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In-vitro Antioxidant Activity of Methanolic Extract in Leaves of *Anisomeles malabarica Linn*

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ABSTRACT

Previous phytochemical analysis of methanolic extract of *A. malabarica L*. has indicated the presence of steroid, flavonoid and terpenoid types of compounds. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, prompted us to check *A. malabarica L*. for possible antioxidant activity by DPPH scavenging activity, reducing power, total antioxidant activity and total phenolic content. The leaf of the plant was air dried, extracted with methanol using Soxhlet apparatus and the dried extract was used for the further studies. The maximum percentage inhibition by DPPH method was found to be 82.08 at a concentration of 250µg/0.1ml when compared with Quercetin and the IC_{50} value was found to be 37.5µg/0.1ml. The reducing capabilities were found to be in dose dependent manner. The total antioxidant activity was expressed as equivalents of ascorbic acid and it was found to be 0.2mg equivalents. The total phenolic content was found to be as 82mg of Pyrocathecol equivalent /gm of extract and the total antioxidant activity was found to be 0.32mg equivalents of ascorbic acid. The above results suggest that leaves of *A. malabarica L* were found to reveal antioxidant potential which supports the use of this plant in traditional medicine.

Keywords: *A. malabarica L*, Quercetin, Total antioxidant, Total phenolic content, Pyrocathecol.

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INTRODUCTION

In the living system, free radicals of different forms are constantly generated for specific metabolic requirement. When the generation of these species exceeds the levels of antioxidant mechanism, they cause extensive damage to the cells leading to oxidative damage of tissues and biomolecules, eventually leading to disease conditions, especially degenerative diseases and extensive lysis. The living system is protected from this by enzymes such as superoxide dismutase, glutathione peroxidase and catalase and certain endogenous antioxidants such as α-tocopherol, ascorbic acid,β-carotene and uric acid. Since the endogenous antioxidants acting as intracellular defense systems protecting cells from free radical damage and extensive lysis, scavenging and diminishing the formation of oxygen-derived species are not 100% efficient, micro nutrients or antioxidants taken as supplements are particularly important in diminishing the cumulative oxidative damages. Various disease conditions are associated with free radical oxidative stress. Herbal drugs containing free radical scavengers like phenolics, tannins and flavonoids are known for their therapeutic activity [1]. Today the evidence is consistent with oxidative stress in rheumatoid arthritis. Therefore the oxidative damage occurs in rheumatoid arthritis is not only due to reactive oxygen species (ROS), the development of arthritis is associated with over production of free radicals or with poor antioxidant activity of synovial fluid. Atherosclerosis, one of the major diseases in the world is mainly due to the platelet aggregation in plasma. Antiplatelet activity becomes a treatment for atherosclerosis. *A. malabarica L* a shrub of family, Labiatae have long been used in folk medicine to treat halitosis, amentia, anorexia, fevers and rheumatic swellings. In the present study, we investigated whether the exhibit plant anti-oxidant activity by the following methods. In the present study, preliminary phytochemical testing showed the presence of high amount of tannins and phenolics. The presence of high amount of tannins and phenolic prompted us to study the free radical scavenging activity of methanolic extract of *A. malabarica L.*

MATERIALS AND METHODS

Collection and extraction of medicinal plant material

The raw material of medicinal plant such as *A. malabarica L* were collected from different regions of Tamil nadu and authenticated by Dr.P.Jayaraman (Botanist) Director PARC West Tambaram. Voucher specimen [NO: PARC/2008/186] deposited in our college herbarium for future reference. The dried powdered of plant materials (leaves) were extracted with methanol using soxhlet apparatus for 48 hrs. The solvent was distilled at lower temperature under reduced pressure and concentrated on water bath to get the crude extract which is stored in desiccator for future use.

Phytochemical Screening

The Phytochemical screening for the plant extract were carried out using standard chemical procedure. [2]

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Determination of DPPH scavenging activity

DPPH scavenging activity was measured by the spectrophotometric method. A stock solution of 25mg of DPPH (150μM) was prepared in 100ml of ethanol. 0.1ml of extract of different concentration (31.25, 62.5, 125, 250, 500, 1000, and 2000μg/0.1ml) and 1.9ml of DPPH was added. Control without test compound was prepared in an identical manner. In case of blank, DPPH was replaced by ethanol. The reaction was allowed to be completed in the dark for about 20 minutes. Then the absorbance of test mixtures was read at 517nm.The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical. Quercetin (31.25, 62.5,125,250,500,1000 and 2000 μg/0.1ml) was used as standard. The percentage DPPH inhibition was calculated from the following formula. The results were tabulated Table.2 and Fig.1.

%DPPH inhibition= [(OD of control-OD of test) ÷ (OD of control)]

Determination of total antioxidant activity

The total antioxidant activity was evaluated by (Prieto et al., 1999). An aliquot of 0.1ml of sample solution / ascorbic acid equivalent to 500μg was combined with 1ml of the reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). In case of blank, 0.1ml of methanol was used in place of sample. The tubes were capped and incubated in a boiling water bath at 95°C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695nm against blank. The antioxidant activity was expressed as equivalents of ascorbic acid (μg/g). [3]

Determination of total phenolic compounds

Total soluble phenolic content of the methanolic leaf extract was determined (Slinkard and Singleton, 1977) with folin-ciocalteu reagent using pyro catechol as standard. 1ml of methanolic extract (500μg) in a volumetric flask was diluted with distilled water (46ml). Folin-ciocalteu reagent (1ml) was added and the contents of the flask were mixed thoroughly. After 3 minutes, 3ml of sodium carbonate (2%) was added then the mixture was allowed to stand for 2 hour with intermittent shaking. The absorbance was measured at 760nm. [4] The concentration of total phenolic compound in the leaf extract was determined as microgram of Pyrocathecol equivalent by using an equation that was obtained from standard curve of pyro catechol.

Absorbance = 0.001 X Pyrocathecol (μg/ml) + 0.003

Determination of reducing power

For the measurement of reductive ability we investigated the Fe^{+3} –Fe^{+2} transformation in the presence of the samples such as leaf extract of A. malabarica L and

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Quercetin at different concentrations (250,500,1000,2000µg/ml) using the method of Oyaizu(1986).Definite amount of the extract and standard drugs,2.5ml phosphate buffer pH 6.6,2.5ml 1% potassium ferrocyanide were incubated at 50ºC for 20 minutes, 2.5ml of 10% trichloro acetic acid(TCA) were added to the mixture and centrifuged for 10 minutes at 3000r.p.m.After centrifugation 2.5ml of the supernatant were diluted with 2.5ml of water and shaken with 0.5ml freshly prepared 0.1% ferric chloride. The absorbance was measured at 700nm [5-6].The control solution was prepared as above, but contained water instead of samples. An increase in absorbance indicated higher reductive ability. The results were tabulated in Table.3 and Fig.2.

RESULTS AND DISCUSSION

The Phytochemical screening for the plant revealed the presence of flavones, flavonones, quinolones, tannins and phenolic compound. The results were tabulated in Table.1.

Table 1: Preliminary phytochemical evaluation of methanolic extract Of leaves of Anisomeles malabarica

S.NO.	CONSTITUENTS	ANISOMELES MALABARICA
1.	Alkaloids	+
2.	Tri-Terpenoids	+
3.	Steroids	+
4.	Coumarins	-
5.	Tannins	+
6.	Saponins	-
7.	Flavones	+
8.	Quinones	-
9.	Flavanones	+
10	Anthocyanins	-
11	Anthraquinones	-
12	Phenols	+
13	Proteins	+
14	Carbohydrates	-
15	Glycosides	+

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Table 2: Determination of DPPH scavenging Activity

Concentration (μg/0.1ml)	% Inhibition	
	Anisomeles Malabarica	Quercetin
31.25	45.58	80.99
62.5	68.60	86.63
125	80.08	86.43
250	82.08	87.18
500	76.22	87.74
1000	68.07	88.02
2000	68.44	84.96
IC_{50}	37.5μg/0.1ml	

Table 3: Determination of reducing power

Concentration (μg/0.5ml)	%Inhibition	
	Anisomeles Malabarica	Quercetin
250	**91.91**	**90.52**
500	**89.66**	**92.83**
1000	**78.39**	**94.70**
2000	**25.80**	**95.95**

DPPH radical Scavenging Activity

Radical scavenging activity of methanolic extract of *A. malabarica L* was tested by its ability to bleach the stable DPPH radical. This method is based on the reduction of alcoholic DPPH solution in the presence of hydrogen donating anti-oxidant(AH) due to the formation of non- radical form DPPH – H by the reaction DPPH+AH→ DPPH –H +A. The remaining DPPH measured after certain time, corresponds inversely to the radical scavenging activity of the anti-oxidant. The sensitivity of the method is determined by the strong absorption of DPPH. This method is rapid, a sample analytical method which takes only 15minutes and little man power, no expensive reagents or sophisticated instruments are required [7]. This assay is being used widely as a preliminary test which provides information on the reactivity of test compound with a stable free radical since odd electron of DPPH gives strong absorption band at 517nm(violet colour) and when it is quenched by the extract , there is a decrease in

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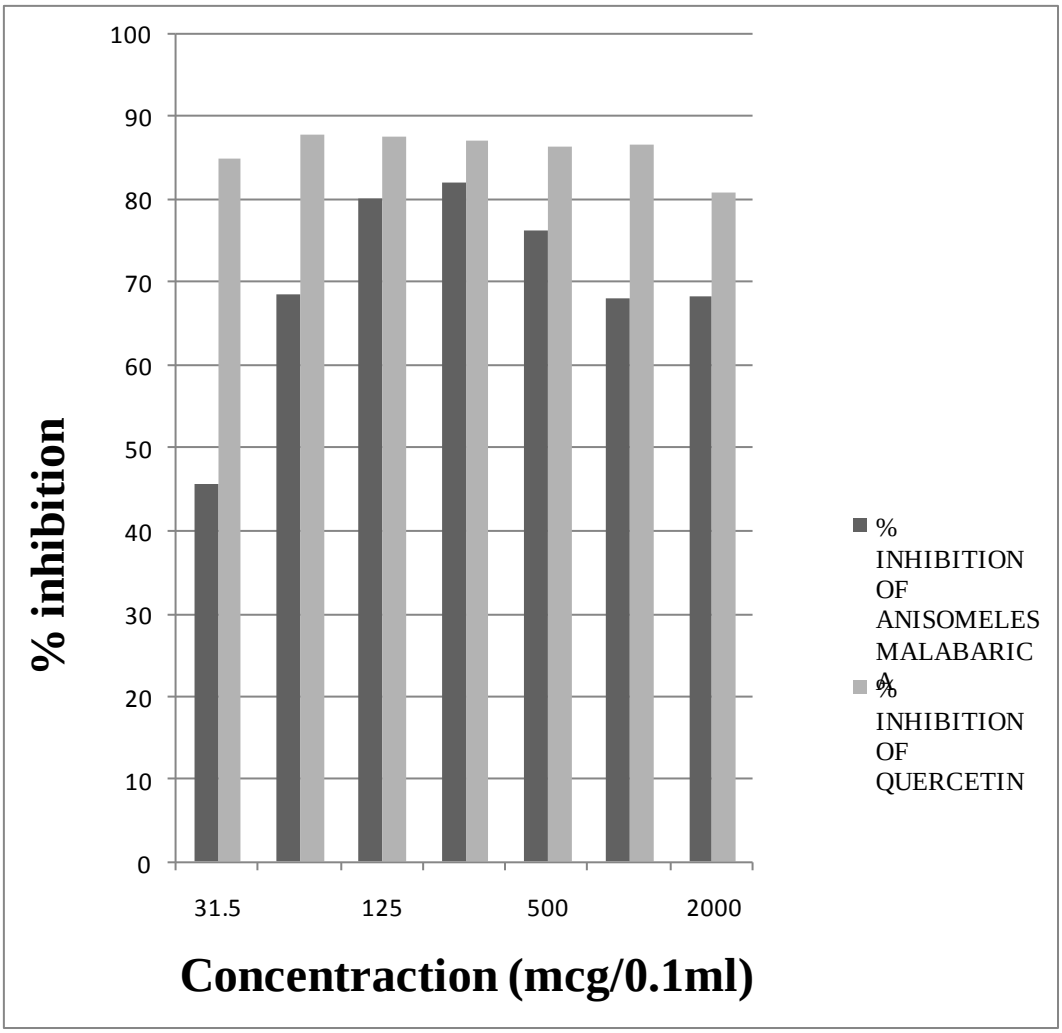
absorbance. Methanolic extract of *A. malabarica L* showed a very good anti-radical activity in scavenging DPPH radical (comparable to the standard, Quercetin) with a maximum inhibition of about 82.08 (IC_{50}=37.5μg/0.1ml) at a concentration of 250mcg/0.1ml.

Figure 1: DPPH Radical Scavenging Activity



Total Antioxidant Activity

The total anti-oxidant activity of methanolic extract of *A. malabarica L* was found to be 0.32mg equivalents of ascorbic acid. This may be due to the presence of alkaloids, steroids, phenolic compounds, proteins and flavonoids [8, 9]. From the results it is made clear that methanolic extract of *A. malabarica L* possess free radical scavenging activity through total antioxidant content.

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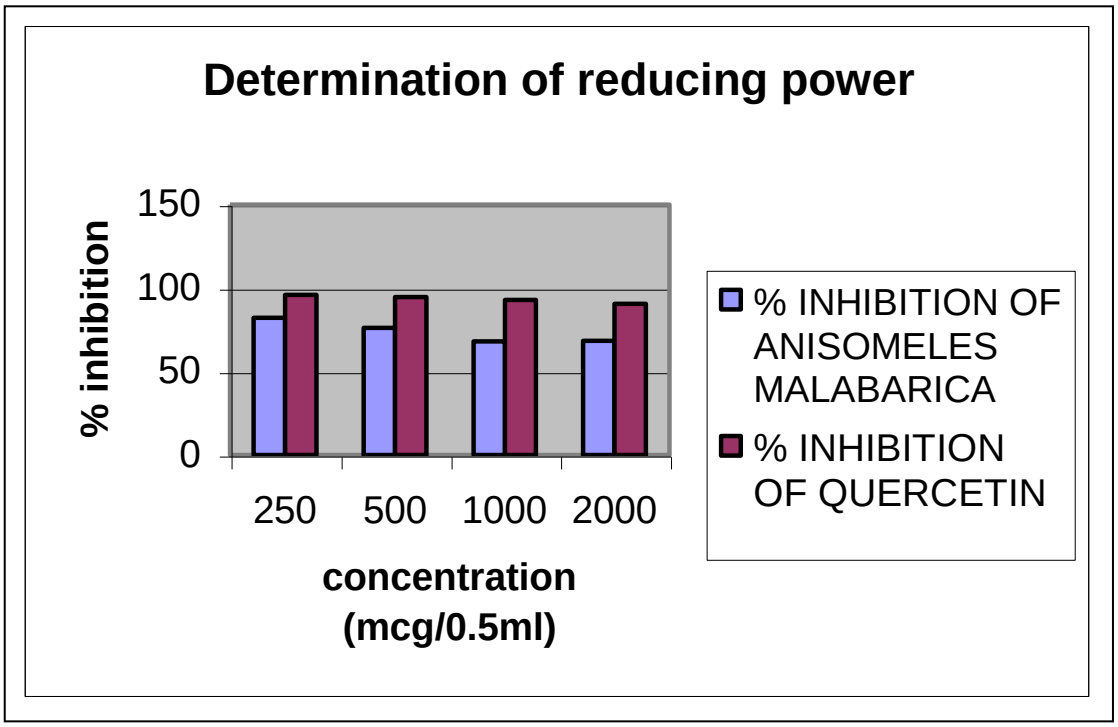
Figure 2: Determination of reducing power



Total Phenolic Content

Phenolics are very important plant constituents because of their scavenging capability due to their hydroxyl groups. It has been revealed that various phenolic antioxidants such as flavonoids, tannins, coumarins, xanthones and more recently procyanidins scavenge radicals dose dependently, thus they are viewed as promising therapeutic drugs for free radical pathologies. In the present study total phenolic compound of methanolic extract of *A. malabarica L* at 500µg was found to 82mg µg of Pyrocathecol equivalent/gram of extract respectively [10 -13].

Reductive Ability

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity . For the estimation of the reductive ability we investigated the Fe^{3+} to Fe^{2+} transformation using the method of Oyaizu, where the change in the optical density of the final mixture is measured at 700nm (Table-2). Increase in optical density indicates higher reductive ability12, 13. The reducing capabilities of the leaf extract of *A. malabarica L* was found to be in dose dependent manner when compared with Quercetin [14-16].

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CONCLUSION

The methanolic extract of the leaves of *A. malabarica L* must contain some active principles, which possess antioxidant property. Hence further investigation and proper isolation of the active principles might help in the findings of new lead compounds which will be effective against free radical mediated diseases.

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