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In-vitro antioxidant potential of solvent extracts from *Anisomeles malabarica*

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Abstract
Anisomeles malabarica (Linn.) Lamiaceae found in Western Ghats of southern India. This plant has traditional medicinal value with phytochemical components which have antioxidant potential. In this present study investigation was done to evaluate *in vitro* antioxidant assay by DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging activity and reducing ability with leaf extracts of *A. malabarcia* using various solvents (methanol and hexane). It was found that methanol and hexane leaf extracts showed good potency of antioxidant activity. Comparative study of these crude extracts revealed that methanol extract was more effective than hexane. Methanol extract showed very good antiradical activity and reductive ability against standard antioxidant (BHA) and standard reductive agent ascorbic acid. Even hexane extract also revealed some good potential comparison with these standards. The result suggests that the methanol extract of *A. malabarica* contains relatively significant quantities of novel active antioxidant compounds compared with hexane extract.

Keywords: *A. malabarica*; antioxidants; free radical scavenging; reducing power.

1. Introduction
In recent years it has been found that human health is also affected by the generation of free radicals. Free radicals which are also known as reactive oxygen species (ROS), reactive oxygen species are small, very reactive, having oxygen containing molecules continuously in the cells as a consequence of both enzymatic and non-enzymatic reactions [1]. Reactive free radicals generally result in degradation of protein, lipid peroxidation and oxidation of DNA, which have been considered to be linked with many chronic diseases, such as diabetes, cancer and atherosclerosis [2]. Excess production of free radicals or decrease in antioxidant level leads to oxidative stress [3]. Oxidative stress is associated with ageing and many age related diseases [4]. Prevention for these free radicals is antioxidants. Antioxidants are the free radical scavengers which protects the human body. There are synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are known to have side effects causing liver damage [5], [6]. Therefore there is a need for isolation and characterization of natural antioxidant having fewer side effects which can be used in foods or medicinal materials to replace damage causing synthetic antioxidants [7]. Plants identified in human dietary have the action of phytochemicals which mainly include; antioxidant activity, detoxification of carcinogens and stimulation of immune system [8].
Anisomeles malabarica (Linn) is one such traditional medicinal plant found in tropical and subtropical regions of India, belongs to family Lamiaceae. It is erect shrub commonly known as 'Malabar catmint', commonly found in Western Ghats from Maharashtra to Kerala in India [9]. The herb is reported to possess anticancer, allergenic, antihelmintic, antiallergic, antianaphylactic, antibacterial, anticarcinomic, anti-inflammatory, antileukemic, antinociceptive, antiplasmodial, and antiseptic, antiperotic properties [10]. In present study, investigation has been made to evaluate the *in vitro* antioxidant activity by DPPH radical scavenging assay and reducing power with the various solvent extracts obtained from the *A. malabarica* leaves. The phytochemical components present in leaves of this plant were reported earlier in the previous study [11]. The presence of flavonoids and tannins in hexane and methanol extract showed potential to conduct further studies to unravel the novel treatment for free radicals.

2. Materials and Methods
Fresh plant material was collected from the various regions of Western Ghats, Karnataka.

Specimen was authenticated by Taxonomist, Dept. of Biotechnology, Kuvempu University and samples were deposited in the same department as herbarium for further reference. Leaves were Separated from the plant and shade dried. Dried sample was crushed and pulverised into powdered form. Powered sample was extracted with standard laboratory grade analytical solvents viz., methanol and hexane. The extracts obtained were evaporated and transferred into vials for further study.

2.1 Antioxidant Assay
The antioxidant activity of *A. malabarica* leaves extract was determined by different *in vitro* methods such as DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging and reducing power assay using standard procedures. Initially the extracts obtained were dissolved in methanol at the concentration of 1 mg/mL (1000 µg/ mL), all these assays were carried out in triplicates and average value was measured and considered.

2.2 DPPH Radical scavenging activity
The free radical scavenging activity was conducted according to standard protocols [12]. About 0.2 mL methanolic solution of each methanol and hexane leaf extracts of *A. malabarica* was taken at different concentration (20 - 100 µg/ mL) was mixed with 0.8 mL of Tris-HCl buffer (100 mM, pH 7.4), 1 mL DPPH (500 mM in methanol) solution was added to above each mixture. The mixture was shaken vigorously and incubated for 30 min in room temperature. Absorbance of the resulting solution was measured at 517 nm UV-Visible spectrophotometer. All the assays were carried out in triplicates with standard synthetic antioxidant BHA (Butylated hydroxyanisole). Blank was prepared without the addition of DPPH and for control 0.2 mL of methanol without leaf extracts was added. Percentage of DPPH radical scavenging determined as follows.

%DPPH radical scavenging =
[(Absorbance (OD) of control* – Absorbance (OD) of test sample) / (Absorbance (OD) of control*)] X 100

*control was the DPPH solution without leaf extract.

2.3 Reducing power
The reducing power assay was carried out with the method as described by [12, 13, 14]. About 1mL of each leaf extract solution was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL (1%) potassium ferricyanide. This mixture was incubated at 50 ^{0}C for 20 min. About 2.5 mL of (10%) trichloroacetic acid was added to the mixture which was then centrifuged at 3000 rpm for 10 min. Finally 2.5 mL of the supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL (0.1%) ferric chloride allowed standing for 10 min (final concentration 100-500 mg/L). The absorbance measured at 700 nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard (concentration 10 mg/mL).

3. Result and Discussions
3.1 DPPH Radical scavenging activity
The antioxidant activity of plants is mainly contributed by the active compounds present in them. The amount of such compounds is deposited in each part of the plant is usually different [15]. Antioxidant activity varies from part to part. Several methods have been used for the antioxidant activity of plants like DPPH radical scavenging assay [16], ABTS decolourisation method [17]. Comparative study indicates that free radical scavenging using DPPH assay is good to find the novel antioxidants. The *In vitro* DPPH assay is widely used technique to evaluate the antioxidant reactivity of a compound extracted. DPPH is considered as a stable free radical and it accepts an electron or hydrogen radical become a stable diamagnetic molecule [18]. Decreased absorbance of the reaction mixture indicates stronger DPPH radical scavenging activity. Decolouration indicates the scavenging potential of the extracted antioxidant compounds, the degree of decrease in the absorbance of DPPH radical caused by antioxidants is visually noticeable [19]. The different leaf extracts of *A. malabarica* at various concentrations induced a rapid decrease in the optical density as discussed below. The % DPPH radical scavenging activity of the methanol and hexane increases with increasing concentration presented in the Figure 1 and Figure 2.

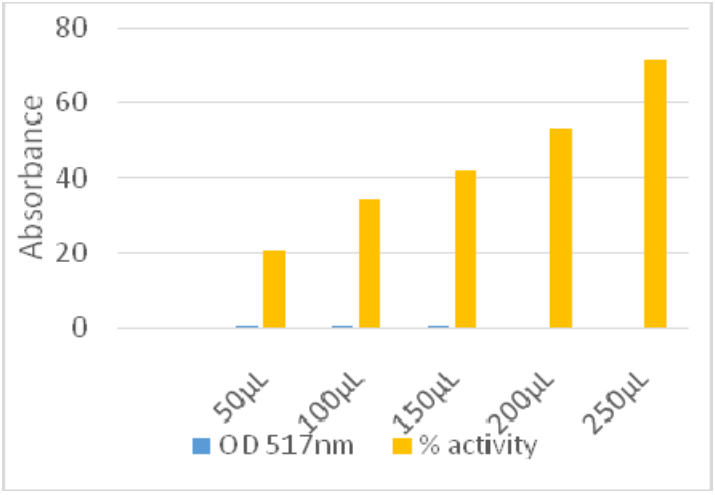

Fig 1: Antioxidant activities of hexane extract comparison with standard

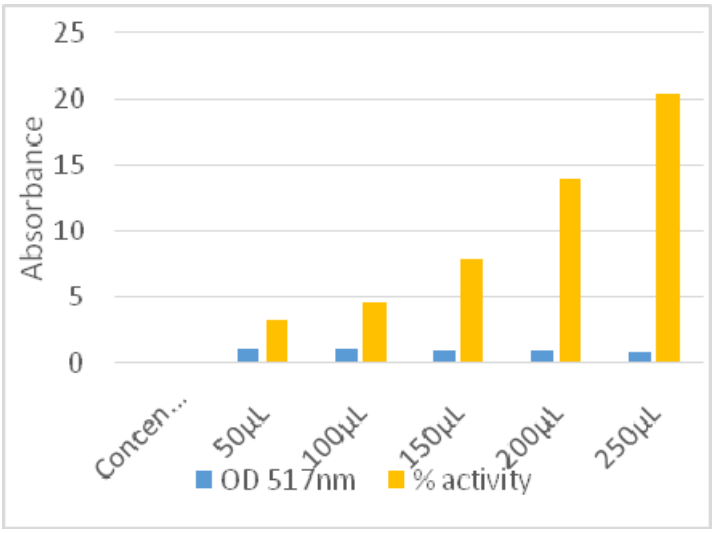

Fig 2: Antioxidant activities of methanol extract comparison with standard

This evaluation revealed that the methanol extract showed very good anti radical activity with maximum percentage of radical scavenging about (71.26) at concentration of (250 µL) which is higher antioxidant potential than that of hexane extract (20.45) percentage radical scavenging activity at the same concentration. The methanol extract of leaves of *A. malabarica* possess good antioxidant activity previous studies have been reported [20]. Although this leaves extracts showed significant antioxidant potential this is lower scavenging activity in comparison to standard BHA.

3.2 Reducing power

The reducing power of the leaf extracts was determined by the method of [21, 12]. High absorbance due to reduction increases optical density indicates higher reductive ability [22, 23]. The reducing ability of the compound depends on the presence of the reductants present in the plant [24]. Presence of reducers causes conversion of the Fe^{3+} complex (ferricyanide) in this method into ferrous form, and it is possible to determine the Fe^{2+} concentration [25]. The reducing capabilities of the leaf extracts of *A. malabarica* was found to be in dose dependent manner when compared with the standard ascorbic acid [26, 27]. The reducing power of methanol and hexane extracts showed increased absorbance with increase in the concentration as showed in (Figure 3 & Figure 4). The reductive ability of the methanol extract (0.96) was more than hexane extract (0.80). The reducing power of methanol extract was relatively equal to the standard ascorbic acid has discussed in our study. The earlier investigation suggests that the different plant extracts of *A. malabarica* has significant antioxidant effect and it has relatively more reducing power than ascorbic acid [28].

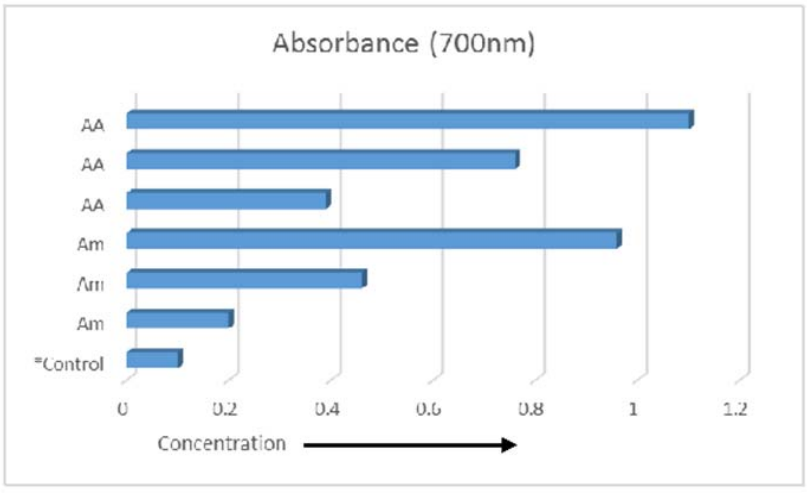

Fig 3: Reducing power of methanol extract of *A. malabarica*

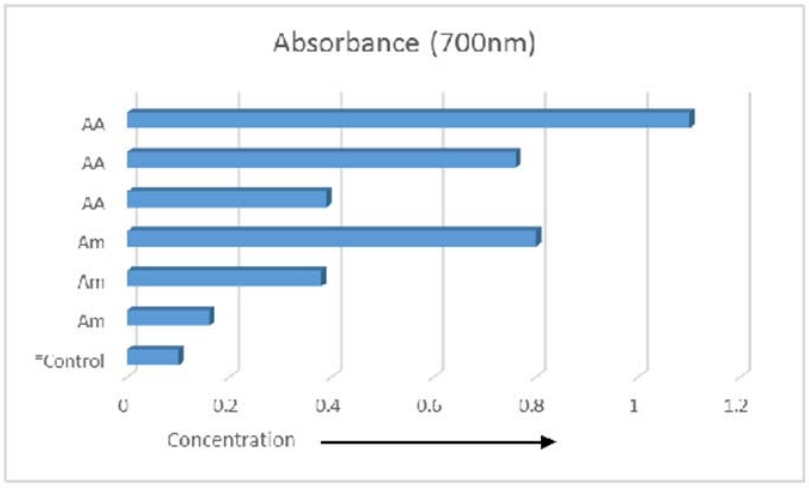

Fig 4: Reducing power of hexane extract of *A. malabarica*

4. Conclusion

The different leaf extracts of *A. malabarica* contains natural active components possess significant antioxidant potential which are relatively equal to synthesized antioxidants. The results of this study clearly promise for development of novel drug molecules. With further crude extracts of this plant will strongly support the effective investigation and proper isolation lead into new findings and encourage developing novel broad spectrum which will lead into harbour new compounds can be effective for mediated ROS radical diseases.

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