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ANTIOXIDANT ACTIVITY OF *MAJIDEA ZANQUEBARICA J.KRIKEX OLIV* (SAPINDACEAE)

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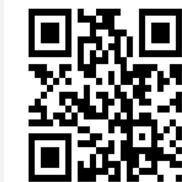

ABSTRACT

Objective: To evaluate the antioxidant capability of the hexane bark extracts of *M. zanquebarica.* **Methods**: In vitro antioxidant activity was evaluated by studying the contents of alkaloids, flavonoids, tannins, Total phenolics, free radical scavenging activity using DPPH, reducing power activity, Superoxide anion radical scavenging assay, Total antioxidant activity. **Results**: Hexane bark extract of *M. zanquebarica have* marked high content of secondary metabolites and also exhibited high DPPH radical scavenging capacity using ascorbic acid as positive control. *M. zanquebarica at* the same concentration showed the dose dependent inhibition of reducing power activity, Superoxide anion radical scavenging assay and Total antioxidant activity. **Conclusion**: The results indicate that the hexane bark extract of *M. zanquebarica has* the potential to scavenge free radicals and act as a good antioxidant for treating various diseases.

INTRODUCTION

There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine. Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health. The main objective of this study is, to search for Indian plants with strong antioxidant activity which could serve as good candidates for the development of standardized phytomedicine. Antioxidant supplements or foods rich in medicinal plants may be used to help the human body in reducing the oxidative damage by free radicals and active oxygen[1]. Since the imbalance between antioxidants and free radicals leads to oxidative stress which may result in tissue injury and subsequent diseases such as atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus, hypertension etc., the development and utilization of more effective antioxidants of natural origin are desired[2]. Phyto medicines are important component derived from various parts of the plants with various applications in pharmaceutical and herbal industry. Development of herbal remedies is more popular now a day due to less side effects and easy availabilities of medicinal plants.[3,4] Presences of bioactive secondary metabolites in the medicinal plants are more

responsible to cure several diseases in mankind. The family Sapindaceae has a widespread distribution with 136 genera and 2000 species[5]. Ethnomedical information revealed that extracts from members of this family are commonly used for the treatment of boils, ulcers, pain, dematological troubles, wounding healing, diarrhea and dysentery[6]. Many species in this family have been reported to possess a number of biological and pharmacological activities[7]. For example, extracts from the leaves, stems and roots of *Paullinia pinnata* showed significant antibacterial and antioxidant activities[8], while the roots and seeds of *Lecaniodiscus cupanoides* were reported to be cytotoxic but possess analgesic and CNS depressant activity[9]. Despite the use of extracts of Sapindaceae species in Indian folk medicine, information on the comparative antioxidant, anticancer and antimicrobial properties of these species are unavailable. This type of information is necessary in the current search for pharmacologically active natural product of plant sources. The choice of this species was based on ethnobotanical information from literature on its uses for the treatment of various ailments such as yaws and ulcers, pain, dermatological troubles and wound healings, disease conditions, in which free radicals have been implicated. Hence an attempt has been made to evaluate hexane bark extract of *M. zanquebarica* for antioxidant activity.

MATERIALS AND METHODS

Direct Extraction with Different Solvents

All the shade dried bark powder of *M. zanquebarica* was alone mixed with hexane separately at 1:10 ratio (w/v) in a clean flask and kept overnight under shaken condition. The process was repeated three times, but using fresh solvent at every extraction. The resultant extracts were filtered through Whatman No.1 filter paper. The solvents were removed by rotary evaporator, under reduced pressure (Buchi, Switzerland) at 40°C to yield thick syrupy extracts and used for further studies.

DETERMINATION OF ALKALOIDS

Determination of alkaloid was done as described[10]. 5 g of plant sample was weighed into a 250 ml beaker and dispersed into 200 ml of 10% acetic acid solution in ethanol. The filtrate was then evaporated to one quarter of its original volume on hot plate. Concentrated ammonium hydroxide was added drop wise in order to precipitate the extract. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

DETERMINATION OF FLAVONOIDS

Total flavonoid content was measured by the aluminum chloride colorimetric assay[11]. An aliquot (1 ml) of extract and standard solution of catechin (100 mg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To this 0.3 ml of 5 % NaNO3 were added. After 5 min, 0.3 ml 10 % AlCl3 was added. Then after 1 min, 2ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DETERMINATION OF TOTAL PHENOLS: Total phenolics were quantified and expressed as gallic acid equivalents[12]. About 3.9 ml of distilled water and 0.5 ml of Folin-ciocalteau reagent were added to 0.1ml of extract in a tube and incubated at room temperature for 3 min after which 2 ml of 20% sodium carbonate was added to this and kept at boiling water bath for 1 min. Phenols react with phosphomolybdic acid in the Folin-Ciocalteau reagent in alkaline medium and produce a blue coloured complex (molybdenum blue) that can be estimated colorimetrically at 650 nm.

DETERMINATION OF SAPONINS

20 g of each sample was dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-

extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin contents were calculated in percentage[13].

DPPH RADICAL SCAVENGING ACTIVITY: One way of estimating antioxidant activity is by the use of the stable free radical DPPH[14]. The DPPH radical scavenging activity was determined according to the method.[15] DPPH offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants[16]. The DPPH assay measures hydrogen atom (or one electron) donating activity and hence provides a measure of free-radical scavenging antioxidant activity. DPPH is a purple-coloured stable free radical and will form yellow color when it was reduced as diphenyl picrylhydrazine complex. Free radical scavenging capacity was evaluated on the basis of the scavenging activity of DPPH by measuring the reduction of absorbance at 517 nm. Various concentrations of extract of the sample (4.0 ml) were mixed with 1.0 ml of methanolic solution containing DPPH being 0.1 mM. The mixture was shaken vigorously and left to stand for 3 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as control. The percentage of DPPH decolorization of the sample was calculated according to the ascorbic acid:

$$\% \text{ activity} = [1-(ABS_{sample}/ABS_{control}] \times 100$$

REDUCING POWER

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity[17]. Reaction mixture contained 2.5 ml of various concentration of extract of the sample, 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 0.2 M sodium phosphate buffer. The control contained all the reagents except the sample. The mixture was incubated at 50ºC for 20 min and were terminated by the addition of 2.5 ml of 10 % (w/v) of trichloroacetic acid, followed by centrifugation at 3000 rpm for 10 min. 5.0 ml of the supernatant upper layer was mixed with 5.0 ml of deionized water and 1.0 ml of 0.1 % ferric chloride. The absorbance was measured at 700 nm against blanks that contained distilled water and phosphate buffer. Increased absorbance indicates reducing power of the sample.

SUPEROXIDE ANION RADICAL SCAVENGING ASSAY

Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress[18]. Numerous biological reactions generate superoxide anions which are highly toxic species. In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen from PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. The assay tubes containing 20 µl of extract, 0.2 ml of EDTA, 0.1 ml of nitro-blue tetrazolium, 0.05 ml of riboflavin and 2.25 ml of phosphate buffer and control tubes were set up without the extract. Similarly the activity of the standard antioxidants was also carried out. The initial optical uniformly with a fluorescent lamp for 30 min at 560 nm was measured again and difference in optical density was taken as the quantum of superoxide production. The percentage inhibition was calculated by comparing with the optical density of the control tubes.

TOTAL ANTIOXIDANT ACTIVITY

The antioxidant activity was determined by ammonium thiocyanate assay[19]. 500 µl of the extract, 200 µl of diluted linoleic acid (25 mg/ml 99

ethanol) and 400 µl of 50 mM phosphate buffer (pH 7.4) was mixed and incubated at 40℃ for 15 min. Aliquot (100 µl) from the reaction mixture was mixed with reaction solution containing 3 ml of 70% ethanol, 100 µL of ammonium thiocyanate (300 mg/ml distilled water) and 100 µl of ferrous chloride (2.45 mg/ml in 3.5% hydrochloric acid). Final reaction solution was mixed and incubated at room temperature for 3 min. Absorbance was measured at 500 nm. Linoleic acid emulsion without extract served as control. Inhibition of linoleic acid oxidation was calculated by using the following formula:

% Inhibition = [(control OD – sample OD)/control OD)] X 100.

RESULTS AND DISCUSSIONS

Quantitative Phytochemical Analysis

Quantitative phytochemical estimation of the phytochemicals have shown that higher amount of phenols (7.2±0.37 mg/g), saponins (22.86 ± 0.3mg/g), flavonoids (7.8±0.43 mg/g), tannins (5.7±0.50 mg/g) and alkaloids (3.62±0.77mg/g) (Table 1) were estimated.

DPPH RADICAL SCAVENGING ACTIVITY

DPPH is a free radical compound that has been widely used to determine the free radical-scavenging ability of various samples[20, 21]. The free radical scavenging activity of the hexane extract of *M. zanquebarica* bark were determined by the DPPH method and the results are shown in Table 2. The DPPH radical has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods. Percentage of DPPH radical scavenging activity of the extracts was dose dependent. The amount of DPPH scavenging activity appeared to depend on the phenolic concentration of the extract. The DPPH radical scavenging activities of the extract was higher when compared to the standard antioxidant ascorbic acid. The highest DPPH scavenging activities were showed by hexane bark extract of *M. zanquebarica* ie. (49.13±0.29 at the concentration of 1000 µg/ml and the IC_{50} was found to be 23.53 µg/ml. In the present study, the order of scavenging activity of sample extracts is hexane > ascorbic acid. This radical scavenging activity of extract could be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability. Our results showed close agreement with antioxidant activity of *Paullinia cupana*[22], *Smilax excelsa*[23]. The results indicated that the extracts with their proton-donating ability, could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants[24].

REDUCING POWER ASSAY: Table 3 depicts the reducing powers of the hexane bark extract of *M. zanquebarica* examined as a function of their concentration. Reducing power is one mechanism of action of antioxidants and may serve as a significant indicator of potential antioxidant activity[25]. Several studies have indicated that the antioxidant effect is related to the development of reductones[26]. Therefore, in this study, reducing activity was determined based on the ability of extracts to reduce a Fe^{3+}/ ferricyanide complex to form a Fe^{3+} ferrous complex. The amount of Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm[27]. In the present study, hexane bark extract of *M. zanquebarica* showed a significantly higher reducing power than the standard ascorbic acid. The data suggest that hexane extract has a good ability

SUPEROXIDE ANION RADICAL SCAVENGING ASSAY: Superoxide is a reactive oxygen species, which can cause damage to the cells and DNA leading to various diseases. It was therefore proposed to measure the comparative interceptive ability of the antioxidant extracts to scavenge the superoxide radical. Several *in vitro* methods are available for generation of superoxide radicals[31]. In the present study, superoxide radicals were generated by auto-oxidation of hydroxylamine in presence of NBT (Nitro Blue Ttrazolium). The reduction of NBT in presence of antioxidants was measured.

Table 1. Quantitative phytochemical estimation

Bioactive compounds	Quantity mg/g
Alkaloids	3.62±0.77mg/g
Phenols	7.2±0.37 mg/g
Flavonoids	7.8±0.43 mg/g
Tannins	5.7±0.50 mg/g
Saponins	22.86 ± 0.3mg/g

Extract	Concentration (µg/ml) and % of inhibition										IC 50 (µg/ml)
	10µg	20µg	30µg	40µg	50µg	60µg	70µg	80µg	90µg	100µg	
Hexane	21.04	25.60	26.60	41.82	47.12	55.59	59.02	66.99	67.72	68.72	53.66 µg
STD	7.59	14.35	26.36	48.54	59.39	66.45	73.18	81.91	92.34	99.87	42.09 µg

Table 2: DPPH radical scavenging activity

Extract	Concentration (µg/ml) and % of inhibition				
	125	150	500	1000	IC_{50} (µg/ml)
Hexane	46.31±0.01	59.11±0.12	71.31±0.20	99.18±0.01	126.88
STD	30.37±0.73	49.98±0.54	70.14±0.73	97.15±0.16	150.06

Table 3: Reducing power activity

Extract	Concentration (µg/ml) and % of inhibition					IC_{50} (µg/ml)
	20	40	60	80	100	
Methanol	14.13±0.03	27.78±0.08	49.98±0.03	76.79±0.05	82.31±0.03	60.02
STD	33.06±0.73	40.68±1.28	52.42±1.58	62.92±1.57	71.45±0.98	57.23

Table 4. Superoxide anion radical scavenging assay

Extract	Concentration (µg/ml) and % of inhibition				
	125	150	500	1000	IC_{50} (µg/ml)
Hexane	27.19±0.51	41.23±0.12	56.77±0.13	88.13±0.59	440.37
STD	21.16±0.63	52.02±0.28	81.16±0.68	99.87±0.24	144.17

Table 5. Total antioxidant capacity

The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture[32]. Different concentrations of bark hexane extract of *M. zanquebarica* (125-1000 µg/ml) were used. bark hexane extract showed strong superoxide scavenging activity (26.31%, 39.21%, 51.31% and 98.18%) (Table 4) when compared to ascorbic acid, the superoxide scavenging activity of the extract was found to be low. This could be due to the presence of reactive concentration of bioactive constituents and mixture of other nutrients in the extract. This result is in accordance with that of *Baccharis grisebachii*[33], *Calendula officinalis*[34], *Plectranthus aromaticus*[35] and *Artemisia nilagirica*[36].

TOTAL ANTIOXIDANT CAPACITY: Total antioxidant activities reflect the capacity of a non-enzymatic, antioxidant defense system. In the phosphomolybdenum method, molybdenum VI (Mo^{6+}) is reduced to form a green phosphate/Mo^{5+} complex at acidic pHs. High absorbance values indicate

that the sample possesses significant antioxidant activity. The total antioxidant capacity was reported as ascorbic acid equivalent. As shown in Table 5, total antioxidant activities of bark hexane extract of *M. zanquebarica* were superior to ascorbic acid and the effects are concentration dependent. The method is utilized for the spectrophotometric quantitation of total antioxidant capacity and employs cost-effective reagents[37]. A similar result was previously observed in plums[38]. Our results are in agreement with previous reports that the phenolic compounds contribute significantly to the antioxidant activity in medicinal plants[39].

CONCLUSION

This research provides information about the antioxidant property of the bark extracts of *M. zanquebarica*. Hence, it is identified that this species can be used as a source for the formulation of drugs of scavenging property. However, large scale *in vivo* studies are required to confirm the scavenging property before going for commercialization.

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