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Antioxidant potential of various extracts from whole plant of *Anisomeles malabarica* (Linn.) R.BR

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ABSTRACT

The objective of the present study was to evaluate the antioxidant potential of whole plant of Anisomeles malabarica. The antioxidant activity was assessed by hydroxyl radical scavenging activity, FRAP assay, nitric oxide radical scavenging activity, estimation of total phenol and total flavonoids. The significant free radical scavenging activity was occurring in methanolic extract of *Anisomeles malabarica* than that of standard. Similar results were not found in other two extracts. The radical scavenging activity of the extract was increased with the increasing concentration. The methanolic extract of *Anisomeles malabarica* was recorded noticeable amounts of flavonoids and phenolic contents than that of other two extracts. These in vitro assays indicate that this plant extracts is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Key words: Anisomeles malabarica, FRAP, Free radical scavenging activity.

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INTRODUCTION

There is extensive evidence to implicate free radicals in the development of degenerative diseases [1, 2]. Almost all organisms possess antioxidant defences that protect against oxidative damage and numerous damage removal and repair enzymes to remove or repair damaged molecules. However, the natural antioxidant mechanisms can be inefficient, hence dietary intake of antioxidant compounds become important [3-5]. Although, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) have been widely applied in food processing, they have been reassessed for their possible toxic and carcinogenic components formed during degradation [6]. In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and incidence of number of human diseases [7-8]. Therefore, search into the isolation of natural antioxidant sources is important.

Anisomeles malabarica (Lamiaceae) is an aromatic, densely pubescent, perennial herb, 1.2-2.0m in height. It is commonly found in Western Ghats from Maharashtra to Karnataka, Andhra Pradesh, Kerala and Tamil Nadu in India [9]. The plant is reported to possess antiperiodic, disphoretic, emmenegogue properties [9, 10]. Ethnobotanically, the leaves of *Anisomeles malabarica* are used against convulsions, in dyspepsia, intermittent fever, colic, boils, tetanus [11-13]. Lack scientific support revealed that the in-vitro antioxidant and free radical scavenging activity of various extract from whole plant of *Anisomeles malabarica* (Linn.) R.Br. In view of the above fact, in the present study the possible to evaluate the in-vitro antioxidant and free radical scavenging activity of various extract from whole plant of *Anisomeles malabarica* (Linn.) R.Br.

MATERIAL AND METHODS

Plant materials

The whole plant of *Anisomeles malabarica* (Linn.) R. Br, were collected from Aadiveeraganallur near srimushnam in Cuddalore District of Tamil Nadu, India. The plant was botanically authenticated. A voucher specimen (AU-6065) of the plant has been deposited at the herbarium of the Department of Botany, Annamalai University. The whole plant of *Anisomeles malabarica* (Linn.) R.Br, were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powered materials were successively extracted with Petroleum ether by hot continuous percolation method in Soxhlet apparatus [14] for 24 hrs. Then the marc was subjected to ethyl acetate for 24 hrs and then marc was subjected to methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

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Evaluation of Antioxidant activity by in vitro methods

Determination of Hydroxyl radical scavenging activity [15]

This was assayed as described by Elizabeth and Rao. The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} -Ascorbate –EDTA –H_2O_2 system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM),0.1 ml EDTA (0.1 mM), 0.1 ml H_2O_2 (1mM), 0.1 ml Ascorbate (0.1mM), 0.1 ml KH_2PO_4-KOH buffer, P^H 7.4 (20mM) and various concentrations of plant extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37^0 C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

FRAP assay [16]

A modified method of Benzie and Strain was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 mMHCl and 20 mM$Fecl_3.\ 6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $Fecl_3\ .6H_2O$. The temperature of the solution was raised to 37^0 C before using. Plant extracts (0.15 ml) were allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Readings of the colored product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $Feso_4$. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Determination of Nitric oxide radical scavenging activity [17]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat. The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25^0C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological P^H spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

Estimation of total phenol [18]

The measurement of total phenol is based on Mallick and Singh (1980). To 0.25g of sample, added 2.5 ml of ethanol and centrifuged at 2^oC for 10 mins. The supernatant was preserved. Then, the sample was re-extracted with 2.5 ml of 80% ethanol and centrifuged. The

pooled supernatant was evaporated to dryness. Then, added 3 ml of water to the dried supernatant. To which added 0.5 ml of Folins phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer.

Estimation of total flavonoids [19]

0.2g of the plant material was ground with ethanol-water in 2 different ratios namely 9:1 and 1:1 respectively. The homogenate was filtered and these 2 ratios were combined. This was evaporated to dryness until most of the ethanol has removed. The resultant aqueous extract was extracted in a separating funnel with hexane or chloroform. The solvent extracted aqueous layer was concentrated 0.5 ml of aliquot of extract was pipette-out in a test tube. 4 ml of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

RESULTS AND DISCUSSION

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals [20, 21]. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals and by their reducing ability.

Hydroxyl radical scavenging activity

The percentage of Hydroxyl radical scavenging activity of various extracts of *Anisomeles malabarica* was presented in Table 1. The IC_{50} values of petroleum ether, ethyl acetate and methanolic extract of *Anisomeles malabarica* were found to be 1335µg/ml, 620 µg/ml and 180µg/ml respectively. Whereas, the IC_{50} value of standard ascorbate was observed 410µg/ml. The methanolic extract of *Anisomeles malabarica* was found significant radical scavenging activity in comparison with standard ascorbate. The moderate scavenging activity was found in ethyl acetate extract of *Anisomeles malabarica* than that of petroleum ether extract.

Table 1: Hydroxyl radical scavenging activity of various extracts of Anisomeles malabarica

Treatment	Concentration (µg/ml)				IC_{50} values (µg/ml)
	125	250	500	1000	
Pet. ether extract	19.55±0.06	21.29±0.10	34.40±0.24	39.43±0.13	1335
Ethyl acetate extract	22.50±0.32	33.50±0.07	47.44±0.12	55.30±0.57	620
Methanolic extract	44.72±0.03	57.65±0.19	64.46±0.25	62.27±0.10	180
Standard	26.87±0.07	30.30±0.05	60.64±0.02	55.23±0.01	410

*All values are expressed as mean ± SEM for three determinations

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Nitric oxide radical scavenging activity

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities [22].

The nitric oxide radical scavenging activity of various extracts of *Anisomeles malabarica* and ascorbate were presented in Table 2. The IC_{50} values of various extracts (petroleum ether, chloroform, ethyl acetate and ethanolic) of *Anisomeles malabarica* were found to be 1390µg/ml, 1125 µg/ml and 230µg/ml respectively. Whereas, the IC_{50} value of standard ascorbate was observed 410µg/ml.

Table 2: Nitric oxide radical scavenging activity of various extracts of Anisomeles malabarica

Treatment	Concentration (µg/ml)				IC_{50} values (µg/ml)
	125	250	500	1000	
Pet. ether extract	11.53±0.08	21.16±0.40	31.19±0.35	36.59±0.26	1390
Ethyl acetate extract	15.82±0.25	22.51±0.08	39.43±0.14	46.63±0.06	1125
Methanolic extract	34.72±1.31	51.02±0.34	56.91±0.27	64.20±0.07	230
Standard	26.87±0.07	30.30±0.05	60.64±0.02	55.23±0.01	410

*All values are expressed as mean ± SEM for three determinations

Based on the above data revealed that the methanolic extract of *Anisomeles malabarica* were observed strong nitric oxide radical scavenging activity than that of other extracts. In comparison of all the three extracts with ascorbate (standard), the methanolic extract of the *Anisomeles malabarica* was showed the strong nitric oxide radical scavenging activity than that of standard.

FRAP Assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Table 3 was illustrated the FRAP values of various extracts of *Anisomeles malabarica* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml). The IC_{50} values of various extracts (petroleum ether, chloroform, ethyl acetate and methanolic) of *Anisomeles malabarica* were found to be 1395µg/ml, 1070 µg/ml and 150µg/ml respectively. Whereas, the IC_{50} value of standard ascorbate was observed 410µg/ml. The methanolic extract of *Anisomeles malabarica* was showed significant reducing capacity than that of other two extracts. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

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Table 3: FRAP Assay of various extracts of Anisomeles malabarica

Treatment	Concentration (µg/ml)				IC_{50} values (µg/ml)
	125	250	500	1000	
Pet. ether extract	18.01±0.21	23.53±0.04	33.61±0.31	36.73±0.40	1395
Ethyl acetate extract	13.77±0.24	25.01±0.38	42.14±0.37	48.82±0.44	1070
Methanolic extract	44.80±0.44	61.10±0.30	67.30±0.09	75.81±0.33	150
Standard	26.87±0.07	30.30±0.05	60.64±0.02	55.23±0.01	410

*All values are expressed as mean ± SEM for three determinations

Total phenol

The total phenolic content of various extract of root of *Anisomeles malabarica* was presented in Table 4. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups [23]. The methanolic extract of *Anisomeles malabarica* was noticed higher amount of phenolic components than that of other two extracts.

Table 4: The total Phenolic content of various extracts of whole plant of Anisomeles malabarica

S.No	Extracts	Total phenol content (mg/g of Catechol) (±SEM)*
1.	Petroleum ether extract of Anisomeles malabarica	1.65±0.03
2.	Ethyl acetate extract of Anisomeles malabarica	2.51±0.01
3.	Methanolic extract of Anisomeles malabarica	4.21±0.03

*All values are expressed as mean ± SEM for three determinations

Total flavonoids

Flavonoids present in food of plant origin are also potential antioxidants [24, 25]. Most beneficial effects of flavonoids are attributed to their antioxidant and chelating abilities [26]. Recent studies showed that many flavonoids & related polyphenols contribute significantly to the total antioxidant activity of many plants [27].The total amount of flavonoids content of various extract of whole plant of *Anisomeles malabarica* was summarized in Table 5. The higher content of flavonoids was found in methanolic extract of *Anisomeles malabarica* than that of other extracts.

Table 5: The total flavonoids content of various extracts of whole plant of Anisomeles malabarica

S.No	Extracts	Total flavonoids content mg/g) (±SEM)*
1.	Petroleum ether extract of *Anisomeles malabarica*	0.64±0.02
2.	Ethyl acetate extract of *Anisomeles malabarica*	1.05±0.01
3.	Methanolic extract of *Anisomeles malabarica*	2.42±0.06

*All values are expressed as mean ± SEM for three determinations

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CONCLUSION

There is a strong need for effective antioxidants from natural sources as alternatives to synthetic food additives in order to prevent deterioration of food, drug and cosmetics. The results of the present study showed that the whole plant of *Anisomeles malabarica* contains higher amount of flavonoids and phenolic compounds which correspond to greater antioxidant activity. A search for its active constituents would be interesting and deserve further studies to discover isolated chemical constituents for antioxidant activity.

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