International Multidisciplinary Research Journal 2011, 1/2:15-19
ISSN: 2231-6302
Available Online: http://irjs.info/
IRMJ-Pharmacology

Antimicrobial activity of various extracts of whole plant of *Anisomeles malabarica* (Linn.) R. Br.

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Abstract: *Anisomeles malabarica* (Linn.) R.Br. is a traditional medicinal plant of Lamiaceae family, distributed throughout India. All the three extracts petroleum ether, Ethyl Acetate, methanol of *Anisomeles malabarica* (Linn.) R.Br. were tested for Antimicrobial efficacy against gram positive and gram negative bacterial and then fungal organisms. The methanolic extract of *Anisomeles malabarica* (Linn.) R.Br. were exhibited maximum antibacterial and anti fungal activity when compared with other two extracts. Hence it can be concluded that the methanolic extract of *Anisomeles malabarica* (Linn.) R.Br. possess a significant Antimicrobial activity. This also stands as a scientific support for the usage of this plant for wound healing.

Key words: *Anisomeles malabarica*, medicinal plant, Lamiaceae, antimicrobial

INTRODUCTION

Anisomeles malabarica (Linn.) R. Br. (Family: Lamiaceae) is a medicinal plant has been used as a folkloric medicine to treat amentia, anorexia, fever, swelling, rheumatism (Chopra et al., 1956). The herb is reported to possess antibacterial, antiallergic, anti-inflammatory, antiseptic, antinociceptive properties (Jeyachandran et al., 2007).

Botanical information

Name	:	*Anisomeles malabarica* (Linn.) R.Br.
Family	:	Lamiaceae.
Local Name	:	Codhara
Habit	:	Herb
Tamil Name	:	Peyimarutti
Telugu Name	:	Moga-biran, Mogabheri
Sanskrit Name	:	Mahadronah
Malayalam Name	:	Perumtumpa, Karintumpa

Description

A small perennial shrub grows up to 1.5 meters in height. Leaves simple, opposite, very thick, aromatic, oblong-lanceolate, acute, pale above, white below, crenate-serrate, softly woolly: flowers purple, in dense whorls of more or less interrupted spikes; fruits nutlets, bearing ellipsoid and compressed seeds.

Received: April 29, 2011; Revised May 21, 2011; Accepted May 21, 2011.

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TRADITIONAL MEDICINE

Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being. Countries in Africa, Asia and Latin America use traditional medicine (TM) to help meet some of their primary health care needs. In Africa, up to 80% of the population uses traditional medicine for primary health care. In industralized countries, adaptations of traditional medicine are termed "Complementary" or "Alternative" (CAM).

ANTIMICROBIAL ACTIVITY

Management of infectious disease is still a major problem for health care administration throughout the world more particularly in third world countries due to socio economic factors even after in event of potent chemotherapeutic agent, immunization techniques and improvement in social factors affecting health of most various terminologies are use to describe to extent, type and severity of infections.

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection (Graybill, 1988) (Dean and Burchard 1996) (Gonzalez et al., 1996). In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections (Sieradzki and Tomasz 1999). The search for compounds with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of

infection by antibiotic-resistance microorganisms (Davis, 1982). Recently, Multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Service, 1995) making it a global growing-problem.

The use of higher plants and their preparations to treat infectious diseases is an age-old practice and in the past possibly the only method available. However, the systematic study of higher plants for detecting antimicrobial activity is of comparatively recent origin (Skinner, 1995). Moreover, many of these plants have been known to synthesize active secondary metabolites such as phenolic compound found in essential oils with established potent insecticidal (Kambu, 1982) and antimicrobial activities, which indeed has formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies (Reynolds, 1996; Lis-Balchin, 1997).

Scope and Plan of Work

The objective of the present work was carried to prepare the the whole plant extract of *A. malabarica* by various solvents like petroleum ether, ethyl acetate and methanol to find out the colour consistency and percentage of extracts and to evaluate the antimicrobial activities of various extracts.

The therapy against infectious diseases is emerging with drug resistance. So search for new drugs always remain a challenge in medical field. The antimicrobial efficacy of various extracts of whole plants of *A. malabarica*. may bring to light, a lead molecule for antimicrobial treatment to save the suffering human population.

MATERIALS AND METHODS

Materials

I. Collection and identification of plants

The whole plant of *A. malabarica* found in chengalpattu, Kanchipuram District of Tamilnadu, The leaves of the plant were collected in the month of August - September. The plant was authenticated by a Botanist Dr. P. Jayaraman, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai-45.

II. Other materials

Soxhlet's apparatus
Rotary vacuum evaporator
Solvent like petroleum ether, Ethyl Acetate, methanol
Microbiological medium.
Microorganism used
Gram Positive microorganisms
Gram Negative microorganisms
Selected fungal species.

Methods

1. Preparation of extracts

The collected plants were washed with distilled water to remove adhering materials. Then it was dried at room temperature not exceeding 50ºC. The dried plant material sliced into small pieces and pulverized by mechanical grinders. The powdered materials extracted with petroleum ether in soxhlet apparatus (Harborne, 1984). The marc was then extracted successively with ethyl acetate and methanol using the same methods. The extract were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

2. Find out colour, consistency and percentage of extracts

The extracts were collected and performed test for the colour consistency and percentage of extract were calculated for different extracts of *A. malabarica*.

3. Anti Microbial Studies

i. Test solution

Test solution of each extract was prepared by dissolving 1gm of each extract separately in 10ml of sterile dimethyl formamide (DMF) in a specific gravity bottle and stored in refrigerator. The solution was removed from the refrigerator one hour prior to each use and allow to warm at room temperature.

ii. Standard solution

The standard antibiotic ciprofloxacin was dissolved in sterile distilled water. This was used as a standard for this study.

iii. Preparation of medium

Nutrient broth was used for preparation of inoculum of bacteria. Nutrient agar was used for preparation of medium for Antimicrobial screening. The composition of nutrient agar medium was as follows.

Peptone	- 5.Og
Beef extract	- 1.5g
Yeast extract	- 1.5g
Agar	- 1.5g
Distilled water	- 1000MI
pH adjusted	-7.2

iv. Preparation of inoculum

Inoculum was prepared by transferring a loopful of stock culture to a 150ml of Erlenmeyer containing 80ml of nutrient broth. The composition of inoculum broth was same as that of stock culture with exception of agar. The inoculum flasks were incubated at 37'c for 24 hrs and used for experiments.

v. Inoculation

The nutrient agar medium was sterilized by autoclaving at 121°C for 15 mints. The petridisc and pipette were sterilized in an oven at 150°C for one hour. About 25ml melted nutrient agar medium (40°-50°C) was poured in each sterilized petridishes and 0.5ml of inoculum broth of bacteria was added to the respective petridishes. The content petridishes were thoroughly maintained at rotary motion. The medium containing inoculum was allowed to solidify at room temperature. After solidification of the medium, fine whattman filter paper disc were made it equal distance. The different concentrated extract of test and standard solution as well as blank were dipped in whattman filter paper disc6 and kept in the petridish and the petridish undisturbed for one hour at room temperature. The petridish were incubated at 37°t/ Vc for 24 hours and the zone of inhibition were recorded in l5mm. The experiment was performed in triplicate and the average readings are recorded.

vi. Determination of zone of inhibition

A suitable dilution of a broth culture or a broth suspension of the test bacterium is flooded on the surface of a solid medium (Mueller Hinton agar). The plate is tilted to ensure uniform spreading and the excess broth pipetted off. Inoculations may also be performed by spreading with swabs. After drying the plates (37°C for 30 minutes) antibiotic discs applied with sterile forceps. After overnight incubation, the degree of sensitivity is determined by measuring the zones of inhibition of growth around the discs.

RESULT AND DISCUSSTION

The whole plant of *A. malabarica* found in chengalpattu, Kanchipuram District of Tamilnadu. The leaves of the plant were collected in the month of August - September. The survey of literature revealed that this plant has not been screened on its Antimicrobial studies. The colour, consistency and yield of extract were recorded and represented in Table 1.

Table 1: The colour, consistency and yield of extract

S.No.	Extracts	Colour	Consistency	% of extract
1.	Pet. Ether	Greenish Brown	Viscous	4.32%
2.	Ethyl acetate	Light yellowish Brown	Viscous	6.31%
3.	Methanol	Brown	Viscous	14.33%

The both bacterial and fungal organisms were screened with Pet. ether, Ethyl acetate and Methanolic extracts of *A. malabarica* were tested for Antimicrobial activities. Ciprofloxacin and Vancomycin used as a reference standard for the present investigation. The result of Antimicrobial activity of different extracts of *A. malabarica* was illustrated in Table2.

Table 2: Antimicrobial activity of various extracts of whole plant of Anisomeles malabarica (Linn.) R.Br.

Gram positive bacterial species:

Zone of inhibition (mm)					
Micro-organisms	Pet. Ether extract (100mg/ml)	Ethyl acetate extract (100mg/ml)	Methanol extract (100mg/ml)	Standard ciprofloxacin (5mg/ml)	Blank DMF
Bacillus cereus	9	8	15	16	0
Bacillus subtilis	8	9	15	17	0
Bacillus pumilis	7	9	18	20	0
Staphylococcus aureus	8	12	14	21	0

Gram negative bacteria species:

Zone of inhibition (mm)					
Micro-organisms	Pet.ether extract (100mg/ml)	Ethyl acetate extract (100mg/ml)	Methanol extract (100mg/ml)	Standard ciprofloxacin (5mg/ml)	Blank DMF
Escherichia coli	9	12	16	15	0
Klebsiella pneumonia	8	10	12	17	0
Pseudomonas aeruginosa	9	13	19	20	0
Salmonella typhimurium	7	11	19	20	0

Fungal organisms:

Zone of inhibition (mm)					
Micro-organism	Pet.ether extract (100mg/ml)	Ethyl acetate extract (100mg/ml)	Methanol extract (100mg/ml)	Standard Vancomycin (30mg/ml)	Blank DMF
Candida albicans	10	12	15	15	0
Aspergillus niger	9	10	14	5	0
Microsporum gypseum	7	13	17	20	0
Trichophyton tonusurans	7	15	18	20	0

Based on the results obtained in the present study was revealed that the methanolic extract of *A. malabarica* were exhibited maximum antibacterial and anti fungal activity when compared with other two extracts. Thus the antimicrobial activity of the methanolic extract of *A. malabarica* will have a positive effect on wound healing.

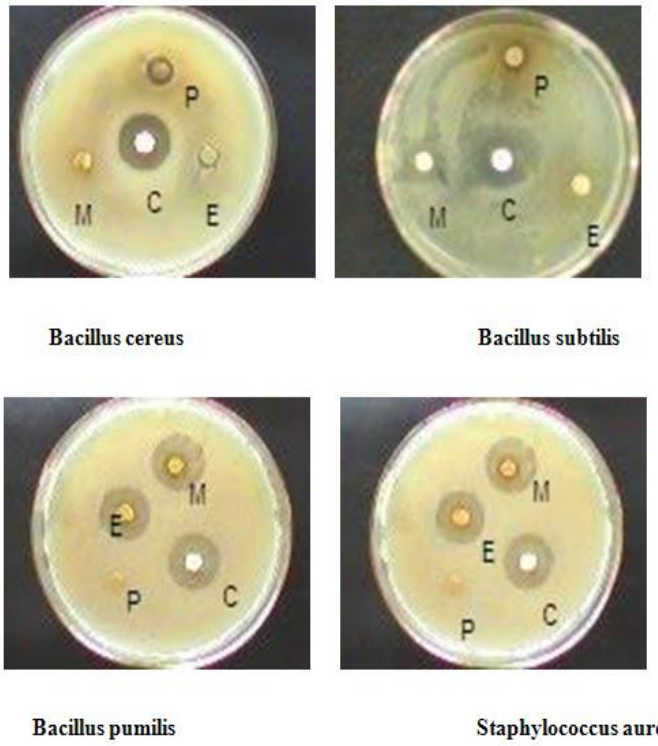

Fig. 1. Antibacterial activity of various extracts of whole plant of *Anisomeles malabarica* (Linn.) R.Br. (Gram positive bacterial species)

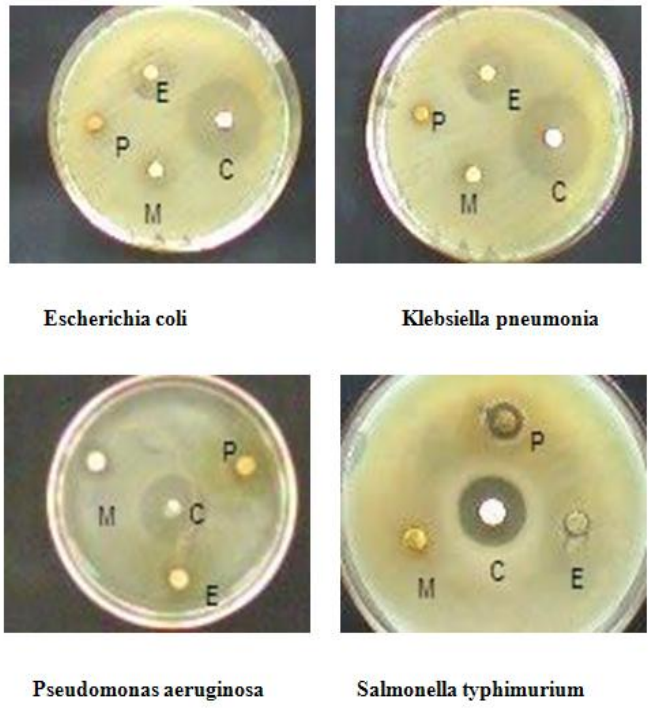

Fig. 2. Antibacterial activity of various extracts of whole plant of *Anisomeles malabarica* (Linn.) R.Br. (Gram negative bacterial species)

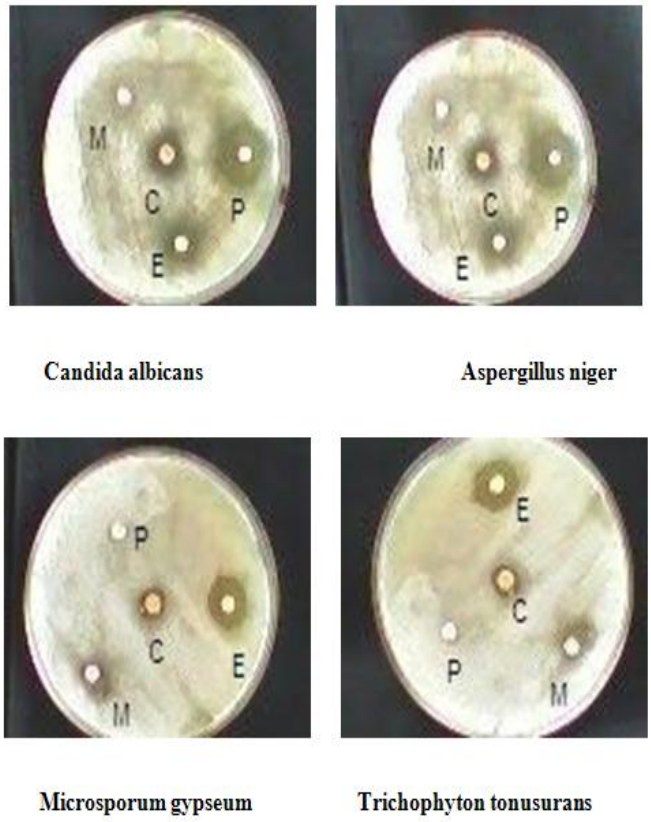

Fig. 3. Antifungal activity of various extracts of whole plant of *Anisomeles malabarica* (Linn.) R.Br.

SUMMARY AND CONCLUSION

The whole plant of *A. malabarica* found in chengalpattu, Kanchipuram District of Tamilnadu. The survey of literature revealed that this plant has not been screened on its Antimicrobial studies. All the three extracts of *A. malabarica* were tested for Antimicrobial efficacy against gram positive and gram negative bacterial and then fungal organisms. The methanolic extract of *A. malabarica* was exhibited maximum antibacterial and anti fungal activity when compared with other two extracts. Hence it can be concluded that the methanolic extract of *A. malabarica* possess a significant Antimicrobial activity. This also stands as a scientific support for the usage of this plant for wound healing.

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