

# Phytochemical Profile and Antioxidant Property of Leaves Extracts of *Anisomeles Malabarica*

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## ABSTRACT

Medicinal plants have been used throughout history for therapeutic purposes. Plants have therapeutic value as they comprise certain bioactive components. Medicinal plants are packed with antimicrobial, antioxidant, anti inflammatory substances that can offer many benefits for the human being. Present study aimed to determine the phytochemical analysis, antioxidant activity and anticancer properties of leaves extracts of *Anisomeles malabarica*. Water was used successively for the extraction. Leaf extracts were screened for phytochemical analysis and for antioxidant activity DPPH assay was used. Anticancer property was studied by MTT assay. Phytochemical analysis revealed the presence of flavonoids, terpenoids, anthraquinones, and oils. The water extract of crude flavonoids showed 91.0 percentage inhibition at at100 µg/ml. The study revealed high antioxidant property of *Anisomeles malabarica*.

**Key words:** MTT assay, DPPH assay, leaf extracts, and flavonoids.

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## INTRODUCTION

Numerous herbal medicines and nutraceutical products have higher safe tolerances. Alkaloids, flavonoids, glycosides, saponins, phenols, terpenes, anthraquinones are major phytoconstituents present in medicinal plants. Medicinal plants can be used for developing new sources for some plant products.

Phytoconstituents prevents and delay the cell damage. Oxidation reactions taking place in human body can form free radicals and these start chain reactions and damage cells. Antioxidants terminate the chain reactions by removing free radical intermediates.

Medicinal plants play a key role in the treatment of various health issues. Medicinal plants because of its least side effects used around the globe (Subramanian S V et al 2006).

*Anisomeles malabarica* of family Lamiaceae generally named as Malabar catmint. Plant is scented, perennial plant which has woolly stems and grows 50 – 150 cm tall. Leaves aromatic white oblong, lanceolate, purple coloured. Leaves are oblong to ovate woolly beneath, thinly hirsute above.



**Fig 1:** *Anisomeles malabarica*

## MATERIALS AND METHODS

### Collection and Identification of Plant

The plant was collected from the Devarayana Durga hills, Tumakuru district, Karnataka, India. The samples were authenticated at Regional Ayurveda Research Institute for Metabolic Disorders (Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Govt. of India) Bengaluru, Karnataka

### Plant Material

The leaves of *Anisomeles malabarica* was washed thoroughly under running tap water followed by distilled water and then air dried under shade at room temperature. The dried leaves were powdered with the help of porcelain mortar and pestle to increase the surface area for absorption of the solvents (Harborne 1973), stored in separate containers in moisture free environment and used for further analysis.

Water was selected for the process of extraction. 30 gm of dried leaves powder of the plant was taken separately and process of extraction using Soxhlet apparatus was performed out in a 250 mL of the solvent. The preliminary phytochemical screening of solvent extract of the plant was subjected to chemical test for different phytochemicals viz. Alkaloids, Flavonoids, Steroids, Terpenoids, Anthraquinones, Phenols, Saponins, Tannins, Carbohydrates and Oil by using standard procedures.

### Quantitative Analysis of Phytochemicals

Determination of flavonoids by Boham and Kocipai- Abyazan method

Plant materials were extracted repetitively using 100 ml of 80% aqueous methanol. The entire solution was filtered using Whatmann filter paper No 42. The filtrate were later transferred into a container and vaporized to dryness above a water bath. The dry materials were weighed.

Weight of total flavonoids:  $W_2 - W_1/W_2$

Where,  $W_1$  = weight of crucible,  $W_2$  = weight of crucible with flavonoids.

### Antioxidant properties

#### DPPH radical Scavenging assay

Determination of DPPH Radical Scavenging activity: 0.1 mm DPPH was added to 3 ml of plant abstract of various concentrations. The absorbance was measured at 517 nm by using spectrophotometer (UV-VS Shimadzu 1700). The all experiments were performed thrice and the results were averaged. Gallic acid was used reference compound.

The percentage inhibition of DPPH radical was calculated as follows:

% inhibition =  $[\text{Abs control} - \text{Abs sample} / \text{Abs control}] \times 100$

### Anticancer activity

MTT cytotoxicity assay for in vitro anticancer study:

The anticancer activity of plant extracts was analysed by MTT assay. 96 well microtiter plates were used for harvesting and inoculation of cells. The medium was aspirated after 72 hrs of incubation MTT solution of concentration 5 mg/ml was added to each well with paltry corrections. The MCF 7 cells were reaped and injected in 96-well microtiter plates. They were cleaned with PBS and the cultured cells were then inoculated with and without the extract. The medium was aspirated after 72 h of incubation the plates were incubated for 4 h at 37°C after adding Ten microliters of MTT solution (5 mg/ml in PBS, pH 7.2). After incubation, 100  $\mu$ l of dimethyl sulfoxide (DMSO) was added to the wells followed by gentle shaking to solubilize the formazan dye for 15 min. 72 hours.

Absorbance was read at 570 nm and the surviving cell fraction was computed. Camptothecin was used as the standard. The suppression of cell viability can be given by

Inhibition activity (%) =  $1 - T/C \times 100$  Where T= Absorbance of the test sample, C= Absorbance of the control sample

## RESULTS AND DISCUSSIONS

In order to establish the preliminary phytochemical profile of plant, the water extract of the plant were subjected to various chemical tests (Table 1).

**Table 1: Phytochemical screening (water extracts)**

Sl. No	Phytochemicals tests	Inference
1	Salkowski's test	+
2	Mayer's test	—
3	Wagner's test	—
4	Brontrager's test	+
5	Lead acetate test	+
6	Alkaline reagent test	+
7	Keller killiani test	—
8	Ferric chloride test	—
9	Molisch's test	—
10	Copper acetate test	—
11	Ninhydrin test	—
12	Frothing test	—
13	Translucent test	+

In order to establish the preliminary phytochemical profile of plants, the water extract of the plant were subjected to various chemical tests and it revealed the presence of flavonoids, terpenoids, anthraquinones and oils. However phenols, saponins, carbohydrates, tannins, steroids and glycosides were found to be absent (Table 1).

### Quantitative Analysis

**Table 2: Quantitative analysis flavonoids**

Solvent	Flavonoids w/w	Flavonoids w/w	Mean
Water	1.65	1.55	1.60

The total flavanoids, present in water extract of *Anisomeles malabarica*, were analyzed. The result showed that the amount of crude flavonoids (1.60% w/w) content (Table 2).

### ANTIOXIDANT PROPERTIES

#### Free Radical Scavenging Activity by DPPH Method

The free radical scavenging activity of different concentration of crude flavonoids of the plant were measured using DPPH method, the percentage inhibition was calculated and the results were compared with standard gallic acid. The DPPH free radical scavenging activity study results showed percentage inhibition of 91% at 100 µg/ml for flavonoids. (Table 4).

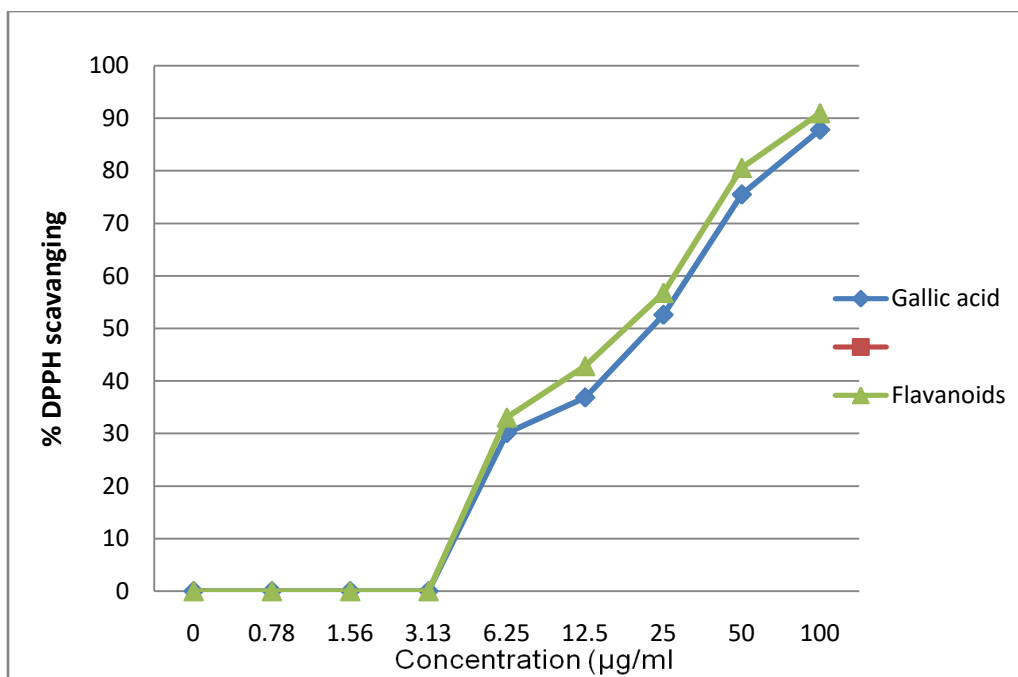
**Table 3: DPPH method of analysis of gallic acid (Standard)**

Conc of sample in µg Gallic acid	Percentage Inhibition
0.000	0.00
6.25	30.08
12.50	36.86
25	52.60
50	75.50
100	87.82

**Table 4: Percentage Inhibition of crude flavonoids by DPPH Assay.**

Conc of sample in µg (Flavonoids) Water extracts	Percentage Inhibition
0.000	0.00
6.25	33.08
12.50	42.86

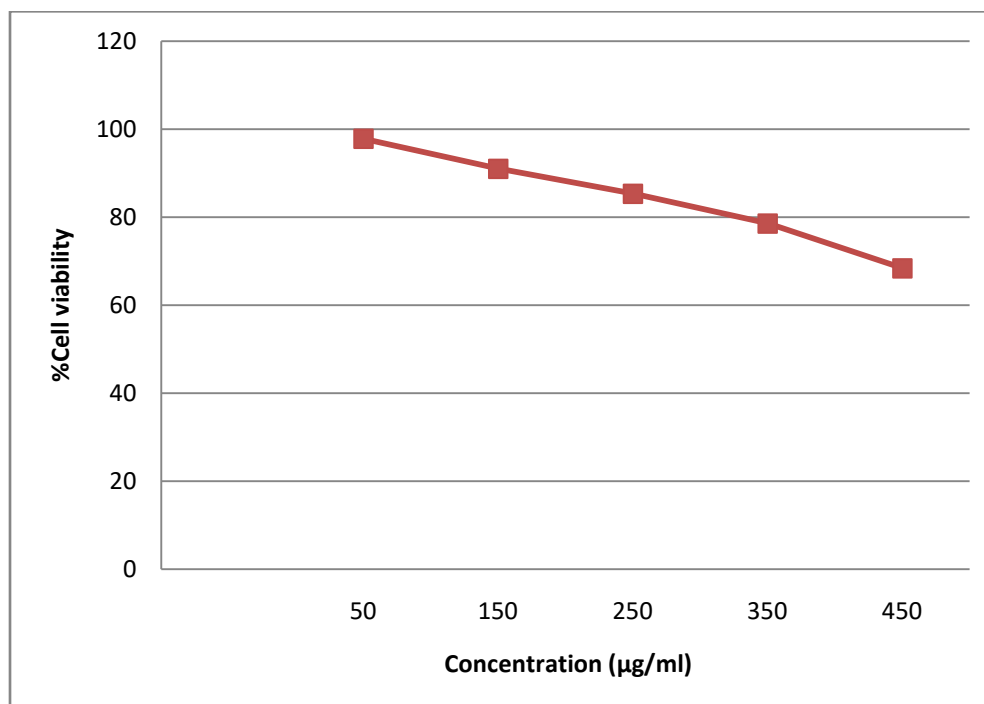
25	56.80
50	80.60
100	91.00



**Graph1: Comparison of antioxidant activity flavonoids of *Anisomeles malabarica* sample with standard gallic acid.**

#### Anticancer activity (Cytotoxic properties)

The cytotoxicity assay was performed according to the microculture MTT method with slight modifications. Flavonoid fraction showed 69.4% viability at 450 µg/ml, the effects of flavonoid fraction on cancer cells are represented graphically (Graph.2).



**Graph 2: Cytotoxic activity of crude flavonoid fractions (water extracts).**

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### CONCLUSION

Herbal medicines have been the oldest forms of health care. In this study *Anisomeles malabarica* leaf extracts have been investigated for antioxidant and cytotoxic properties. Flavanoid crude fractions obtained after water extraction showed good scavenging activities. *Anisomeles malabarica* showed poor anticancer activity, 69.4% viability at 450 µg/ml when compared to standard camptothecin showing 54.09% cell viability at 13.2 µg/ml. Thus we can conclude that the plant showed poor anticancer properties. Hence, it is interpreted that leaves of *Anisomeles malarica* plant can be used as good antioxidant agent further, in vivo studies are essential to enumerate its medicinal use and prove its efficacy in therapeutic applications.

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