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Research Article

ANTIMICROBIAL ACTIVITY OF LEAVES OF *AZIMA TETRACANTHA* AGAINST CLINICAL PATHOGENS

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ABSTRACT

The antimicrobial activity of leaves of the medicinal plant *Azima tetracantha* collected from the regions of Ambalathara, Kerala, South India was checked against the clinical pathogens by agar well diffusion method. *Azima tetracantha* showed highest antimicrobial activity on ethanolic extracts. The phytochemical evaluation showed the presence of alkaloids, saponins and tannins. With the help of column chromatography, the methanolic crude extract was purified and highest antimicrobial activity was observed in 2:8 concentration of methanol: water. By thin layer chromatography the compounds were separated and the R_f values obtained are 0.6, 0.714, 0.913. From the study it was revealed that the *Azima tetracantha* had a wide activity against clinical pathogens.

Keywords: *Azima tetracantha,* Antimicrobial activity, Saponins, Tannins, R_f value.

INTRODUCTION

Medicinal plants are relied upon by 80% of world's population, and in India, the use of medicinal plants as therapeutic agents remains an important component of the traditional system [1]. According to World Health Organization, medicinal plants are the best source to obtain a variety of new herbal drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to understand their properties, safety and efficacy. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant [2]. Plants are still widely used for ethno medicine around the world and phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including those caused by opportunistic pathogens. Microorganisms have been developing resistance to many antibiotics due to the indiscriminate use of antimicrobial drugs, increasing clinical problems in the treatment of infections[3].

Antibiotic resistance has become a global concern in recent years. This problem is of great significance especially in developing countries because infectious diseases are one of the major causes of mortality in these countries[4]. Various plant species have been serving as the best natural source of drugs and medicines since the beginning of civilization. Most of the plant constitutes, particularly the secondary metabolites possess potent antibacterial and antifungal activity. Among the different plant derived secondary metabolites, alkaloids proved to be the most important group of compounds that showed wide range of antimicrobial activity [5].

Azima tetracantha belongs to the family Salvadoraceae. It occurs naturally in central, eastern and South Africa as well as in the Indian Ocean islands, and extends through Arabia to tropical Asia. In East Africa the pounded roots of *Azima tetracantha* are applied directly to snakebites and an infusion is taken orally as a treatment for them, while in Zimbabwe a mixture of roots and leaves is used similarly. The Bajun people of the Kenyan coast use a root decoction to treat stomach disorders. In Madagascar an infusion of the leaves is used to treat venereal diseases. In the Cape Province of South Africa the juice of the berries is applied directly into the ear to treat earache and the dried root is ground, put in cold water and given to cows to facilitate difficult parturition. The Zulu people of South Africa apply the sap of the plant directly to treat toothache and bleeding gums after tooth extraction and also as a disinfectant. In India and Sri Lanka the root, root bark and leaves are added to food as a remedy for rheumatism. The plant is considered diuretic and is also used to treat dropsy, dyspepsia, chronic diarrhoea and as a stimulant tonic. In western India juice of the leaves is applied as eardrops against earache and crushed leaves are placed on painful teeth. The present study focus on to evaluate the antimicrobial activity and phytochemical analysis of the leaves of medicinal plant *Azima tetracantha* collected from the regions of Ambalathara, Kerala, South India used for the treatment of clinical infections caused by pathogenic microorganisms.

METHODOLOGY

Collection of plant materials

The medicinal plant *Azima tetracantha* free from diseases were collected from the regions of Ambalathara, South India. The collected plant parts were removed, washed thoroughly with running tap water and again washed with sterile distilled water to remove dirt prior to drying process. The leaves of *Azima tetracantha* were shade dried at room temperature for a week to remove the moisture content and powdered using mixer grinder.

Preparation of plant extract

The air dried finely ground leaves (1 gm) were taken separately in air tight bottles and 10 ml of different solvents (ethanol, methanol, acetone, chloroform and distilled water) were added and kept under dark. After two days, the contents were stirred and filtered using Whatmann no: 1 filter paper. The filtrate was collected and stored in sterile glass beakers for further study.

Collection of test organisms

Seven clinical microbial cultures namely *Staphylococcus aureus* (Pus), *Klebsiella* sp. (Sputum), *Escherichia coli* (Urine), *Pseudomonas* sp. (Pus), *Enterococci* sp. (Urine), *Serratia* sp. (Sputum) and *Proteus* sp. (Sputum) were used in this study was collected from Travancore Medical College, Kollam. The pathogenic cultures were grown in nutrient broth at 37ºC, maintained in nutrient agar slants, and stored at 4ºC for determining the antimicrobial activity of these selected medicinal plants.

Antimicrobial activity assay

The antimicrobial activity of selected medicinal plants against clinical pathogens was determined by using agar well diffusion method[6]. The Mueller Hinton Agar medium was prepared and poured onto 100 mm petriplates (15-20 ml/plate) still molten. After solidification, 24 h nutrient broth grown pathogenic cultures were swabbed on the molten medium using sterile cotton swabs. Wells of 6 mm diameter were punched over the agar plates using a sterile gel puncher. 50 µl of each extract were poured into the wells and the plates were incubated at 37ºC for 24 h. After incubation, the

antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well. All the solvents under study were used as negative control and the commercial antibiotics such as tetracycline, penicillin and chloramphenicol are used as positive control. All the experiments were performed in triplicates and mean of the triplicate values were calculated.

Phytochemical analysis

Phytochemical analysis for major phytoconstituents of the plant extract was performed as follows [7].

Detection of alkaloids (dragendroff's test)

Filtrates were treated with Dragendroff's reagent (Solution of Potassium Bismath Iodide). Formation of an orange precipitate indicates the presence of alkaloids.

Detection of glycosides

Extracts were treated with 1ml of glacial acetic acid and a few drops of ferric chloride. To this few drops of conc. sulphuric acid was carefully added. Formation of reddish brown colour at the junction of two layers and bluish green colour in the upper layer indicates the presence of glycosides.

Detection of saponins (foam test)

Extracts were shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phenols (ferric chloride test)

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

To 0.5ml of plant extracts, 1ml of distilled water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for catecholic tannins.

Detection of flavonoids (alkaline reagent test)

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Detection of proteins and amino acids (xanthoproteic test)

The extracts were treated with few drops of conc. nitric acid. Formation of yellow colour indicates the presence of proteins.

Detection of resins

Extracts were treated with acetone. Small amount of water were added and shaken. Appearance of turbidity indicates the presence of resins.

Detection of reducing sugar

To 0.5 ml of plant extracts, 1 ml of distilled water and 5-8 drops of Fehling's solution was added and heated over water bath. Formation of brick red precipitate indicates the presence of reducing sugar.

Detection of phytosterols (salkowski`s test)

Extract were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Biochemical analysis

Column chromatography

Column chromatography was performed by packing a glass column with silica gel (1:2). A column may be packed wet by pouring solvent adsorbent slurry into the tube or dried by filling it with dry adsorbent. One ml of plant extract is then dissolved with appropriate solvent and added carefully at the top of the column, so as not to disturb the packing. Fractions are developed by adding different concentration (1:10 to 10:1) of the solvent (methanol: water) to the column packed with silica gel and collecting the fractions of eluent in separate eppendorf tubes, which was further subjected to antimicrobial activity.

Thin layer chromatography

To determine the purity of collected samples by column chromatography, the active fractions were again subjected to TLC on silica gel plates (silica gel G 60; Merck). The Rf value of each macromolecule was noted using the formula:

$$Rf = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent}}$$

RESULTS AND DISCUSSION

Azima tetracantha leaves are good source of natural phenolic compounds. Ekbote *et al*. [8] reported that *Azima tetracantha Lam.* belongs to Salvodoraceae and known as Kundali in Ayurvedic medicine. The plant is reported to possess antidiarroheal, antimicrobial activity in fruits, diuretic and rinderpest in leaf. The juice of the leaves is used in tooth and ear ache. The ethanolic extract of the *Azima tetracantha* leaves showed better free radical capacity against different reactive oxygen /nitrogen species, among other extracts although with different efficiencies. The high content of antioxidants like phenolic compounds, flavonoids and vitamins found in these extracts, may impart health benefits by combating the free radicals in synergistic manner along with other compounds and thus constitute part of the basis for the ethno pharmacological claim. Thus *Azima tetracantha* shall further be subjected to systematic *in vivo* studies for the preventive action against cancer, cardiovascular and neurological disorders[9]. In the present study, an attempt was made to find out suitable phytoremedy, which can effectively inhibit the growth of clinical pathogens.

Antimicrobial activity assay

Antimicrobial activities of five solvent extracts (ethanol, methanol, acetone, chloroform and distilled water) were tested against seven clinical pathogens such as *Staphylococcus aureus* (Pus), *Klebsiella* sp. (Sputum), *Escherichia coli* (Urine), *Pseudomonas* sp. (Pus), *Enterococci* sp. (Urine), *Serratia* sp. (Sputum) and *Proteus* sp. (Sputum). Among the five solvents tested, ethanolic extracts of *Azima tetracantha* showed higher significant activity against the pathogenic organisms such as *Proteus* sp. (Sputum), *Serratia* sp. (Sputum), *Pseudomonas* sp. (Pus) followed by *Escherichia coli* (Urine), *Staphylococcus aureus* (Pus) and *Klebsiella* sp. (Sputum) (Table 1). According to Duraipandiyan *et al*. [3] reported that traditionally, *Azima tetracantha* has been used to treat many diseases. Hexane, ethyl acetate and methanol extracts were tested against fungi. Hexane extract showed some activity against tested fungi. This study supported the present report that the leaves of *Azima tetracantha* are used for determining the antimicrobial activity against the clinical pathogens.

Table 1: Antimicrobial activity of *Azima tetracantha* against clinical pathogens

S. No.	Tested organism	Zone of Inhibition (mm)				
		Methanol	Acetone	Chloroform	Ethanol	Distilled water
1	*Klebsiella* sp	10	-	-	10	-
2	*Escherichia* coli	11	10	10	12	-
3	*Proteus* sp	15	-	14	15	-
4	*Serratia* sp	14	-	10	15	-
5	*Enterococci* sp	-	-	-	-	-
6	*Staphylococcus aureus*	10	-	8	11	-
7	*Pseudomonas* sp	15	-	11	13	-

Phytochemical evaluation

Ethanolic extracts of *Azima tetracantha* which showed highest antimicrobial activity was subjected to phytochemical evaluation. Phytochemical analysis of ethanolic extracts revealed the presence of secondary metabolites such as alkaloids, saponins and tannins. The plant extracts with effective antibacterial properties were also subjected to phytochemical analysis. There are few reports on phytochemical composition of the plant *Azima tetracantha* indicates the presence of dimeric piperdine alkaloids azimine, azacarpaine, carpaine, triterpenoids, isorhamnetin 3- rutinoside, and novel fatty acids. Presence of neoascorbinogen and glucosinolates, has also been reported.

Column chromatography

Ethanolic extracts of *Azima tetracantha* was purified using column chromatography. Column chromatography was done with the help of stationary phase and mobile phase. The mobile phase was prepared in different concentration and the fractions (1ml) were collected. The eluents were subjected to antimicrobial activity and highest activity was obtained in 2:8 fractions against the organism *Proteus* sp., *Serratia* sp. and *Pseudomonas* sp.

Thin layer chromatography

The fraction which showed highest activity in column chromatography was subjected to TLC. The R_f value of the separated compound was 0.6, 0.714, 0.913. Further research is needed to characterize the compounds obtained and their mechanism of action on the bacterial pathogens. Researches on the pharmacological properties of the plant extracts have several limits, due to unknown composition of all the components of the plant source investigated. All studies on this area need for other confirmations and require additional research. Thus, the present study reflects a hope for the development of novel drugs.

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