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Preliminary Phytochemical Screening and Antibacterial Activity of Anisomeles Malabarica Roots

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Abstract:

The present study was carried out to evaluate phytochemical screening, characterization and In-vitro antibacterial activity of Anisomeles malabarica roots. The antibacterial activities of A.Malabarica roots were assessed by disc diffusion method against four bacterial strains S.aureus, B.subtilis, Escherichia coli and Pseudomonas aeruginosa. The preliminary phytochemical analysis of A.Malabarica roots using the solvents like n-Hexane, Ethyl acetate and Methanol revealed the presence of alkaloids, flavonoids, tannins, saponins, and glycosides. The antibacterial activities show that the methanol extracts exhibited an active antibacterial activity at 50µg/ml and produced 6.5 mm zone of inhibition against B.subtills and P.aeruginosa 7 mm zone of inhibition in the same concentration. The hexane and methanol extracts exhibit a positive antibacterial activity which produced 6 mm and 5.5 mm (both two bacterial strain) zone of inhibition. The resulting sample was analyzed by FT-IR to find out the functional group present in the extracts. The FT-IR spectrum confirmed the presence of alkanes, alcohol, ethers, carboxylic acid, amines and phenyl ring substituted the bond.

Keywords: Anisomeles Malabarica roots, phytochemical screening, antibacterial activity and FT-IR spectrum.

Full Length Original Research Paper

Introduction

Medicinal plants are the richest bio-resources of drugs of the traditional system of medicine, modern medicine pharmaceutical intermediates and chemical entities for synthetic drugs (1). Plants are the main source of food. Over the centuries, societies around the world have developed their own tradition to make sense of medicinal plants and other use. The exploitation of plant by man for the cure of diseases has been in applied for a very long period. They are also rich in compounds that have pain-relieving and remedial ability. From initial times itself, plants were used for behavior of the disease without knowledge about the compounds present and their mode of action (2).The World health organization estimated that 80% of the population of development countries rely on traditional medicine mostly plant drugs for their primary health care needed. Medicinal plant has no side effect (3). Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the moving components by using selective solvents. During extraction, solvents concentrate into the solid plant material and solubilize compounds with related polarity (4). Medicinal plant extract used as the tinctures (liquid extracts) to be incorporated in any dosage form such as tablets and capsules. These products contain the complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, and flavonoids (5). Anisomeles Malabarica (Malabar catmint), Nepeta Malabarica, Family- Lamiaceae is a medicinal plant are used as a folk medicine to treat amentia, anorexia, fevers, swellings, rheumatism (6).The medicinal plants A.Malabarica is reported to possess anticancer, allergenic, anthelmintic,

antiallergic, antianaphylactic, antibacterial, anticarcinogenic, anti ecdemic, antihistaminic, anti-inflammatory, antileukemic, anti plasmodial, antiseptic and antibiotic properties (7-13).The present study was aimed for phytochemical screening of three different extracts and evaluate the antibacterial activity.

Materials and Methods

Plant collection

The raw material of medicinal plant such as A. Malabarica is collected from Government College of engineering, Salem district, Tamil Nadu. The fresh plant roots material was air dried at room temperature and then grained to get homogenize fine powder as shown Fig.1 A and B.

Fig.1A) Anisomeles malabarica roots

B) Anisomeles Malabarica roots Powder

Extraction of Anisomeles Malabarica Roots

The dried powdered of plant material was extracted with different solvents like n-Hexane, ethyl acetate and methanol using Soxhlet apparatus for 8 hrs. The solvent was concentrated with a rotary evaporator at reduced pressure the crude extract which was stored in desiccators for future use.

Phytochemical Screening

The Phytochemical screening for the plant extract was carried out using standard chemical procedure (14)

FT-IR (Fourier transform infra-red) spectroscopy

A single-beam FT-IR spectrometer (FT-IR-7600, Lambda Scientific). The FT- IR spectra were recorded using KBr disc for the successive extracts.

Antibacterial activity

The extracts obtained were screened In-vitro for their antibacterial activity against gram positive and gram negative bacterial strains by sop for Disc Diffusion method using cultivated on a suitable agar medium under optimal incubation conditions to obtain a fresh overnight grown culture (Bauer et-al 1966) (15). The bacterial strains used for the determination of antibacterial activity are Escherichia coli, Staphylococcus aureus, and Bacillus subtilis Pseudomonas aeruginosa.

DAY 1

1. The solutions of the extracts were prepared at 5ml in dimethyl sulfoxide (DMSO).

DAY 2

2. Harvest a number of distinct colonies from the fresh grown plate culture to suspend in a tube containing broth until turbidity (visually) corresponding to 1.0 McFarland standard is reached. Using a sterile cotton swab dipped into the adjusted culture medium and squeezed. Then made a lawn culture on Muller – Hinton Agar media. Allow to dry the plates for max. 15 minutes. Longer drying times allow pre-incubation of the cells that should be avoided. Plates should be incubated as soon as possible after the application of the discs. Using sterile forceps, the discs (Antibiotic or tested compound

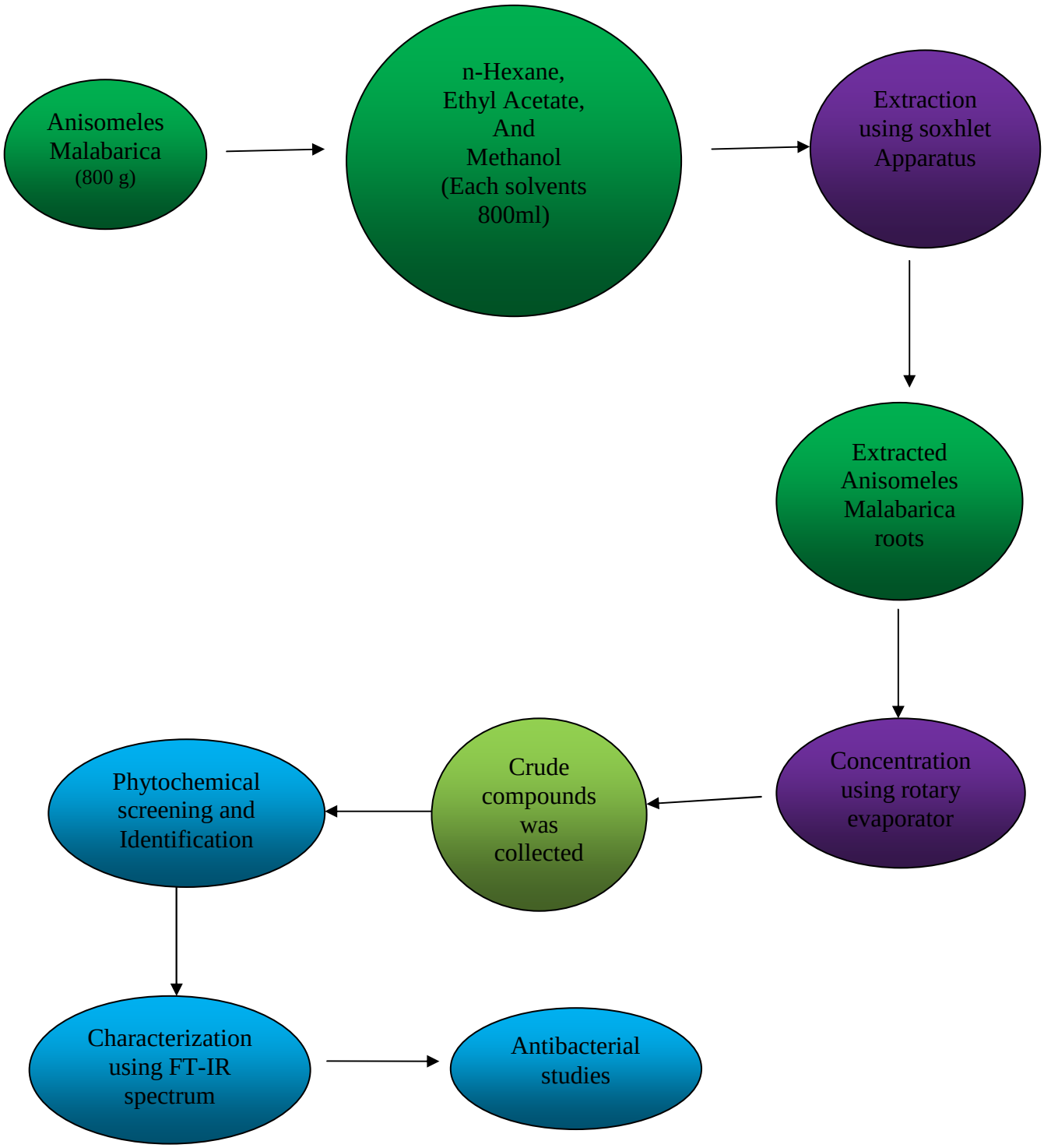
loaded) are applied onto the agar surface. Discs must not be relocated once they have made contact with the agar surface. Incubate the plates under optimal incubation conditions.

DAY 3

3. The diameter of the inhibition zones are measured to the nearest mm from the point of rapid inhibition of growth (using calipers).

FLOWCHART

EXTRACTION OF ANISOMELES MALABARICA



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Results and Discussion

The present study has been carried out to evaluate the plant of A. Malabarica for three different solvents extraction. Phytochemical analyses of the plant extract were carried out, and the results were summarized.The results of the crude extracted compound obtained by using FT-IR, Biological studies. The performance of three different solvents extraction for antibacterial activity was compared.

Phytochemical Screening

The chemical group tests were performed, and the results are mentioned in table .1. Results indicated that alkaloids, saponins, tannins, flavonoids, amino acid, and carbohydrate were detected in the common three different solvents extract. The phytochemical analysis revealed that the plant contains bioactive substances that are connected with the antibacterial properties in plants.

Table 1: Results of phytochemical screening of roots extracts of A. Malabarica

S. No	Test	HEAM	EAAM	MEAM
1	Test for alkaloids			
	a. Dragendraffs test	+	+	+
	b. Wagner test	+	-	-
	c. Hagers test	+	+	+
2	Test for Flavonoids			
	a) Lead acetate test	-	-	+
	b) NaOH	-	-	+
3	Test for Phenols			
	a) $FeCl_3$	-	-	-
4	Test for Tannins			
	a) $FeCl_3$	-	-	+
	b) $K_2Cr_2O_7$	-	-	+
	c) Lead acetate	+	+	+
5	Test for saponin			
	a) Foam test	+	+	-
6	Test for Amino Acid			
	a) Xantho proteic test	-	+	+
	b) Biuret Test	-	-	-
7	Test for Coumarin	+	-	+
8	Test for Starch (Iodine test)	-	-	-
9	Test for Quinone	-	-	+
10	Test for Carbohydrates			
	a) Fehling test	-	-	-
	b) Benedict test	-	-	-
	c) Molishs test	-	+	+
11	Test for glycosides			
	Killer –Killani test	-	-	-
12	Test for terpenoids			
	a) Salkovaski test	-	-	-
	b) Lieberman's test	-	-	-
13	Test for phytosterol			
	a) Salkovaski test	-	-	-
	b) Lieberman's test	-	-	-
14	Test for Anthraquinone			
	a) Extraction $+NH_4OH$	-	-	-
	b) Benzene Test	-	-	-

+ Present, - Absent

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It is clearly indicated from the table that the other phyto-constituents like phenols, terpenoids and glycosides were absent in all the three solvent extracts. These results suggest the presence of primary bioactive metabolite that acts as the precursors for the synthesis of secondary metabolites. These turns help in the development of new bio products for future.

FT-IR – Spectrum

FT-IR spectrum of A.Malabarica roots extracts was taken for plant material. The spectroscopy can also be usefully contributed to structural elucidation when new compounds are encountered in plants.FT-IR spectra were taken for hexane, ethyl acetate and methanol extract of A.Malabarica roots. The FT-IR spectrum profile is illustrated in the Figures .2, 3 and 4. The spectrum was recorded in the wavelength region between400cm^{-1} to 4000cm^{-1}.The spectrum shows peaks at 3435cm^{-1}, 3393cm^{-1}, and 3431cm^{-1} which indicates the presence of O-H stretching of the carboxyl group and N-H stretching of secondary amides. Further, the peaks observed at 2924cm^{-1}, 2059cm^{-1} represents the C- H stretching bonds of alkanes. The peak observed at 17446cm^{-1}, 1642cm-1, 1640cm^{-1}, 1350cm^{-1}and 1383cm^{-1}, 1593cm^{-1}, 1588cm^{-1} ,1462cm^{-1}, 1383cm^{-1} represent the C-H scissoring and bending aromatic conjugates. The sharp peak at 1258cm^{-1} and 1268cm^{-1} is assigned to C-O that indicates that alcohols and ethers carboxylic acids and esters. The peak observed at 722cm^{-1} which is representing the presence of C-H phenyl ring substitutions bonds.

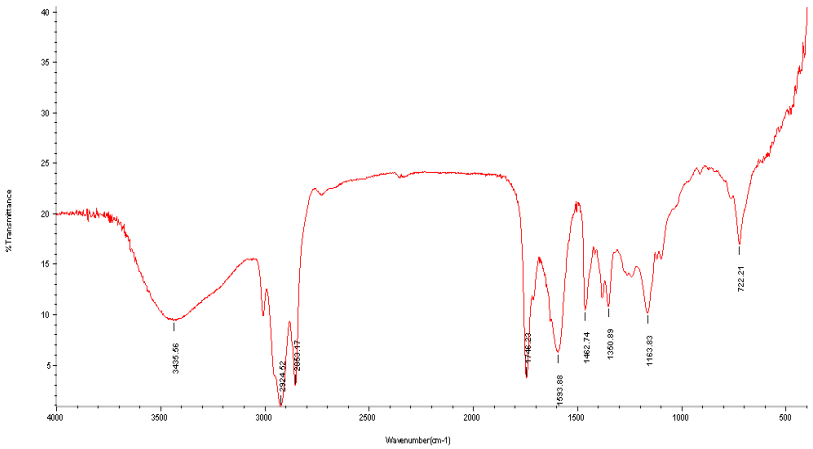

Fig 2: FT-IR Spectrum of Hexane Extraction of a Malabarica roots

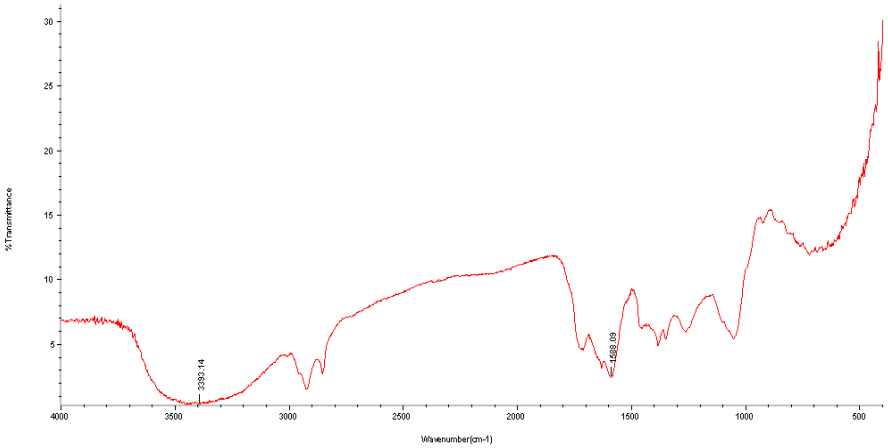

Fig 3: FT-IR Spectrum of Ethyl acetate Extraction of A.Malabarica roots

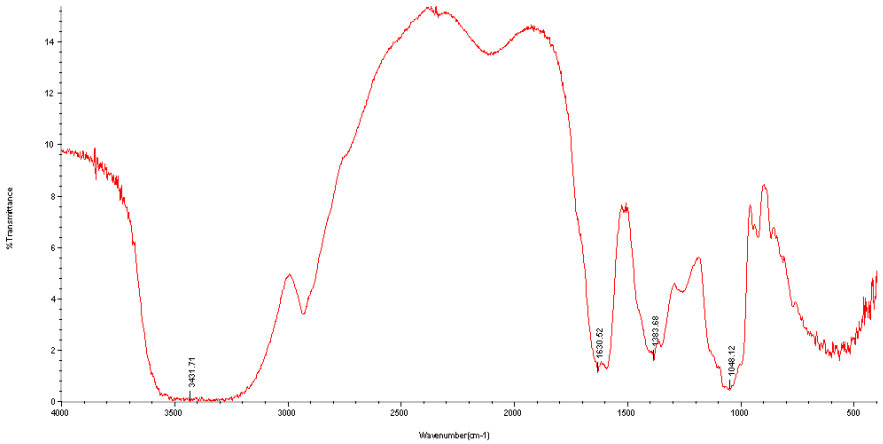

Fig 4: FT-IR Spectrum of methanol extraction of a Malabarica roots

Antibacterial Activity

Antibacterial activity of A.Malabarica was examined by four bacterial strains, such as Streptococcus aureus, Bacillus subtilis are gram +ve and Pseudomonas Auregenosa and Escherichia coli are gram -ve bacteria.

B) P.aeruginosa

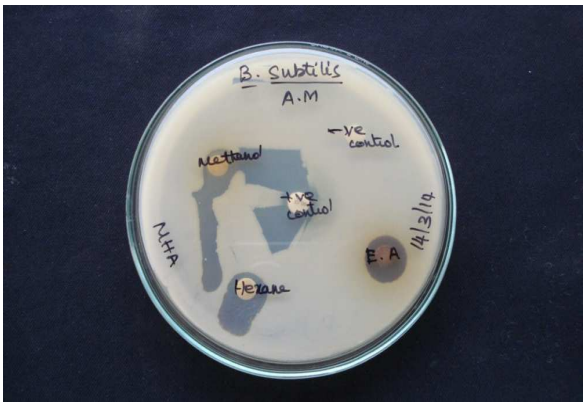

Figure 5: Zone of inhibition of a Malabarica roots extracts using different solvents against Bacterial Pathogens. A) B. Subtillis

Table 2: Antibacterial activity of various roots extracts of A.Malabarica. (Zone of inhibition in mm)

S. No	Extracts	Concentration (ml)	Diameter of zone of inhibition(mm)			
			G+ve		G-ve	
			S.aureus	B.subtilis	P.aeruginosa	E.coli
1	HEAM	50µg	NA	5mm	5.5mm	NA
2	EAAM	50µg	NA	6mm	6mm	NA
3	MEAM	50µg	NA	6.5mm	7mm	NA

The gram +ve bacterial strain using A.Malabarica roots of methanol extract was shown to be effective Zone of inhibition is 6.5 mm ethyl acetate shown to be zone of inhibition 6.0 mm followed by hexane shown to be zone of inhibition 5.0 mm. The gram –ve pathogens A.Malabarica roots methanol, ethyl acetate and hexane extracts were shown the zone of

inhibition values are 7.0 mm, 6.0 mm and 5.5 mm. The order of the antibacterial activities is Methanol > Ethyl acetate > Hexane extract the results are indicated that figure-5. The results clearly show that alkaloids, tannins and Amino acids which were in large quantities found in Methanol, Ethyl acetate and Hexane extracts were responsible for the antibacterial activity of A.Malabarica roots. The antibacterial activity results are given in table 2.The significant antibacterial activity of the all these three extracts of A.Malabarica roots. The extracts might be attributed to the presence of the secondary metabolites in the extracts.

Conclusion

It can be concluded that the extracts of A.Malabarica possess significantly good antibacterial activity. The A.Malabarica roots extracts contain a number of pharmaceutically important phytochemical constituents like alkaloids, saponins, carbohydrates, tannins, flavonoids and amino acid. Biological activities shows that the methanol extracts exhibited 6.5 mm and 7mm zone of inhibition against B.subtills and P.aeruginosa. The FT-IR studies confirmed the presence of the secondary amide, alcohols, ethers, carboxylic acids and esters in the plant extracts. However, further studies are to purify, characterize and test the active molecule for its bioactive compound.

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References

1) Chopra RN, Nayar SL, Glossary of Indian medicinal plants, CSIR, New Delhi, 1956.
2) Jeyachandran R and Mahesh A, International journal of Canadian Research, 3(4), 2007.
3) Govindachari TR, Joshi BS, Viswanathan N, The phenolic constituents of semecarpus and cardium Linn, Indian journal of chemistry, Vol.9,1971.
4) Tomoko N., Takashi A., Journal of Health Science. 48, 273–276. 2002.
5) Chopra RN., Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi 19. 1956.
6) Jeyachandran R., Inter. J Can Res, 3 (4), 174-179. 2007.
7) Odebiyi EO., Journal of Chemistry Social.Nig.261. 1978.
8) Waterman PG, Methods in plant Biochemistry, Academic. vol. 8, 1993.
9) Kavitha, Nelson R. Scholars Research Library Journal Microbiology. Biotech. Res., 2 (1)-1-5, 2012.
10) R Lavanya, S Uma Maheshwari, In-vitro Antioxidant Activity of Methanolic Extract in Leaves of A.Malabarica, RJPBCS, 4, 737, 2010.
11) Vijayalakshmi, Antioxidant potential of various extracts from the whole plant of A.Malabarica, RJPBCS, Vol. 3, 2012.
12) Remya Mohanraj, Bioactivity Studies on A.Malabarica, Journal of Biotechnology and Bio therapeutics, 2, 9.2012.
13) Devi, In vitro antibacterial and antifungal activities of M.Tinctoria leaf in different solvents, 2013.
14) Harborne J B. Phytochemical methods, Chapmann & Hall, London, 1973.
15) Bauer AW., Kirby E., American Journal of clinical. Pathology, 45, 493-496, 1966.

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