



PRELIMINARY PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF LEAVES OF *AZIMA TETRACANTHA* LAM.

*Anbukumaran.A, Ambikapathy, V and Panneerselvam, A.

AVVM Sri Pushpam College (Autonomous), Poondi, 613503, Thanjavur.

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*Corresponding Author

Anbukumaran A.

AVVM Sri Pushpam

College (Autonomous),

Poondi, Thanjavur.

ABSTRACT

In the present investigation the antibacterial activity of leaves of the medicinal plant *Azima tetraantha* was studied. The leaf extract of test plant against the clinical pathogens were performed by agar well diffusion method. *Azima tetraantha* showed highest antibacterial

activity on different concentration of ethanol when compared to methanol and water extracts. The maximum zone of inhibition was observed in *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus* ranges from 9-17 mm in all concentration of extracts, but the positive results ranges from 22-37mm in all types of clinical pathogen. The phytochemical evaluation showed the presence of aminoacids, flavonoids, carbohydrates, alkaloids, terpenoids and glycosides in *Azima tetraantha*.

KEYWORDS: *Azima tetraantha*, clinical pathogen, Mueller Hinton agar medium.

INTRODUCTION

Medicinal plants are used in major parts of developing countries as traditional medicinal systems to cure many infectious diseases with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many parts of the world (Sukanya, *et al.*, 2009). Plants are potent biochemists that have been used as components of phytomedicine since time immemorial. All the plants may produce some secondary metabolites such as alkaloids, flavonoids, phenols, glycosides, tannins, resins, carbohydrate, protein, fat, lipid, terpenoid, steroid, xanthoxylines, coumarin etc. Plant based natural constituents can be derived from various parts of the plant like bark, leaves, flowers, roots, stems, fruits and seeds that may contain active components (Tiwari,*etal.*,2011).

Azima tetracantha Lam. (Family: Salvadoraceae) locally known as “Mulsangu”, is a rambling spinous shrub flowering throughout the year found in Peninsular India, West Bengal, Orissa, African Countries and extends through Arabia to tropical Asia. The juice of the leaves is said to relieve the cough phthisis (pulmonary tuberculosis) and asthma. Plant pacifies vitiated kapha, vata, bronchitis, cough, asthma, and is a good expectorant. It is also used in diabetes, diarrhea and arthritis (Ayurvedic Medicinal Plants, 2015). The survey showed that *Azima tetracantha* Lam. has diuretic property, treating rheumatism, chronic ulceration treatment, eye conjunctivitis treatment, rheumatic fever, anti-inflammatory activity, antiulcer property, good antibacterial activity against *Staphylococcus aureus*, *Streptococci mutans* and *Salmonella typhi* showed good antifungal activity against *Aspergillus niger*, *Candida albicans* (Nayar, *et al.*, 1956, The Wealth of India, 1985, Anonymous, 2001 and Tiwari., 2001).

MATERIALS AND METHODS

Collection of plant material

Plant leaves of *Azima tetracantha* was collected from Point Calimere Wildlife Sanctuary, east coast of Tamilnadu, India.

Preparation of extracts

The collected leaves of *Azima tetracantha* were washed well, shade dried and powdered. The air dried finely ground leaves (1 gm) were taken separately in air tight bottles and 10 ml of different solvents (ethanol, methanol, and 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

Four clinical microbial cultures namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus cereus* were collected from Department of Microbiology, K.A.P. Viswanathan, Government medical college, Tamilnadu, India. The pathogenic cultures were grown in nutrient broth at 37°C, maintained in nutrient agar slants, and stored at 4°C for determining the antimicrobial activity of *Azima tetracantha*.

Antimicrobial assay

The antimicrobial activity of *Azima tetracantha* against clinical pathogens was determined by using agar well diffusion method (Bauer, *et al.*, 1966). The Mueller Hinton Agar medium was

prepared and poured into 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 diameters were punched over the agar plates using a sterile gel puncher. Different concentration of 50, 75 and 100 μ l of each extract were poured into the wells in addition to Positive control (streptomycin) and negative control (solvent) were injected in respected well and the plates were incubated at 37°C for 24 h. After incubation, the antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well. All the experiments were performed in triplicates and mean of the triplicate values were calculated.

Phytochemical analysis

Screening of various phytochemical constituents like Proteins, Aminoacids, Flavonoids, Saponins, Glycosides, Carbohydrates, Steroid, Terpenoids, Alkaloids and Phenols were carried out using standard methods (Harborne, 1973).

RESULT AND DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Tona, *et al.*, 1988). Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Behera, *et al.*, 2005 and Govindarajan, *et al.*, 2007).

Azima tetracantha leaves are good source of natural phenolic compounds. Ekbote *et al.* (2010) reported that *Azima tetracantha* Lam. belongs to Salvadoraceae and known as Kundali in Ayurvedic medicine. In the present study, antibacterial activity revealed that the different concentration of ethanol extract of the *Azima tetracantha* leaves showed better results of clinical pathogen such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus cereus*. The positive controls were compared to the test organism, its better results shown but the negative control also no activity against test organism. All the three solvents at higher concentration (100 μ l) act as one of the inhibitory agent (Table 1).

According to Duraipandiyan *et al.* (2006) *Azima tetracantha* has been used traditionally to treat many diseases. Hexane, ethyl acetate and methanol extracts were tested against *Candida albicans*. Similarly Hema, *etal.*, (2012) reported that ethanol extract of *Azima tetrecantha* showed significant effect in controlling the pathogenic organisms. *Azima tetracantha* showed

highest antimicrobial activity on ethanolic extracts. 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), *Serratiasp.* (Sputum) and *Proteus* sp. (Sputum).

Negi, *et al.* (2011) reported that the secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic, antileprosy activities etc.

In the present study, the phytochemical screening were studied with ethanol, methanol and water extract of the leaves of *Azima tetraacantha*. The result revealed with aminoacids, flavonoids, glycoside, carbohydrate, terpenoids and alkaloids were present in all the solvents of *Azima tetraacantha* leaves (Table 2).

Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory and immunomodulatory properties (Rabi, *et al.*, 2009 and Wagner and Elmadfa, 2003). In addition, terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties as well (Sultana and Ata, 2008). Glycosides also have vast therapeutic efficacy as they are found in almost every medicinal plant.

Table 1: Antibacterial activity of *Azima tetraacantha* against clinical pathogens

Test organism	Zone of inhibition (mm)														
	Ethanol(µl)					Methanol(µl)					Water (µl)				
	50	75	100	P	N	50	75	100	P	N	50	75	100	P	N
<i>E.coli</i>	12	10	11	35	-	-	-	5	32	-	-	-	8	35	-
<i>P.aeruginosa</i>	9	15	17	37	-	10	10	15	35	-	9	11	11	32	-
<i>B. cereus</i>	11	15	15	25	-	15	11	15	33	-	-	-	7	23	-
<i>S.aureus</i>	10	13	14	25	-	15	9	15	24	-	4	5	5	22	-

P – Positive control (Streptomycin) N-Negative Control (Solvent)

Table 2: Qualitative phytochemical analysis of *Azima tetraacantha* leaves

S.No	Testname	Ethanol	Methanol	Water
1	Protein	+	-	+
2	Aminoacid	+	+	+
3	Flavonoid	+	+	+
4	Saponin	-	+	-

5	Glycoside	+	+	+
6	Carbohydrate	+	+	+
7	Steroid	+	+	-
8	Terpenoid	+	+	+
9	Alkaloid	+	+	+
10	Phenol	+	+	-

P – Positive N-Negative

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