Original article

Therapeutic properties of extracts of Leucas aspera and Anisomeles malabarica

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ABSTRACT

Leaves of two plant extracts from Leucas aspera and Anisomeles malabarica are studied for their therapeutic activity. These plants have been evaluated for antibacterial property against Bacillus cereus and Pseudomonas aeruginosa. The plant extracts showed low antibacterial effects on the selected microbes. However, the methanol leaf extracts showed significant antibacterial activity. The determination of anticancer activity showed that the plants are poor in substances with appreciable therapeutic potential.

1. Introduction

Leaves of plants with varied therapeutic potential have known to benefit humans. These plants are selected as they are known to possess useful properties like anti-inflammatory, anti-cytotoxic, hepato-protective effects, etc.

Leucas aspera (Wild.) Linn. belonging to the famiy of Lamiaceae is popularly known to grow all over India. This beneficial herb rises to an altitude of 15-60 cm with stout and hispid acutely quadrangular branches and stems [1]. Its leaves are found beneficial in treatment of psoriasis, chronic rheumatism and other chronic skin eruptions. L. aspera plant is found to be useful for cure of respiratory tract disorders, gastrointestinal disorders, edema, and used as an antidote to poison [2]. Antiplasmodial activity of the leaf extract of plant against chloroquine-sensitive (3D7) strain of Plasmodium falciparum has been discovered [3].

The methanol extract of L. aspera flowers and its fractions has been found to show the good antibacterial activity. The expressed flower juice and alkaloidal residue also exhibited good antibacterial activity [4]. The L. aspera root ethanolic extract is found to inhibit acetic acid induced writhing in mice at the doses of 250 mg/kg and 500 mg/kg. The extract revealed an important free radical scavenging activity with an IC50 of 8 μg/ml [5].

Anisomeles malabarica is a highly scented plant belonging to the family Lamiaceae (Labiatae) and is commonly well-known as Malabar catmint. It is a perennial plant which has woolly stems and grows 50 – 150 cm tall. The leaves and roots are useful as an astringent, a carminative, febrifuge and a tonic. It is found to be used or the treatment of amentia, colic, fever, dyspepsia, etc.

The plant leaves are found active against convulsions, colic, for dyspepsia in intermittent fevers, boils, tetanus, inflammation, cough, cold, stomachache, itches and in uterine affections. Inflorescence and leaves extracts have been found to control mosquitoes. This plant extract have known to possess antibacterial and anticancer properties.

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Antibacterial property

An antimicrobial agent is a substance which kills or prevents the development of microorganisms. Due to continuous use of antibiotics, the microorganisms have developed resistance. Sometimes industrial antibiotics are associated with side effects such as low immunity and allergic reactions [6]. Hence, plant materials remain an important resource to fight severe infections in the world. A scientific study has been conducted on plants to regulate their antimicrobial activity in compounds, is a reasonably new field approach.

Anticancer Activity

Cancer is a devastating disease which occurs with no specific symptoms and no specific age. There is a constant demand for new therapies to treat and prevent this life-threatening disease. The disease is characterised by cells in the human body continually multiplying with the inability to be controlled or stopped. The cells consequently form malignant tumours with the potential to be metastatic [7]. Current treatments include chemotherapy, radiotherapy and chemically derived drugs. Treatments such as chemotherapy can put patients under a lot of strain and further deteriorate their health. Therefore, there is a focus on using alternative treatments and therapies against cancer particularly from plant origin [8].

In the present study, Leucas aspera and Anisomeles malabarica leaves extract are tested for their potentials as antibacterial and anticancer agents. Antibacterial property is evaluated by agar well diffusion method (Mixed culture method) and the anticancer potential is estimated by MTT Assay.

MATERIALS AND METHODS

Plants Material

The plants, Leucas aspera and Anisomeles malabarica were collected from Devarayana Durga Hills, Tumkur District. 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.

Preparation of plant extracts

Leaves of plants are collected separately, washed thoroughly and dried under the shade. These leaves are coarsely powdered and packed in Soxhlet apparatus and is continuously extracted individually using solvents such as ethanol, methanol, water, chloroform till all the constituents are extracted. Later, rotary vacuum evaporator was employed to concentrate the extracts.

Phytochemical Analysis of the Extracts

The leaves extracts of Leucas aspera and Anisomeles malabarica were subjected to phytochemical examinations as per the standard methods adopted as per Brain and Turner (1975) and Evans (1996).

Determination of Anti Bacterial Activity

The antibacterial activity was determined by agar well diffusion method [11] against Bacillus cereus, Pseudomonas aeruginosa.

Petri plates containing 20 ml Nutrient Agar Medium were seeded with 24 hr culture of bacterial strains B. cereus and P. aeruginosa. Wells were cut and 50 µl of the plant extracts were added with different concentrations (1 mg/ml, 5 mg/ml, 10 mg/ml, standard- Ciprofloxacin 10 µg/ml). The plates were then incubated at 37 °C for 24 hours. The antibacterial activity was recorded by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993) [9, 10, 11].

Anticancer activity

MTT cytotoxicity assay for in vitro anticancer study

The cytotoxicity assay was performed according to the microculture MTT method with slight modifications [12]. The cancer cells MCF-7 obtained from Merck, which are cultured and harvested (1.5 x 104 cells/well) and inoculated in 96-well microtiter plates. They were washed with phosphate-buffered saline (PBS) and the cultured cells were then inoculated with and without the extract. After 72 h of incubation, the medium was aspirated. Ten microliters of MTT solution (5 mg/ml in PBS, pH 7.2) was added to each well and the plates were incubated for 4 h at 37 °C. After incubation, 100 µl of dimethyl sulfoxide (DMSO) was added to the wells followed by gentle shaking to solubilize the formazan dye for 15 min. Absorbance was read at 570 nm and the surviving cell fraction was calculated. Camptothecin was used as the reference standard for anticancer activity.

The inhibition of cell viability calculated using the formula: [12]

Inhibition activity (%) = 1-T/C X 100

Where T= Absorbance of the test sample, C= Absorbance of the control sample

Results

Antimicrobial Activity

The zone of inhibition was measured after treatment of bacteria with plant extracts.

Figure 3: Petri dishes showing the zone of inhibition of extracts (crude).
Table 1: Antibacterial activity of methanolic extracts of Leucas aspera and R2 is Anisomeles malabarica. R1; Leucas aspera, R2; Anisomeles malabarica, M; methanolic extract.

<table>
<thead>
<tr>
<th>Plant Extract (Crude)</th>
<th>Zone of Inhibition (mm)</th>
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<tbody>
<tr>
<td>R1M</td>
<td>80.00 60.00 9.5 34</td>
</tr>
<tr>
<td>R2M</td>
<td>80.00 10.5 13 35</td>
</tr>
</tbody>
</table>

Then Agar well diffusion method was carried out for R1M (alkaloids), R1M (flavonoids), and R2M (alkaloids) R2M (flavonoids), the concentration of the extracts used for both alkaloids and flavonoids were 1 mg/ml, 5 mg/ml, 10mg/ml and that of standard Ciprofloxacin (Standard antibacterial agent) 10 µg/ml. The experiment was carried out in triplicates.

Fig. 4. Antibacterial activity of methanolic extracts of Leucas aspera (R1) and Anisomeles malabarica (R2). Fla; flavanoids; Alk; alkaloids.

Anticancer activity

The cytotoxicity assay was performed according to the microculture MTT method with slight modifications. The results are tabulated (data not shown). R1M alkaloid fraction showed an IC50 of 647.54, and R2M alkaloid fraction showed IC50 462.90. R1M flavonoid fraction showed an IC50 of 564.42, and R2M flavonoid fraction showed IC50 623.74. The effects of plant extracts alkaloid fraction on cancer cells is represented graphically (Fig.3 & 4). The influence of plant extracts flavanoid fraction on cancer cells is represented graphically (Fig.5 & 6).

Fig. 4. Effect of alkaloid extract (R2M) on MCF cell viability.

Fig. 5. Effect of flavanoid extract (R1M) on MCF cell viability

Fig. 6. Effect of flavanoid extract (R2M) on MCF cell viability.
Conclusion

Herbal medicine is known as botanical medicine or phytomedicine. It refers to use of plant seeds, roots, berries, leaves, bark and flowers for medicinal purposes. Recently, the World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care. Herbal medicines have been the oldest forms of health care. In this study, Leucas aspera and Anisomeles malabarica plant extracts have been investigated for antibacterial and anticancer properties. These plant extracts showed poor antibacterial and anticancer properties when treated as crude extracts. However, alkaloid and flavonoid fractions obtained after methanol extraction showed appreciable antibacterial and anticancer activities.

Conflict of Interest

Authors declare no conflicts of interest.

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