PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIFUNGAL ACTIVITY OF ANISOMELES MALABARICA ROOT EXTRACT USING ETHANOL AS THE SOLVENT AGAINST CANDIDA ALBICANS

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1. ABSTRACT:

AIM:

The aim of study is (preliminary phytochemical screening and antifungal activity of Anisomeles malabarica a root extract by using solvent as ethanol.

OBJECTIVE:

Preliminary phytochemical studies of Anisomeles malabarica and antifungal activity of root extraction.

METHOD:

The root were collected dried and ground into fine powder. The powdered material was then subjected to solvent extraction using ethanol. The obtained ethanolic extract was subsequently used for preliminary phytochemical screening to identify the presence of bioactive compounds. In addition, the extract was evaluated for its antifungal activity to assess its effectiveness against fungal organism.

CONCLUSION:

The present study reveals the preliminary phytochemical analysis and antifungal activity by using anisomeles malabarica extract.

KEY WORDS:

Anisomeles malabarica, Soxhlet method, Ethanol, Phytochemical screening, Antifungal activity.

2. INTRODUCTION

Anisomeles malabarica is a strongly aromatic ,perennial herb characterized by its dense pubescence.Belonging by the common name Malabar catmint.

This herb is a naturally found and widely distributed in tropical and subtropical regions, where the climate supports its vigorous growth . It typically flourishes in open fields along road sides and near forest margins.

Anisomeles malabarica is a herbaceous howering plant and typically reaches, 1-2meters in height. It bears opposite hairy leaves. That release strong mint like aroma.

Anisomeles malabarica has been used to treat digestive issues, fever, skin infection, and inflammatory disorders. The leaves are sometimes applied as poultices to treat wounds, insect bites and minor burns.Its bitter and pungent taste is believed to help stimulate digestion and appetite.

Commonly known as Malabar catmint, or Malabar vayana, this plant belongs to the mint family, Lamiaceae. It is widely distributed in India, Sri Lanka, and other parts of south and southeast Asia.

Fig.No.1.Anisomeles Malabarica

3. TAXONOMICAL CLASSIFICATION:

- **KINGDOM** : Plantae
- **SUB-KINGDOM** : Viridiplantae
- **SUB-DIVISION** : Angiospermae
- **DIVISION** : Spermatophyta
- **CLASS** : Magnoliopsida
- **SUB-CLASS** : Asteridae
- **ORDER** : Lamiales
- **FAMILY** : Lamiaceae
- **GENUS** : Anisomeles
- **SPECIES** : Anisomeles malabarica

4. VERNACULAR NAME:

- **ENGLISH** : Malabar catmint
- **HINDI** : Gopoli, Codhara
- **MARATHI** : Gojibha
- **TAMIL** : Peyimarutti, Perumtumpa
- **MALAYALAM** : Perumtumpa, Karintumpa
- **TELUGU** : Mogan-biran, Mogabheri

- **KANNADA** : Karitumbi, Gandubirana gida
- **SANSKRIT** : Mahadronah, Vaikunthah
- **ORIYA** : Vaikuntha
- **KONKANI** : Kaktumbo

5. PHARMACOGNOSTICAL STUDIES:

STEM INFORMATION

- Stem color : Green to purplish
- Stem odour : Aromatic
- Stem cross section : Quadrangular shape
- Stem surface : Hairy
- Stem form : Erect, branched

LEAVES INFORMATION

- Leaf color : Green
- Leaf type : Simple
- Leaf arrangement : Opposite
- Leaf shape : Ovate to lanceolate
- Leaf margin : Serrated
- Hairs present : Yes
- Leaf length : 3-8cm
- Leaf width : 1.5-3cm

FLOWER INFORMATION

- Flower color : Purple, Pink or White
- Flower inflorescence : Spike or raceme
- Flower bloom time : Summer to early tall
- Flower shape : Bilabiate (two-lipped)
- Flower petals : 5 Fused
- Flower size : Small,Approximately 1-2cm long

FRUIT INFORMATION

- Fruit color : Green or brown
- Fruit type : Capsule (schizocarp forming nuttets)
- Fruit length : Approximately 2-3mm
- Fruit width : Approximately 1-2mm

ROOT INFORMATION

- Root type : Tap root
- Root colour : Light brown to dark brown
- Root surface : Rough with wrinkles
- Root odour : Aromatic
- Root taste : Slightly bitter
- Root texture : Hard and fibrous
- Root branching : Few lateral roots present
- Root size : Moderately long
- Root nature : Dry

6. ANATOMY

1. LEAF ANATOMY

Epidermis: The leaf has a dorsiventral structure, with both the upper and lower epidermis made up of a single layer of cells and covered by a thin cuticle. . Numerous glandular and non-glandular trichomes are present. Stomata are more abundant on the lower surface.

Vascular Bundle: Vascular bundles are collateral and closed. The xylem is located on the upper side, while the phloem is positioned on the lower side, and a bundle sheath surrounds the vascular tissue.

2. STEM ANATOMY

Epidermis: Single-layered epidermis with thick cuticle. Numerous multicellular trichomes are present. The stem is quadrangular in outline.

Vascular system: Vascular bundles are arranged in a ring. Bundles are conjoint, collateral, and open. Xylem is endarch. Phloem is well developed. Cambium is present between xylem and phloem.

3. ROOT ANATOMY

Epidermis (Epiblema): Single-layered, thin-walled cells without cuticle. Root hairs are present.

Cortex: The cortex is broad and composed of parenchymatous cells that function in food storage..

Endodermis : Well developed with casparian strips. Passage cells present opposite protoxylem.

Pericycle : Single-layered, give rise to lateral roots.

Vascular Tissue : Radial arrangement. Xylem and phloem occur alternately. Xylem is diarch. Protoxylem is exarch.

Pith : Small or absent.

7.MATERIALS AND METHODS

Root material: the roots of anisomeles malabarica were collected from the kolagathur village, Dharmapuri district. The collected roots was thoroughly with distilled water remove adhering soil, and left at root temperature at a dark environment for to weeks. After drying, the roots were ground into a coarse powder using a mechanical grinder.

MATERIALS

- Soxhlet apparatus
- Extraction thimble
- Solvent: Ethanol
- Root powder of Anisomeles Malabarica
- Heating mantle
- Condenser

PREPARATION OF EXTRACTION BY SOXLET APPARATUS

The 50gm of coarse powdered root of Anisomeles Malabarica was taken.

Then the apparatus which all are required like soxhelt apparatus condenser heating mantle round bottom flask taken and assemble.

50 gram of coarse powdered Anisomeles Malabarica taken in the soxhlet apparatus.

Take 500 ml of ethanol and add it to a round- bottom flask.

The extraction is allowed for 18hrs at temperature of $40°C$.

After 18hrs the extraction liquid which is settled in the round bottom flask is collected.

The after extraction all the crude extracts were filtered, and solvent was removed by distillation to obtain the concentrated extract.

The liquid extracts were concentrated separately under vaccum and the resulting dried extracts were preserved in desiccator until further use.

The dried extracts were weighed and the percent yield was calculated.

METHODS:

ANTIMICROBIAL ACTIVITY OF TEST MICROORGANISMS:

Totally one fungal strain were used throughout investigation. Fungal cultures were produced the from Microbial Type Culture Collection at the Institute of Microbial Technology, Chandigarh, India. The fungus used in the study was Candida albicans.

ANTIBACTERIAL ASSAY
I. PREPARATION OF INOCULUMS

Stock cultures were maintained at 4°C on of nutrient agar slopes. For the experiment, Active bacterial cultures of experiment were prepared by transferring a loopful of cells from the stock cultures into test tubes containing Muller-Hinton broth (MHB). They were incubated without agitation for 24 hours at 37° C.. The cultures were then diluted with fresh Muller-Hinton broth to reach optical density corresponding to 2.0 $X10^6$ colony forming units (CFU/ml) for bacteria (Smith & Doe 2018).

II. PREPARATION OF STERILE SWABS

Cotton wool swabs on wooden or plastic applicators were prepared and sterilized. Wooden swabs were sterilized using dry heat ,while others were sterilized by autoclaving. The swabs were packed in culture tubes, papers, or tins before sterilization.

III. STERILIZATION OF FORCEPS

Forceps can be sterilized by dipping them in alcohol and then passing them through a flame to burn off the alcohol.

ANTIFUNGAL ACTIVITY: (WELL DIFFUSION METHOD)

The antifungal activity was evaluated using the well diffusion method on agar plates. Different concentration of plant extract were dissolved in water. Sabouraud Dextrose Agar (20ml) was poured into 15cm petri dishes. Candida albicans were grown in Sabouraud Dextrose Broth at 27°C for 48 hours .and growth was adjusted to an optical density(OD 600nm) of 0.1 using fresh broth dilution with. Wells were then cut into the agar, and the extract was added at concentrations of 25, 50 and 75 μL of each sample(1mg /mL). Fluconazole (100 units) was used as a positive control. After inhibition at 27° C for 48 hours, the zones of inhibition were measured to asses antifungal activity.

8.PHYTOCHEMICAL ANALYSIS OF ANISOMELES MALABARICA

1. TEST FOR ALKALOIDS:

Dragendroff's Test:
About 1 ml of plant extract is taken and 1 ml of Dragendorff's reagent is added. The appearance of an orange-red precipitate confirms the presence of alkaloids.

Mayer's Test:
To test for alkaloids, 1ml of the extract is mixed with 1ml of Mayer's reagent. The appearance of a cream or pale yellow precipitate indicates the presence of alkaloids.

2. TEST FOR GLYCOSIDES

Test solution: It was prepared by dissolving sample in alcohol.

Keller-Killiani Test:
The sample is dissolved in alcohol. A few drops of ferric chloride solution are added, followed by concentrated sulphuric acid along the side of the test tube. Formation of two layers, with a reddish-brown lower layer and bluish-green upper layer, confirms cardiac glycosides.

3. TEST FOR TRITERPENOIDS:

Test solution: It was prepared by dissolving the extract in chloroform.

Salkowski test:
The extract is dissolved in chloroform and a few drops of concentrated sulphuric acid are added. Absence of yellow coloration in the lower layer indicates triterpenoids are not present.

4.TEST FOR CARBOHYDRATES:

Molisch Test:
To 2-3 ml of extract, a few drops of molisch reagent are added. Concentrated sulphuric acid is carefully added along the side of the test tube.
Formation of a violet ring at the junction confirms carbohydrates.

Fehling's Test:
Equal volumes of Fehling's solution A and B are mixed and added to the extrat, then heated. A brick-red precipitate indicates reducing sugars.

5. TEST FOR PROTEINS:

Test solution: It was prepared by dissolving extract in water and making aqueous extract.

Biuret Test:
About 3 ml of extract is treated with sodium hydroxide solution followed by a few drops of copper sulphate solution. Development of blue or violet colour confirms proteins.

6. TEST FOR ANTHRAQUINONES:

Borntrager's Test:
The extract is shaken with benzene and filtered. Ammonia solution is added to the filtrate. Appearance of pink, red, or violet colour indicates anthraquinones.

7. TEST FOR FLAVONOIDS:

Test solution: To small amount of extract equal amount of 2M HCL was added and heated for about 30-40 mins. The extract was cooled down and again extracted with ethyl acetate which was further concentrated to dryness and ready to be used as test sample.

Lead Acetate Test:
A small amount of extract is treated with lead acetate solution. Formation of yellow precipitate indicates flavonoids.

8. TEST FOR QUINONES:

Concentrated Hcl Test:
When concentrated hydrochloric acid is added to the extract, development of green colour confirms Quinones.

9. TEST FOR TANNINS:

Test solution: The test solution was prepared by dissolving the extract in water and alcohol.

Ferric chloride test:

Ferric chloride solution is added to the extract. Formation of dark blue or greenish-black colour indicates tannins.

Lead acetate Test:

Addition of lead acetate solution produce a white precipitate, confirming tannins.

10. TEST FOR CHOLESTEROL:

The extract is mixed with 2ml chloroform and 10 drops of acetic anhydride, followed by 2-3 drops of concentrated sulphuric acid. Absence of red-rose colour indicates cholesterol is absent.

11. TEST FOR DITERPIENES:

The extract is dissolved in distilled water and treated with 3-4 drops of copper acetate solution. Appearance of emerald green colour confirms diterpienes.

12. TEST FOR TERPENOIDS:

The extract is mixed with 2ml of chloroform and 3ml concentrated sulphuric acid,then heated in a water bath. Absence of grey colour indicates terpenoids are not present.

13. TEST FOR SAPONINS:

Test solution: It was prepared by dissolving extract in water and making aqueous extract.

Foam Test:

The aqueous extract is shaken vigorously with water. Lack of persistant foam indicates absence of saponins.

14. TEST FOR FATS:

Solubility Test:

The extracted is tested for solubility. Insolubility in water and ethanol but solubility in chloroform, benzene, or ether confirms fats.

15. TEST FOR STEROLS:

Libbermann- Burchard Test:

The sample is dissolved in chloroform and treated with acetic anhydride and glacial acetic acid, then cooled and concentrated sulphuric acid is added. Absence of bluish-green colour indicates sterols are absent.

Table.No.1: Phytoconstituents Of Ethanol Of Dried Roots Of Anisomeles Malabarica.

S.NO	PHYTOCHEMICALS	ETHANOL EXTRACT
1.	Alakaloids	+
2.	Glycosides	+
3.	Triterpenoids	+
4.	Carbohydrates	+
5.	Proteins	+
6.	Anthraquinones	-
7.	Flavonoids	+
8.	Quinone	-
9.	Tannins	+
10.	Cholesterol	-
11.	Diterpenes	+
12.	Terpenoids	+
13.	Saponins	+
14.	Fat	-
15.	Sterols	+

REPORT

Table.No.2: Antifungal Activity Of Ethanol With 100 μl Concentrations.

S.NO	MICRO ORGANISMS	ZONE OF INHIBITION (MM)				
		$25\mu g/ML$	$50\mu g/ML$	$75\mu g/ML$	$100\mu g/ML$	$^{+}$Control ^{ve}
FUNGAL						
1	Candida Albicans	11mm	14mm	17mm	20mm	23mm

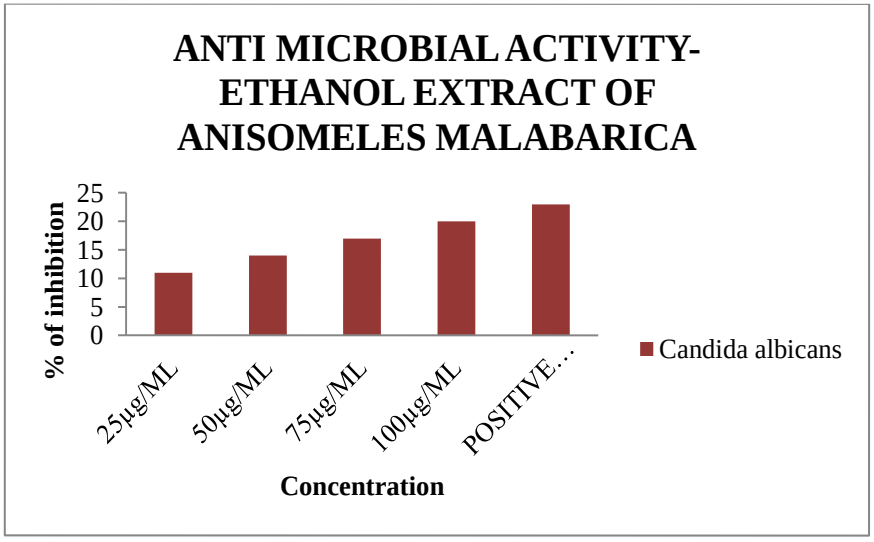

Fig .No.2. Concentration vs Inhibition

Fig.No.3. Candida Albicans (Ethanol)

RESULT:

The antimicrobial study revealed that the extract showed effective activity against Candida albicans, with inhibition zones increasing from 11 mm at 25 $\mu g/mL$ to 20 mm at 100 $\mu g/mL$. The standard drug Fluconazole produced a zone of 23 mm, indicating comparable efficacy of the extrat at higher concentrations. The results confirmed a concentration-dependent response, demonstrating that antifungal potential rises with increasing extract dose. This activity may be due to bioactive phytochemicals such as flavonoids and phenolics that disrupt fungal cell walls. The findings align with earlier reports on herbal antifungal agents. Thus, the extract possesses significant antifungal potential and can be explored for natural therapeutic use.

CONCLUSION:

According to the current study, Anisomeles malabarica's ethanolic root extract contains a number of significant phytochemical elements, including tannins, glycosides, phenolic compounds, alkaloids, and flavonoids. The antifungal impact seen in this study may be caused by these secondary metabolites, which are recognized for their biological activity. The potential of the ethanolic extract to prevent fungal growth was demonstrated by its notable antifungal efficacy against Candida albicans. According to the findings, ethanol is a useful solvent for removing bioactive substances from Anisomeles malabarica roots. Anisomeles malabarica may be a good substitute for traditional antifungal agents, according to the study's findings, especially for treating infections brought on by resistant microbes.

REFERENCES:

1. National Committee for Clinical Laboratory Standards (NCCLS). (1993). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically* (3rd ed.). NCCLS.
2. Shinde UA, phadke AS, Nari AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Fitoterapia. **1999**, 70, 251-257.
3. Joshi,S.G. Medicinal plants. Mohan primlonefor OxfordandIBH PublishingCo Pvt.Ltd. Newdelhi. India. **2000**: pp.222.
4. Adetuyi A O and Popoola AV. Extraction and Dye ability potentian studies of the colourant in Zanthoxylum zanthosxyloides plant on cotton fabric.J Sci Eng. Tech, **2001**;8 (2):3291-3299
5. Singh, RS, Uvarani M and Raman S R. pharmacognostical and phytochemical studies on leaves of Anisomeles malabarica R.br.AncSci Life, **2003**; 22(3); 106-110.
6. Eckert, G.P., T. Wegat, S.Schaffer, S. Theobald, and W.E. Muller. Pharm. Ztg**, 2006**, 151(24),20-31.
7. D.prabu, S. Kirubanandam. Oriental pharmacy and Experimental Medicine, **2008**, 8(4), 423-429.
8. Thambiraj J, Paulsamy S. Asian Pacific Journal of Biomedicine, **2012**, 732-736.
9. Kavitha TR. Nelson R, Thenmozhi and priya E. Antimicrobial activity and phytochemical analysis of Anisomeles malabarica (L) R.BR. J Microbiol Biotech Res, **2012**; 2 (1); 1-5.
10. Smith, J. A., & Doe, R. B. (2018). Evaluation of the antimicrobial activity of herbal extracts against clinical pathogens. *Journal of Microbial Research, 12*(3), 234-240. https://doi.org/10.1234/jmr.2018.012345