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SCREENING OF PHYTOCONSTITUENTS IN THE HYDRO ETHANOLIC EXTRACT OF *A. GALANGA* AND ITS POTENCY TO ANTIBACTERIAL ACTIVITY

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ABSTRACT The rhizome of *A. galanga* has been used as a traditional remedy for various ailments. In the present study, hydro ethanolic extract of *A. galanga* rhizome were investigated to explore the bio active principles residing in it. The fractions were first subjected to phytochemical analysis followed by evaluation of their antimicrobial potential. It was observed that the rhizome possess several antioxidants and is devoid of phytosterols and saponins. The extract was subjected to antimicrobial studies and was observed to exhibit potent anti-bacterial effect towards the selected gram positive and gram negative strains.

KEYWORDS : *A. galanga,* phytoconstituents, antibacterial potency

INTRODUCTION:

Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. Medicinal plants and derived medicine are widely used in traditional cultures all over the world and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemicals. In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects [1].

Alpinia galanga belongs to the *Zingiberaceae* family is a rhizomatous herb. Rhizomes of *Alpinia galaga* are used for cough, indigestion, dysentery and food poisoning. Fresh juice of its rhizomes is also used for the treatment of ringworm. Seeds of *Alpinia galangal* is used for colic, diarrhea and vomiting. The rhizome of *Alpinia galanga* is used against rheumatism, bad breath and ulcers, whooping colds in children, throat infections, to control fever. It is a potent antioxidant. It is used as a snuff to treat cold and flu symptoms. It has also been used as a digestive aid. The rhizome is also used as anti-microbial, anti-bacterial [2], anti-inflammatory and flavoring agent [3]. In this context, the present study aims to screen the phytochemical constituents and to evaluate its antibacterial activity.

METHODS:

Preparation of plant extracts: The *A. galanga* rhizome were chopped and dried under shade for a week then they were coarsely powdered. The powdered (rhizome) was macerated with 50% ethanol for 6 hr at 60°C.

A. Qualitative Phytochemical analysis

A systematic and complete study of crude drugs should include a thorough investigation of both primary and secondary metabolites. The qualitative chemical tests provide the prime source of information in establishing profiles of the extract for their nature of chemical composition. In the present study, the hydro ethanolic extract of *A.galanga* was subjected to the following qualitative chemical tests for the identification of various phytochemicals [3-8].

I. Tests for Alkaloids

1. Wagner's test: To the extract, Wagner's reagent was added. Formation of reddish brown precipitate indicates the presence of alkaloid.
2. Mayer's test: To the extract, 1 or 2 ml of Mayer's reagent was added. Formation of dull white precipitate indicates the presence of alkaloid.
3. Hager's test: To the extract, 3 ml of Hager's reagent was added. Yellow precipitate indicates the presence of alkaloid.

II. Tests for Carbohydrates

1. Molisch test: To the extract, 1 ml of -naphthol solution was added and conc. sulfuric acid was added along the sides of test tube. Purple or reddish violet color at the junction between the two liquids indicates the presence of carbohydrates.
2. Fehling's test: To the extract, equal quantities of Fehling's solution A and B was added. Upon heating gently, a brick red precipitate indicates the presence of carbohydrates.
3. Benedict's test: To 5 ml of Benedict's reagent, 8 drops of solution under test was added mixed and the mixture was boiled vigorously for two minutes and cooled. A red precipitate indicates the presence of carbohydrates.

III. Tests for Proteins

1. Biuret test: To the extract, 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulfate solutions were added. A violet color indicates the presence of proteins.

IV. Test for Amino Acids

1. Ninhydrin test: Two drops of freshly prepared 0.2% Ninhydrin reagent was added to the extract and heated. Development of blue color indicates the presence of proteins, peptides or amino acids.

V. Tests for Steroids and Sterols

1. Salkowaski test: The extract was dissolved in chloroform and an equal volume of conc. sulfuric acid was added. Bluish red to cherry red color is observed in chloroform layer, whereas the acid layer assumes marked green fluorescence indicating the presence of steroids.

VI. Tests for Glycosides

1. Legal test: The extract was dissolved in pyridine or sodium nitroprusside solution added to it and made alkaline. Pink red or red color indicates the presence of glycosides.
2. Baljet test: To the extract, freshly prepared sodium picrate solution was added. Yellow to orange color indicates the presence of glycosides.

VII. Test for Flavonoids

1. Shinoda test: To the extract, magnesium turnings were added, followed by the addition of conc. hydrochloric acid. A red color indicates the presence of glycosides.

VIII. Tests for Tannins

1. Ferric chloride test: To the extract, ferric chloride was added. Dark blue or greenish black color indicates the presence of

tannins.

2. Lead acetate test: To the extract, lead acetate solution was added. A precipitate indicates the presence of tannins.

B. Microbiological assay

Antibacterial activity of the plant extract was analyzed by performing the standard agar diffusion method [9] where the sample solution of different concentrations were poured into the wells, which were made in the nutrient agar media seeded with the gram negative and gram positive organism. Ciprofloxacin (100 µg/well) was used as the standard antibiotic.

RESULTS AND DISCUSSION:

A. Phytoconstituents studies of the extract:

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the screening of the phytochemicals. Phytochemical compounds are the major compounds which imparts medicinal value of the plant. Qualitative phytochemical analysis of hydro ethanolic extract of *A. galanga* showed the presence of majority of the compounds including terpenoids, flavonoids, alkaloids, tannins, saponins and glycosides. The results of the phytoconstituents are depicted in table -1.

Phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, it might be responsible for the potent antioxidant capacity of the plant. The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value.

From this study, the presence of phenolic compounds such as terpenoids, steroids (phytosterols i.e. ß-sitosterol) in hydroethanolic extract of *Alpinia galanga* may contribute to the antioxidant properties benefited by traditional medicine use. For many years now, it has been known that plant polyphenols (steroids, terpenoids, flavonoids etc) are antioxidants *in vitro* [10]. These antioxidants are compounds that reduce the formation of free radicals or react with and neutralize them thus potentially protecting the cell from oxidative damage [11]. The tannins and resins in the extract are employed as astringent both in the gastrointestinal tract and on skin abrasions by traditional medicine. The flavonoids and phenolic compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, and anti-carcinogenic activity which are detected in the extract [12].

Table – 1 Analysis of *Alpinia galanga* extracts:

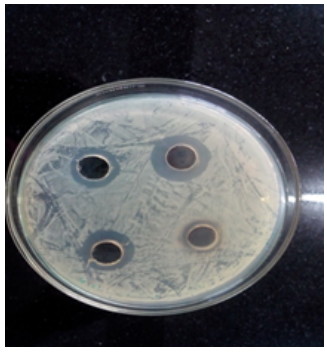
Tests	Results
Alkaloids	+
Carbohydrates	+
Flavonoids	+
Glycosides	+
Phytosterols	-
Proteins and amino acids	+
Saponins	-
Phenolic compounds and Tannins	+

(+) denotes the presence of the respective class compound
(-) denotes the absence of the respective class compound

A. Microbiological assay:

The hydroethanolic extract of *A. galanga* were evaluated for their inhibitory potential by comparing their respective zone of inhibition against four bacteria by using agar diffusion assay are presented in Fig 1.

Fig-1: Agar diffusion assay



The results are based on the zone of inhibition which is obtained. As the concentration of the drug increases the width of the zone of inhibition also increases. The extract of the plant part studied showed weak inhibition as they inhibited the bacteria. The inhibitions against bacteria exhibited similarity in their antimicrobial potential. The extract showed moderate antimicrobial activity against *Micrococcus leuteus* with the greatest diameter of inhibition zone of 13 mm followed by 8 mm against *Staphylococcus epidermis*. Antimicrobial activities of the *Alpinia conchigera* and *Alpinia galanga* against a wide spectrum of microorganisms were demonstrated from many previous studies [13] [14] also evaluated the ethanol extracts of the *Zingiberaceae* family *Alpinia galanga* for antimicrobial action on *Staphylococcus aureus* 209P and *Escherichia coli* NIHJ JC-2 by using an agar diffusion assay. The galanga extract had the strongest inhibitory effect against *M.leuteus.*

CONCLUSION

The medicinal value of *A.galanga* lies in bioactive phytochemical constituents such as alkaloids, phenolic compounds, essential oils, flavonoids, tannins, terpenoids, saponins, etc that produce definite physiological action on the human body. They have been serving as potential sources of new compounds of therapeutics value and also as sources of lead compounds in the drug development. It was observed that the rhizome possess potent anti-bacterial effect.

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