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Research Paper

Phytochemical profiling and Evaluation of Antimicrobial Potential of Non-Polar Extracts of *Arisaema nilamburens*

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ABSTRACT

Dried aerial parts of *Arisaema nilamburens* was powdered to obtain a coarse powder and placed inside a thimble, which is loaded into the main chamber of the Soxhlet extractor. The extraction of weighted leaf powder with respective volume of the solvent was carried out with its boiling point. Selected solvents were carried for the extraction depending on their increasing polarity. The phytochemicals of these extracts are examined and identified by qualitative tests and antimicrobial assessment was done through disc diffusion method. The extracts showed significant antimicrobial activity..

INTRODUCTION

Arisaema nilamburens is a monocot herb which is located in the Western Ghats of Kerala and Tamilnadu.. The germination time of the above mentioned plant is during the month of June - September. Habitat-Single large compound leaf, a cobra-like flower (spathe and spadix). Phytoconstituents present in the aerial parts of the plant are -Alkaloids, Phenolic compounds, terpenes and triterpenoids, Flavonoids, Saponins and glycosides, Lectins and sterols. These compounds are associated with antimicrobial, antioxidant, anticancer, and other pharmacological activities in several *Arisaema*

species. The non polar solvents used for the study are Petroleum ether and Chloroform. Various bioactive constituents are present in these extracts.

MATERIALS AND METHODS

Collection of plant material – The plant was collected from the Western Ghats of Kerala during the month of May-June

Authentication of plant material – The authentication of the plant material was performed from Kerala Forest Research Institute, Thrissur, Kerala

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1. Phytochemical investigations

The phytochemical investigations of a plant may involve the following.

- Extraction of Plant Material.

The plant material was dried in the shade. Then the shade dried plant material was subjected to get coarse powder and it was extracted in Soxhlet Apparatus using various solvents according to their polarity.

- Identification of the Phytoconstituents by chemical tests.-The investigation was performed for various Phytochemical constituents include-, Phenol, Tannin, Flavonoid, Saponin, Terpenoids, Alkaloid, Glycoside, Steroid, Quinones, Fatty acid

Carbohydrate, Protein, Amino acid, Beta cyanin, Anthocyanin, Coumarin, Phytosterol¹⁻⁹

- ### 2. Antimicrobial activity- Anti microbial activity was performed by Disc Diffusion Method.

Preparation of Inoculum

- To Prepare a fresh bacterial culture.

Inoculation of Agar Plate

- Pour sterilized Mueller–Hinton agar into Petri plates and allow to solidify.
- Using a sterile swab, evenly spread the bacterial suspension over the entire agar surface (lawn culture).

Preparation of Discs

- Soak sterile filter paper discs in plant extract
- Allow discs to dry under sterile conditions.

Placement of Discs

- Place extract-impregnated discs on the inoculated agar surface using sterile forceps.
- Include:
 - Positive control disc (Ampicillin)

- Negative control disc (DMSO)

Incubation

- Incubate plates at **37°C for 18–24 hours**.

Measurement

- Measure the diameter of the **zone of inhibition (in mm)** around each disc.
- Larger zones indicate stronger antimicrobial activity.
- MIC (Minimum Inhibitory Concentration) is the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism after incubation.¹⁰⁻¹³

Qualitative Phytochemical analysis

Phenols

To 2ml of test sample added 1ml 1% lead acetate solution and formation of white precipitate indicated the presence of phenolic compounds.

Flavonoids

Test sample (2ml) was treated with 2ml of 10% Lead acetate solution. Appearance of yellowish green colour indicated the presence of flavonoid.

Alkaloids

To 2ml of test sample, added 2ml of Wagner's reagent and the test tubes were observed for the appearance of reddish-brown precipitate.

Tannins

The samples (0.5g) were boiled in 20ml of distilled water in a test tube, filtered and added 1ml 5% FeCl₂ the filtrate. Appearance of brownish green coloration showed the presence of tannins.

Glycosides



To 5ml of test sample added 2ml glacial acetic acid, one drop of 5% FeCl_3 and conc. H_2SO_4 and appearance of brown ring indicated the presence of glycosides.

Two ml of the leaf extract was added with three ml of chloroform and 1 ml of 10% ammonium solution was added. Formation of pink colour indicates the presence of glycosides

Saponins

To 1ml of each test sample added 2ml of distilled water and shaken vigorously with a few drops of olive oil. Foam which persisted was taken as evidence for the presence of saponins.

Plant extract (0.5 g) was dissolved in 2 ml of boiling water in a test tube, allowed to cool and shaken well to mix thoroughly. The appearance of foam indicates the presence of Saponins.

Steroids

About 1ml of extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer chloroform layer turning red and sulphuric acid layer showing yellow with green fluorescence indicated the presence of steroids.

Terpenoids

To 1ml of test sample added 1ml of chloroform followed by a few drops of concentrated sulfuric acid. Reddish brown colour precipitate indicated the presence of terpenoids.

Quinones

About 2ml of each test sample was mixed with 3 or 4 drops of concentrated HCl . A yellow colour precipitate indicated the presence of quinones.

To 1ml of the leaf extract, add 1 ml of concentrated sulphuric acid. Formation of red colour indicates the presence of quinones.

Fatty Acids

About 0.5 ml of test sample was added to 5ml of ether and allowed it to evaporate on filter paper. Then the filter paper was dried and the appearance of transparency on filter paper is the indication of presence of fatty acids.

Cardiac Glycosides

To 1 ml of each extract, 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added, formation of brown ring at the interface indicates the presence of cardiac glycosides in the sample extract.

Carbohydrate

Molisch test: Add Molisch reagent (alpha-naphthol) to the sample, then slowly add concentrated sulfuric acid down the side of the tube. A purple or violet ring forms at the junction of the two layers.

Benedict's test: Tests for reducing sugars (monosaccharides and some disaccharides). Add Benedict's reagent to the sample and heat in a boiling water bath. A color change from blue to green, yellow, orange, or red precipitate forms, depending on the sugar concentration.

Fehling's test: Test for reducing sugars. Mix the sample with equal amounts of Fehling's solution A and B, then heat. Formation of a reddish-brown precipitate of cuprous oxide indicated positive result.

Iodine test: Detects the presence of starch and other polysaccharides like glycogen. Add a few drops of iodine solution to the sample and mix. A blue-black or dark color indicated the presence of starch.



Seliwanoff's test: Differentiates between aldoses (like glucose) and ketoses (like fructose). Add Seliwanoff's reagent and heat the solution. A deep red color will be formed quickly in the presence of ketoses and a slower, pale red or yellow color formed by aldoses.

Protein

Biuret test: The test works by adding a Biuret reagent, which is a solution of copper sulfate in a strong alkaline solution, to a protein sample. If proteins are present, the copper ions in the reagent will react with the peptide bonds and form a violet or purplish color, indicating a positive result. To 2 ml of the test solution, 5 drops of 1% copper sulphate solution and 2 ml of 10% NaOH was added and were mixed thoroughly. Presence of proteins was indicated by formation of purple or violet color.

Aminoacid

Ninhydrin test: Detects free amino acids and primary amines. In this reaction amino acids react with ninhydrin (a powerful oxidizing agent) reagent to give a purple coloured complex (Ruhemann's purple) while, imino acids (proline and hydroxyproline) react with ninhydrin to produce yellow colour. Ninhydrin reagent (2% w/v): Prepare by dissolving 2g of Ninhydrin powder in 100 mL acetone.

Coumarins

One ml of the plant extract (0.5 g) was taken in a small test tube and covered with filter paper moistened with 1 N NaOH. The test tube was placed for few minutes in boiling water. Then the filter paper was removed and examined in UV light for yellow fluorescence to indicate the presence of Coumarins.

Anthocyanin and Betacyanin

To 2 ml of the leaf extract, 1 ml of 2N sodium hydroxide was added and heated for 5 minutes at 100°C. Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of betacyanin.

Phytosterols

Salkowski's test: Extract was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated H₂SO₄ and shakes, allow standing, appearance of golden red indicates the positive test.

Liebermann-Burchard test: Add a small amount of test sample to a dry test tube. Add approximately 2 mL of chloroform to dissolve the extract. Add a few drops of acetic anhydride and boil the mixture, then cool it. Carefully add a few drops of concentrated sulfuric acid along the side of the tube. The development of a bluish-green color confirms a positive result.

RESULT AND DISCUSSION

The aerial parts of *Ariseama nilamburens* was performed for the extraction process using Soxhlet Method. The non polar solvents such as petroleum ether and Chloroform were used for the extraction. The phytochemicals present in the petroleum ether extract are terpenoids, alkaloids, quinones, Coumarins and Phytosterols. Where as in the Chloroform extract, besides these bioactive constituents, Anthocyanins also present.

Table 1-Phytochemicals in Petroleum ether and Chloroform extract

Phytoconstituents	Sample code	Sample Code
	PE	CE
Phenol	-	+
Tannin	-	-
Flavonoid	-	-
Saponin	-	-



Terpenoids	+++	+++
Alkaloid	+	+++
Glycoside	-	-
Steroid	-	-
Quinones	++	+++
Fatty acid	++	+
Carbohydrate	-	-
Protein	-	-
Aminoacid	-	-
Beta cyanin	-	-
Anthocyanin	-	+
Coumarin	++	+++
Phytosterol	++	++

N. B+/- indicated presence/absence of the tested phytochemical.

Heavily present: +++; Slightly present: ++;

Present: +; Absent: -

Antimicrobial Activity

The antimicrobial activity of Arisaema nilamburens extracts were the most prominent against some bacteria such as *S. typhi*, *S. aureus* and *P. aeruginosa* and fungus like *C. albicans*.

Chloroform and Petroleum ether extract showed significant activity against *S.*

typhi and *C. albicans*; moderate activity against *P. aeruginosa* and no inhibitory effect against *S. aureus* and *K. pneumonia*-(Table-2)

Table-2 Minimum Inhibitory Concentration of Sample 1(petroleum ether) and Sample2(Chloroform)

Extract No.	<i>S. typhi</i> MIC Value (µg/ml)	<i>P. aeruginosa</i> MIC Value (µg/ml)	<i>Staph. aureus</i> MIC Value (µg/ml)	<i>C. albicans</i> MIC Value (µg/ml)	<i>K. pneumoniae</i> MIC Value (µg/ml)
1	512	256	>1024	256	>1024
2	64	512	>1024	256	>1024

CONCLUSION

The dried aerial parts of Arisaema nilamburens was extracted by means of nonpolar solvents like Petroleum ether and Chloroform and the phytochemicals present in these extracts were identified and these extracts were significant in antimicrobial activity.

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