Antimicrobial activity and phytochemical analysis of *Anisomeles malabarica* (L) R.BR.

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ABSTRACT

*Anisomeles malabarica* [L] is a medicinal plant has been used as a folk medicine to treat amentia, anorexia, fevers, swelling, and rheumatism. The herb is reported to posses' anticancer, anti-allergic, anti-anaphylactic, anti-bacterial anti-carcinogenic anti-inflammatory properties. The present study was carried out to evaluate in vitro antibacterial activity of leaf extracts of *A. malabarica* against *E.coli, S.aureus, P.mirabilis, P.aeruginosa, k.pneumonia*. The preliminary phytochemical analysis of both Ethanolic and Diethyl ether extracts revealed the presence of alkaloids, flavonoids, tannins, safonins, and glycosides. It was found that the ethanolic extract exhibited a maximum antibacterial activity at 200µg/ml and produced 25mm zone of inhibition against *S.aureus* whereas Diethyl ether extract produced 30mm zone of inhibition in the same concentration. The results provide justification for the use of *A. malabarica* to treat various infectious diseases.

Key words: *Anisomeles malabarica*, phytochemical analysis, Antibacterial activity.

INTRODUCTION

In recent years multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminative use of commercial antimicrobial drugs commonly used in the treatment of diseases. This situation forced the scientists for searching new antimicrobial substances from various sources of novel antimicrobial chemotherapeutic agent.

Some plant is known as medicinal because they contain active substances that cause certain reaction from relenting to the cure of diseases on the human organism [1]. Plants are the main source of food. They are rich in nutrients. They are also rich in compounds which have pain relieving and healing abilities. From earliest times itself plants were used for treatment of disease

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without knowledge about the compounds present and their mode of action. Over the centuries societies around the world have developed their own tradition to make sense of medicinal plants and their use.

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds [2].

Anisomeles malabarica (L.) (Malabar catmint) Cogn. Syn. Nepeta malabarica L., (Family: Lamiaceae) is a medicinal plant has been used as a folkloric medicine to treat amentia, anorexia, fevers, swellings, rheumatism [3]. The herb is reported to possess anticancer, allergic, anthelmintic, antiallergic, antianaphylactic, antibacterial, anticarcinomic, antiedemic, antihistaminic, antiinflammatory, antileukemic, antinociceptive, antiplasmodial, antisepticand antiperotic properties [4].

MATERIALS AND METHODS

Collection of plant materials
Fresh leaves of Anisomeles malabarica [L] free from disease was collected from our college campus in january2011 .The taxonomic identification of this plant was determined by Dr.V.Veeragurunathan the taxonomist at the department of Botany, J.J College of Arts and Science Pudukkottai.

Preparation of plant extract
- The leaves of Anisomeles malabarica was washed thoroughly 2-3 times with running water and air dried in shade for five days and then powdered with the help of warring blender
- 20gram of shade dried powder filled in thimble and extracted with Ethanol and Diethyl ether solvents in Soxhlet apparatus.
- The solvent extracts were concentrated under reduced pressure and stored in airtight bottle until further use

Phytochemical Analysis
Phytochemical analysis of Ethanol, diethyl ether extracts were carried out by the standard methods provided[ 5] and [6] for the presence and absence of metabolites such as alkaloids, glycosides, flavonoid, tannins, saponins was carried out.

Glycosides
Glycosides are compounds which up on hydrolysis give rise to one or more sugars and compound which is not a sugars.10cm³ of 50 % H₂SO₄ was added to 1cm³ of the extract of the test tube. The mixture was heated in a boiling water bath for 15 minutes.10cm³ of Fehlings solution was added and the mixture was boiled and observed for brick red precipitate.

Test for Tannins
(a) 10cm³ of freshly prepared 10%KOH was added to 1cm³ of each of the extract and observed for dirty white precipitate.
(b) 2drops of 5% FeCl₃ was added to 1cm³ of the extracts and observed for green precipitate.

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Test for Saponins (frothing test)
2cm$^3$ of each extract in a test tube was vigorously shaken for two minutes and observed for persistent foaming.

Test for flavonoids
To 3cm$^3$ of each extract was added 1cm$^3$ of NaOH and observed for yellow coloration.

Test for Alkaloids
1cm$^3$ of Hcl was added to 3cm$^3$ of each extract in a test tube. The mixture was heated for 20 minutes, cooled and filtered. 2 drops of wagner’s reagent was added to 1cm$^3$ of the filtrate and observed for reddish brown precipitate.

Inoculum Preparation
The inoculums were prepared using nutrient broth and the 24 hours old cultures were used for the antimicrobial studies using Agar well diffusion method.

Agar Well Diffusion Method [7].
The antibacterial activity of ethanol extract and Diethyl ether extract of leaves of *Anisomeles malabarica* were determined by well diffusion method. Plates were prepared by pouring sterile Muller Hinton agar (Himedia) into sterile Petri dishes that were previously autoclaved. Sterilized cotton swabs were dipped in the bacterial culture in nutrient broth and then swabbed on the agar plates. Wells of equal size were cut with proper gaps in the medium and the plant extracts were added into it. Then the plates were incubated at 37ºC and observed for zones of growth inhibition after 24 hours.

**RESULTS AND DISCUSSION**

The phytochemical analysis revealed that the plants contain bioactive substances which are connected with antimicrobial properties in plants (Table 1). These agents are alkaloids, saponins, tannins, flavonoids, and Glycosides. Flavanoids have been reported to expert multiple biological effects such as anti-inflammatory, anti allergic, anti-viral and anti-cancer activities. The one of the chemicals produced by plants are the alkaloids and their amazing effect on humans has let to the development of powerful pain killer medicine[8].Tannins has important roles such as antioxidants[9].Herbs having tannins are astringent in nature and are used for the treatment of diarrhea and dysentery[10]. This investigation forms a primary platform for further phytochemical and pharmacological studies.

**Antimicrobial Sensitivity Test**
Antibacterial activity of field grown *Anisomeles malabarica* was screened against gram positive and gram negative bacteria such as *E.coli, S.aureus, P.mirabilis, P.aeruginosa, k.pneumonia*. Both extracts showed varying of inhibitory effects. The inhibitory effects of extracts were directly proportional to the increasing concentration of field grown leaf. The ethanolic extract produced good antimicrobial activity at 200µg/ml. The ethanolic extracts showed a 25mm zone of inhibition against *S.aureus* and 24mm zone of inhibition against *P.aeruginosa* and 23mm against *E.coli, k.pneumonia, P.mirabilis* (Table2). Diethyl ether extracts produced 30mm, 25mm, 24mm, 23mm, 25mm against *E.coli, S.aures, P.mirabilis P.aeruginosa, k.pneumonia*
respectively (Table3). Reports from other workers also support the present study. It was reported that the methanolic extract of *Anisomeles malaborica* showed inhibitory activity for Gram-positive and negative bacteria [11]. Some others works also support the present study [12] and revealed microbiological activity of *Anisomeles malaborica* on *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus pumilus* and *Staphylococcus aureus*.

**TABLE-1 Preliminary phytochemical screening of Anisomeles malabarica (L) R.Br**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Metabolites</th>
<th>Ethanolic extract</th>
<th>Diethyl ether extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ indicates presence of particular metabolites. - indicates absences of particular metabolites.

**TABLE -2 Antibacterial activity of ethanolic extract of Anisomeles malabarica**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Test microorganism</th>
<th>Zone of inhibition(mm) in different concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>control 50µg/ml 100µg/ml 150µg/ml 200µg/ml</td>
</tr>
<tr>
<td>1</td>
<td><em>S. aureus</em></td>
<td>C 20 22 24 25</td>
</tr>
<tr>
<td>2</td>
<td><em>E. coli</em></td>
<td>C 23 20 21 23</td>
</tr>
<tr>
<td>3</td>
<td><em>P. aeruginosa</em></td>
<td>C 20 20 22 24</td>
</tr>
<tr>
<td>4</td>
<td><em>P. mirabilis</em></td>
<td>C 21 22 21 23</td>
</tr>
<tr>
<td>5</td>
<td><em>K. pneumonia</em></td>
<td>C 18 19 21 23</td>
</tr>
</tbody>
</table>

**TABLE -3 Antibacterial activity of diethyl ether extract of Anisomeles malabarica**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Test microorganism</th>
<th>Zone of inhibition(mm) in different concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>control 50µg/ml 100µg/ml 150µg/ml 200µg/ml</td>
</tr>
<tr>
<td>1</td>
<td><em>S. aureus</em></td>
<td>C 15 15 25 30</td>
</tr>
<tr>
<td>2</td>
<td><em>E. coli</em></td>
<td>C 15 15 21 25</td>
</tr>
<tr>
<td>3</td>
<td><em>P. aeruginosa</em></td>
<td>C 15 15 22 24</td>
</tr>
<tr>
<td>4</td>
<td><em>P. mirabilis</em></td>
<td>C 30 20 20 23</td>
</tr>
<tr>
<td>5</td>
<td><em>K. pneumonia</em></td>
<td>C 15 20 21 25</td>
</tr>
</tbody>
</table>

**REFERENCES**


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