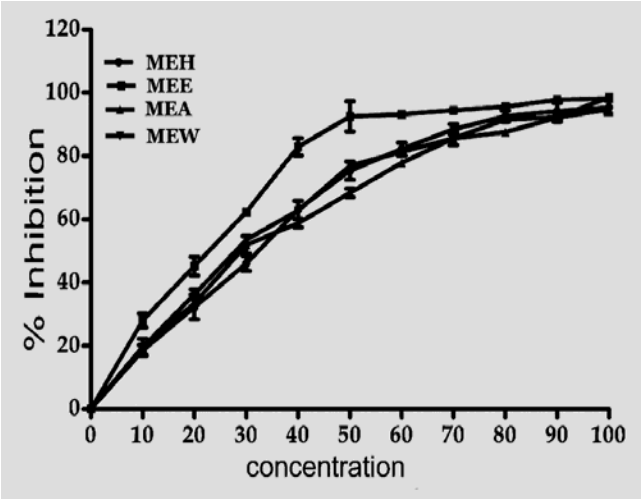

Fig 3: DPPH scavenging activity of different *M. emerginata* leaf extracts

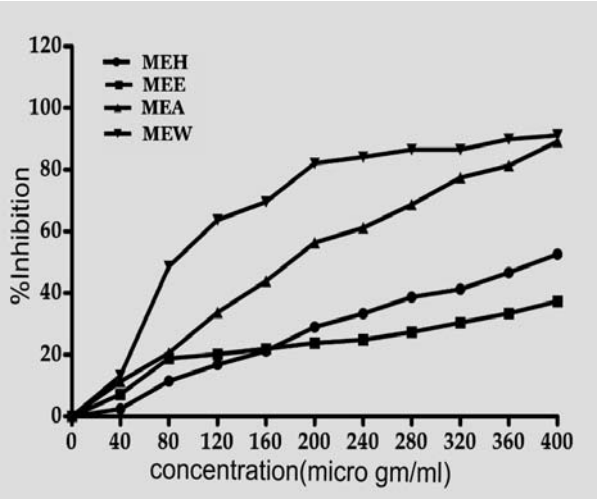

Fig 5: Superoxide scavenging activity of different *M. emerginata* leaf extracts

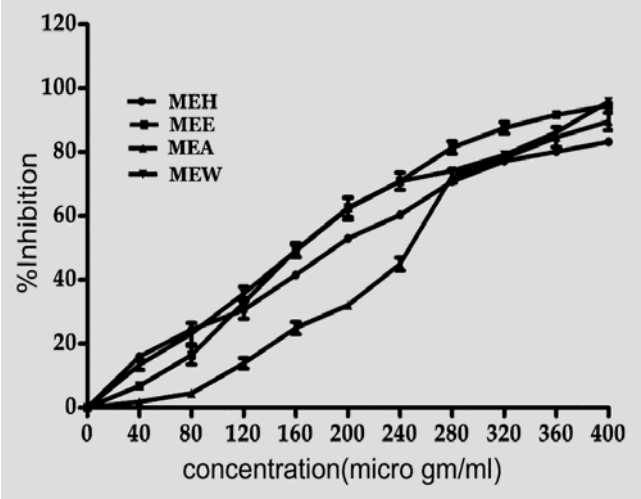

Fig 4: Nitric oxide scavenging activity of different *M. emerginata* leaf extracts

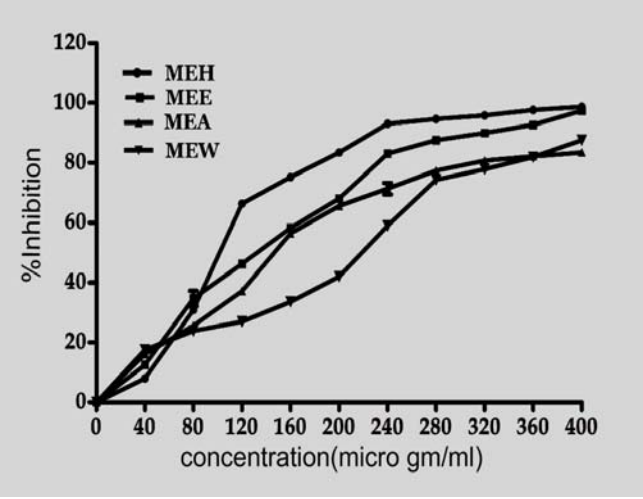

Fig 6: Hydroxyl radical scavenging activity of different *M. emerginata* leaf extracts

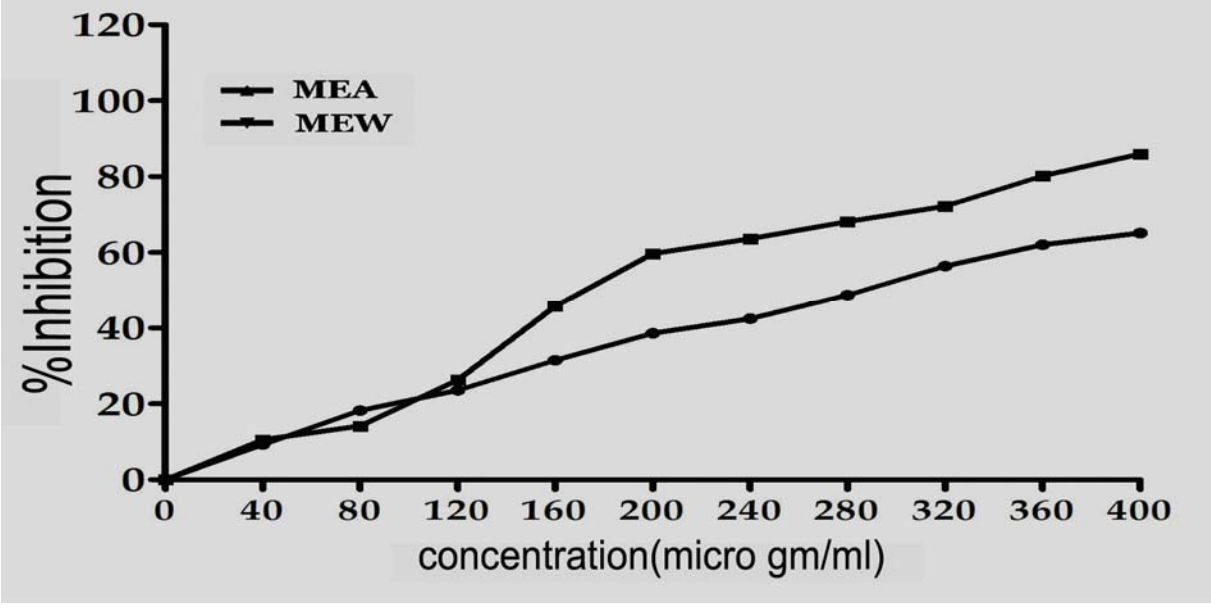

Fig 7: α-glycosidase inhibitory activity of different *M. emerginata* leaf extracts

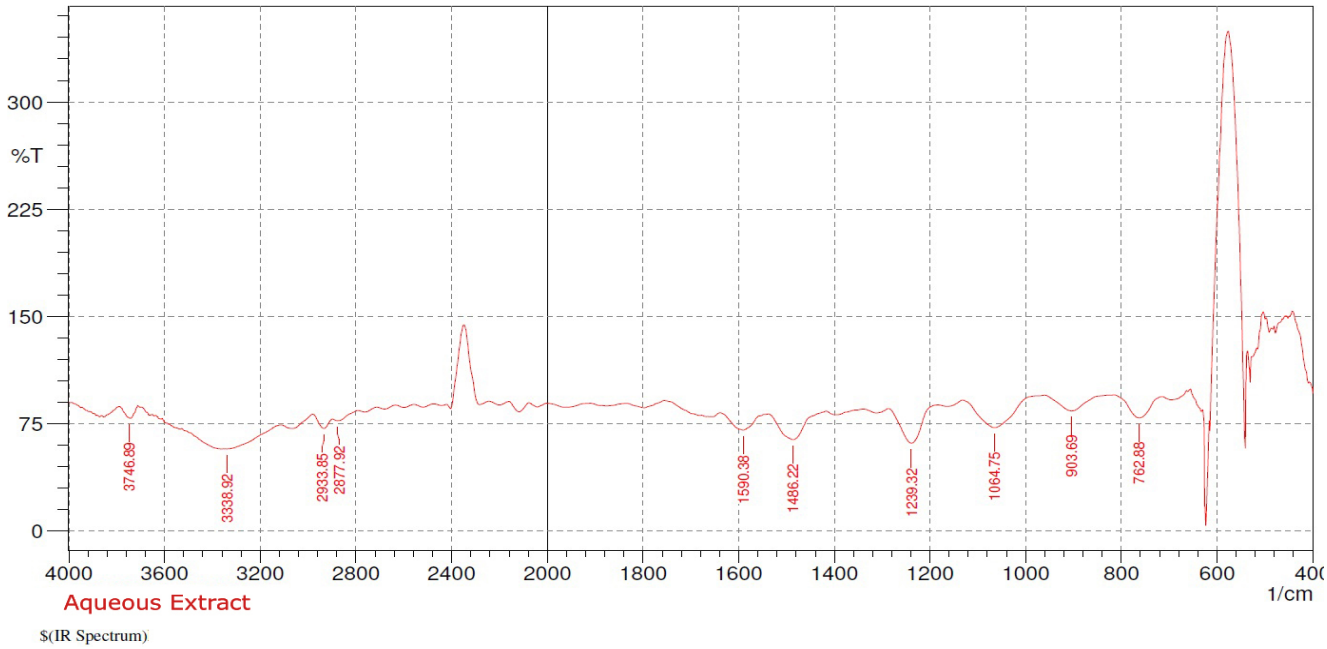

Fig 8: IR spectra of Aqueous extract of *M. emerginata* leaf

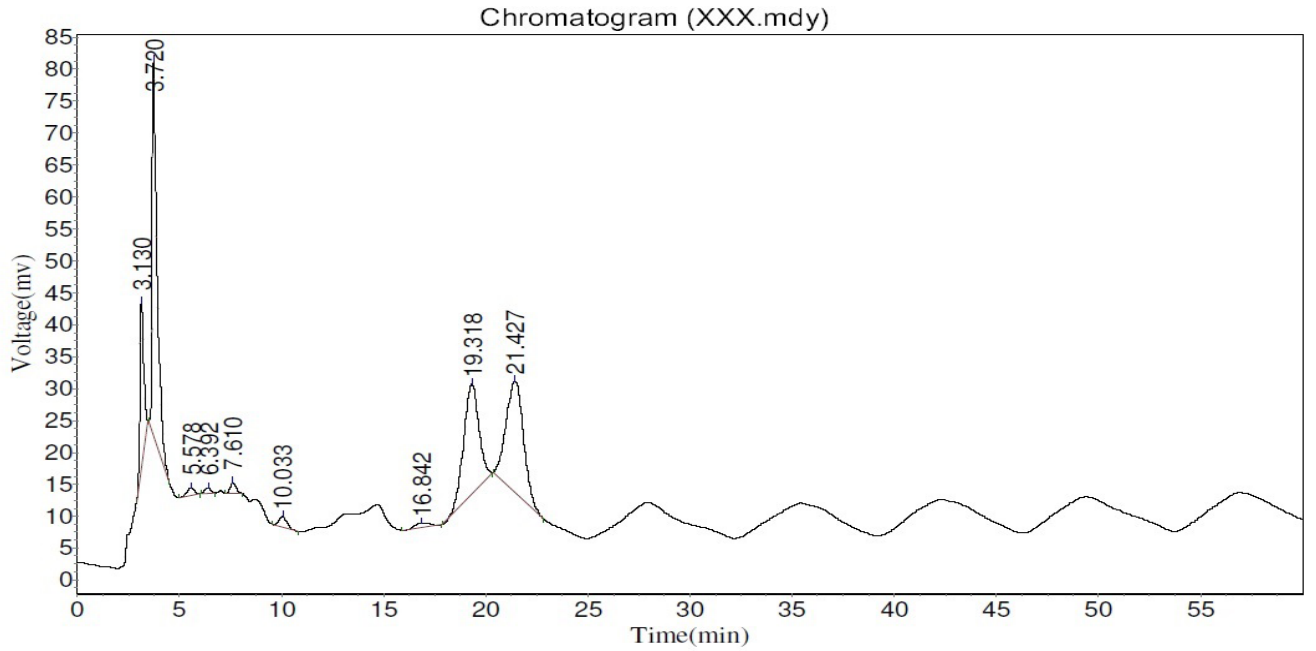

Fig 9: HPLC Chromatogram of Aqueous extract of *M. emerginata* leaf

Statistical Analysis

The % of inhibitions of radicals, peroxidation of lipids and α-glucosidase inhibitory activities of an extracts were calculated by using the following formula:

Percentage inhibition = (Acontrol − Asample)/ Acontrol × 100.

All the full length experiments were performed in triplicates and experimental results were expressed as mean ± standard deviation of mean (SEM) of three replicates. IC50 value (the concentration of the extracts required to scavenge 50% of radicals) was calculated for different extracts of *M. emarginata* Graph pad prism 5software was used for statistical analysis and to prepare the graphical representation of results.

Conclusion

In the conclusion, the results of this present study fairly indicate that the *M. emarginata* has shown powerful and in effable radical scavenging activity against various oxidative provided systems particularly *in vitro*. Different antioxidant properties of this potent plant may be reliable to its components effectiveness as scavengers of free radicals, reductive capacity, as well as metal chelating capacity. The free radical-scavenging property may be one of the mechanisms by which this plant is attributed as useful for traditional medicine. Hence, our results support that the *M. emarginata* as an available and accessible source of natural antioxidants.

Competing Interests
"The authors declare that they have no competing interests."

Authors' contributions
DB framed the study and protocol, carried out all the experimentation, analyzes the data and drafting of the manuscript. NBS provided technical support and involved in the drafting and revision of the manuscript. Both the authors read carefully and approved the final manuscript.

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