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Research Article

DETERMINATION OF PHYTOCHEMICALS FROM THE LEAVES OF *AZIMA TETRACANTHA* LAM AND ANTIMICROBIAL POTENTIAL AGAINST SOME CLINICAL PATHOGENS

Regalakshmi T., Vasantha S., Panneerselvam A and Ambikapathy V

Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi- 613 503, Thanjavur (Dist), Tamilnadu, India

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ABSTRACT

Azima tetracantha Lam. (Salvadoraceae) is widely used in folklore herbal medicine practices in the villages of Avidanallavijayapuram Orathanadu Taluk Thanjavur District. The plantpossess anti-inflammatory, antiperiodic, analgesic and wound healing properties. The medicinal plant *Azima tetracantha lam* leaf extract was analysed for the determination of phytochemicals and antimicrobial activity. The phytochemicals evaluation showed the presence of alkaloids, terpenoids, phenols, tannins, saponins, quinines, protein and steroid in various solvents like ethyl acetate, chloroform, methanol and aqueous extracts. The *In-vitro* antimicrobial activity was studied against human pathogens of gram positive strains such as *Bacillus subtillis, and Staphylococcus aureus,* and gram negative strains *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and three fungal strains: *Aspergillus niger*, *A. fumigatus* and *A. flavus* using disc diffusion method. The effect of these extracts were compared with standard drugs penicillin and streptomycin. The phytochemical screening of the extracts revealed the presence of secondary metabolites like alkaloids, flavonoids, tannins, triterpenoids, saponins etc.

INTRODUCTION

Medicinal plant are still major parts of traditional medicinal systems in developing countries. Many infectious disease are known to be treated 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 developing countries (Sukanya *et al.,* 2009). Medicinal plants which form the backbone of traditional medicine have in the last few decades been the subject of very intense pharmacological studies. 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 (Prusti, *et al.,* 2008). 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 (Duraipandiyan, *et al.,* 2006). In many parts of the world medicinal plants are used for antibacterial, antifungal and antiviral activities a plant derived drugs serve as a prototype to develop more a effective and less toxic medicinal. Tribal medicine has not been studied extensively. Many of the plants used today were known to the people of ancient cultures throughout the world and they were valued for their preservative and medicinal powers. *Azima tetracantha* is known as 'Esanku' in Malayalam, 'Mulsangu' in Tamil and 'Kundali' in Sanskrit, respectively. Its root, root bark and leaves are used with food as a remedy for rheumatism (Kritikar and Basu, 1984). It is a powerful diuretic given in rheumatism, dropsy, dyspepsia and chronic diarrhoea and as a stimulant tonic after confinement (Nadkarni, 1976). *Azima tetracantha* as efficient acute phase anti-inflammatory drug is traditionally used by Indian medical practitioners (Ismail *et al.,* 1997). The plant is used to treat cough, phthisis, asthma, small pox and diarrhea. The decoction of the stem bark is considered astringent, expectorant and antiperiodic (Chelladurai, 1983). Its leaves were found to possess azimine, azecarpin, carpine and isorhamnitine-3-Orutinoside (Rail *et al.,* 1967, Williams and Nagarajan, 1988). Friedelin, lupeol, glutinol and β sitosterol have been isolated from the leaves of *A. tetracantha* (Rao and Rao, 1978). Recently, some novel fatty acids were isolated from seeds of this plant (Daulatabad *et al.,* 1991). There are few reports on phytochemical composition of the plant like presence of dimeric piperdine. alkaloids, azimine, azacarpaine,

Corresponding author:* **Regalakshmi T
Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi- 613 503, Thanjavur (Dist), Tamilnadu, India

carpaine (Rall, *et al.,* 1967) triterpenoids (Venkatrao and Prasad Rao 1978), isorhamnetin 3-rutinoside (Vasikaran Williams and Nagarajan.1987). Presence of neoascorbinogen and glucosinolates has also been reported. The present study was aimed at evaluation determination of Phytochemical and antimicrobial activities using various extracts of *Azima tetracantha* Lam leaves.

MATERIALS AND METHODS

Plant collection

The fresh plants were collected from Avidanallavijayapuram Village, Orathanadu Taluk Thanjavur District of Tamilnadu, India. The taxonomic identity of the plant was confirmed by the Botanists Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur. The collected plant parts were 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* lam. Were shade dried at room temperature for a week to remove the moisture content and powdered using mixer grinder and packed in a zip lock cover and labelled.

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 (ethylacetate, aqueous, methanol and chloroform) were added and kept Soxhlet apparatus. The organic solvent was removed by evaporation using rota vapor (Helidolph-Hei-VAP [HB/ HL/G1] Germany) at not more than 40°C. The residue was then placed in an oven at 40°C for about 48 h to remove the water. The resulting dried mass was then powdered, packed into a glass vial and stored in desiccators over silica gel until further use.

Phytochemical Screening of Plant materials

The extracts was tested for the presence of bioactive compounds by using following standard methods (Kokate *et al.,* 1994).

Test for Saponins

To 0.5g of plant extracts, distilled water was added and heated for few minutes. Foam formation indicated the presence of saponins.

Test for Tannins

To 0.5g of plant extracts, 10ml of distilled water was added and filtered. To the filtrate 0.1% of Ferric chloride solution was added. Formation of brownish green indicated the presence of tannins.

Test for Steroids

To 0.5g of plant extracts, 2ml of acetic anhydride and 2ml of Sulphuric acid was added. Formation of violet-blue colour indicated the presence of steroids.

Test for Flavonoids

To 0.5g of plant extracts, few drops of acetone was added and heated in a water bath until the acetone evaporated and then filtered. The filtrate was cooled and 5ml sodium hydroxide was added. Presence of yellow colour indicated the presence of flavonoids.

Test for Alkaloids

To 0.5g of plant extracts, 3ml of methanol was added with 10% acetic acid and ammonium hydroxide was added. Formation of precipitate indicated the presence of alkaloids.

Test for Phenol

To 0.5g of plant extracts, distilled water was added and heated, to that 2ml of ferric chloride was added. Blue/Green colour formation indicated the presence of phenol.

Test for Glycosides

0.5g of plant extracts, 1ml of Glacial acetic acid was added, and then Ferric chloride and 1ml of sulphuric acid was added. Reddish brown colour appeared at the junction of two layers and the upper layer turned bluish green which indicated the presence of glycosides.

Test for Carbohydrates

300mg of 50% alcoholic extracts was dissolved in water and filtered. The filtrate was treated with concentrated Sulphuric acid and then with Molisch's regent. Appearance of pink or violet colour indicated the presence of carbohydrates. The filterate was boiled with Fehling's and Benedict solution. Formation of brick red precipitate in Fehling's and Benedict's solution is the positive result for reducing sugars and non-reducing sugars respectively.

Test for Triterpenoids

Five ml of each extracts was mixed in 2ml of chloroform, and 3ml of concentrated Sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive result for the presence of terpenoid.

Test for Protein

Leaf extracts were treated with 1 ml 10% sodium hydroxide solution separately and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation of purple violet colour might be indicated the presence of protein.

Antimicrobial Screening

Microorganisms used for in vitro studies

Five bacterial strains and three fungal strains were slected in the present investigation. The clinical isolates were obtained from the Center for Bioscience and Nanoscience Research (CBNR) lab Eachanari, Coimbatore. The five bacterial species used in this study were the gram positive strains: *Bacillus subtilis, and Staphylococcus aureus* and gram negative strains: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and three fungal strains: *Aspergillus niger*, *A. fumigatus* and *A. flavus* were analysed for antimicrobial activities. They were identified according to standard phenotype tests.

Antibacterial and Antifungal assays

Antibacterial activity

The extracts and the standard drugs were dissolved in minimum quantity of DMSO and adjusted, to make up the

volume with sterile distilled water to get 50 and 100µg /ml concentrations. The procaine penicillin was used against gram positive and streptomycin was used against Gram negative bacteria as standard drugs. The antibacterial activity tests were performed by cup plate method, (Chattopadhya and Khore. *et al.,* 1969). The fresh cultures of bacteria, *Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were cultured by inoculating into peptone broth and incubated at 37ºC ± 2ºC for 24 hours. This culture was mixed with nutrient agar media and poured into petri dishes by aseptic techniques. After solidification of the media, five bores were made at equal distance by using sterile steel cork borer (8 mm diameter). Into these well different concentrations of test extracts and standard drugs were introduced. DMSO was used as a control. After introduction of standard drugs and the extracts to be screened, the plates were placed in a refrigerator at 8 10ºC for proper diffusion of drugs into the media. After two hours of cold incubation, the petriplates were maintained in an incubator at 37ºC for 24hrs.The plates were observed for clear zone formation around the well and the experiment was carried out in triplicate. Antibacterial activities were expressed in millimetre (Table 2).

Anti-fungal activity

The antifungal activity was studied by cup plate method as described above. The fresh cultures of *Aspergillus niger*, *A. fumigatus* and *A. flavus* were introduced into Potato-Dextrose Agar media and poured into petriplates. After solidification five bores (8mm) were made with the help of sterile cork borer. Standard drug Griseofulvin (50µg /ml) and extract solutions (50µg /ml and 100 µg /ml) were prepared in DMSO separately and introduced into the wells. Only DMSO was introduced into a well, which served as control. The test plates were incubated at 25ºC for 24 hrs and zone of inhibition were measured, and the results were tabulated (Table 3).

RESULTS AND DISCUSSION

In the present investigation, the phytochemical screening and antimicrobial activities were studied with the solvent of ethyl acetate, chloroform, methanol and aqueous extract of the leaves of *Azima tetracantha*. The result revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, protein, glycosides, terpenoids and phenol in leaves (Table 1).

Table 1 Preliminary phytochemical screening of leaf extracts of *Azima tetracantha* lam

S.no	Phytochemical Test	ethylacetate	chloroform	methanol	aqueous
1	Carbohydrates	-	-	-	-
2	Terpenoids	-	+	+	-
3	Alkaloids	+	+	+	+
4	Saponins	-	-	+	+
5	Protein	+	+	+	-
6	Steroids	-	-	+	+
7	Phenol	+	+	+	+
8	Flavonoids	-	+	+	-
9	Tannins	+	+	+	+
10	Glycosides	-	-	+	+

(+ present; - absent)

Antimicrobial activity of the leaves of Azima tetracantha

The antimicrobial activity of different concentration of aqueous, chloroform, ethyl acetate and methanol extract of the leaves of *Azima tetracantha* against bacterial and fungal pathogens were recorded in (Table 2 and Table 3).

Antibacterial activity

The results of the plant extracts viz., ethylacetate, chloroform, methanol and aqueous extracts were subjected to antibacterial activity revealed only chloroform and methanol extracts showed encouraging results and are tabulated (Table2). Both the chloroform and methanol extracts possessed antibacterial activity in a concentration dependent manner against the test organisms at concentrations of 50 and 100µg /ml and are comparable with the standard drug Streptomycin and Procaine penicillin. The effect of the extract was however found to be lower than the reference drugs at concentrations studied. In the chloroform extract, maximum zone of inhibition was recorded by *E. coli* at both the concentrations (10 and 12 mm) while *Klebsiella pnemoneae* recorded minimum zone of inhibition (07 and 09 mm) at similar concentrations indicating its susceptibility. In regard to methanol extracts, zone of inhibition found to be maximum at 100µg /ml concentration where in similar to chloroform extract, *E. coli* was found to be most sensitive (14 and 17 mm) followed by *Pseudomonas aerugenosa* (13 and 15 mm) where as *Klebsiella pnemoneae* recorded lowest zone of inhibition values (11 and 13 mm).

Antifungal activity

In antifungal experiments, among the various solvents used. only methanol extract has showed maximam zone of inhibition in a dose dependent manner (Table 2). *Aspergillus flavus* exhibited 04 and 10 mm of inhibition at 50 and 100 µg /ml concentration respectively, while *Aspergillus niger* showed 12 and 17 mm of clear zone at similar concentrations thus indicating sensitivity. However the zone of inhibition was less when compared to the reference standard.

Table 2 Antibacterial activity of leaf extracts of *Azima tetracantha* Lam

S.no	Name of the bacteria	Zone of Inhibition in diameter (mm)					
				Chloroform		Methanol	
		Streptomycin 50µg /ml	Procaine penicillin 50µg /ml	50µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
1	*Bacillus subtilis*	4	19	08	11	11	14
2	*Staphylococcus aureus*		18	08	10	12	14
3	*Escherichia coli*	15		10	12	14	17
4	*Klebsiella pneumoniae*	17		07	09	11	13
5	*Pseudomonas aeruginosa*		20	09	12	13	15

Table 3 Antifungal activity Leaf extracts of *Azima tetracantha* Lam

S. no	Name of the fungi	Zone of Inhibition in diameter (mm)				
		Fluconazole. 50µg /ml	Chloroform		Methanol	
			50µg /ml	100µg /ml	50µg /ml	100µg /ml
1	*Aspergillus flavus*	15	05	08	04	10
2	*A. niger*	21	09	13	12	17
3	*A. fumigatus*	17	05	11	05	12

DISCUSSION

The present study revealed that the Methanol extracts of *Azima tetracantha* leaves contains flavonoids, triterpenoids, alkaloids, steroids, phenolic compounds and tannins were analysed. Specifically, tannins and alkaloids present in the extract may be attributed to the antimicrobial properties (Ghosh *et al*., 2006). Interest in plants with antimicrobial properties has revived as a result of current problems such as resistance, associated with the use of the antibiotics. The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternative, offering profound therapeutic benefits and more affordable treatment. Over the past several years, intensive efforts have been made to discover clinically useful antimicrobial drugs, which have been reviewed by many researchers (Athnasiadou *et al*., 2001). The current investigation revealed that the methanol and chloroform extracts of *A. tetracantha* leaf possess potent anntibacterial activity. The results of zone of inhibition study revealed that the test extracts possessed antibacterial activity in a higher concentration dependent manner against the test organisms at concentrations of 50 and 100 µg/ml and are comparable with the standard drugs. The activity could be attributed to the presences of phytoconstituents viz., flavonoids, triterpenoids, alkaloids, steroids, phenolic and tannins compounds which have multiple biological effects, including antioxidant, wound healing properties. Which are toxic to the microorganisms. The flavonoids, phenolic compounds in the test plant are important for the plant growth and defense mechanism against infection and injury. These compounds while exhibiting antioxidant property are usually also act as excellent antimicrobial agents and anti-inflammatory activities (Cos *et al*., 2001). The underlying mechanisms could be enzyme inhibition by oxidation (McGaw, *et al*., 2002). However, the variation in antimicrobial sensitivity may be due to the differences in the phytochemical nature of the plant materials. (Kotze and Eloff 2002). Thus the present activity produced by *Azima tetracantha* Lam. might be because of these constituents.

CONCLUSION

Results of this study revealed the presence of biologically active constituents which might be responsible for the antibacterial and antifungal activity. The antimicrobial activities of the different solvents of plant extract prove the usage of the plant as a traditional medicine. Multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune supervision and allergic reactions. This situation forced scientists to search for new antimicrobial substances to search for new antimicrobial substances. Our future plan of work is to isolate and identify the active compounds present in this extract against various diseases.

References

Athnasiadou S, Kyriazakis F, Jackson. And Coop. (2001) *"Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: In vitro and in vivo studies. Vet. Parasitol". 99: 205-219.*

Chattopadhya M K and Khore RL (1969) "*Ind. J. Pharm".*; 31, 104.

Chelladurai V. and Minnikizhangu-(1983) An unique folk medicinal plant from the adivasis (tribals) of point Calimere, Tamil Nadu. "*Bull Med Ethnobot Res*."4:148-153.

Cos P, Calomme M, Sindambiwe JB, De Bruyne T, Cimanga K, Pieters L, Vlietinck AJ and Vanden Berghe D. (2001)*."Cytotoxicity a dlipid peroxidation inhibiting activity of flavonoids. planta Medica"* 67;515-519.

Daulatabad CD, Desai VA, and Hosamani KM 1991. Novel fatty acids in *Azima tetracantha* seed oil. "*J am oil chem soc".*; 68:978-979

Duraipandiyan, V. Muniappan, A. Savarimuthu, I. (2006); Antibacterial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. "*Complementary and alternative medicine".* 10, 6-35.

Ghosh T, Maity TK, Bose A, Dash GK and Das M. *(2006) "A study on antimicrobial activity of Bacopa monnieri* Linn*. Aerial parts. J. Nat.Rem". 6(2):170- 173.*

Ismail TS, Gopalakrishnan S, Begum VH and Elango V (1997) "*Anti- inflammatory activity of Salacia oblonga* Wall *and Azima tetracantha* Lam*... J. Ethnopharmacol.*" 56(2), 145-152.

Kirtikar KR, Basu BD and An ICS (1984) "*Ind. Med. Plants".* 1 & 2, 3nd Edn. Bishen Singh Mahendra Pal Singh, Dehra Dun. pp: 582- 1541

Kokate CK, Khandelwal KR, Pawar AP, and Gohale SB. (1994) "Practical Pharmacognosy", 3rd ed. Nirali Prakashan, Punc., 137-139.

Kotze M and Eloff JN. (2002). *Extraction* of antibacterial compounds from *Compretum microphyllum* (Combretaceae*). "South African Journal of Botany." 68: 62-67.*

McGaw LJ, Jager AK and Van Staden J. *(*2002)*.Variation in antibacterial activity of Schotia Species. "South African Journal of Botany".* 68: 41-46.

Nadkarni KM (1976) "Indian Meteria Medica", 3rd Edn. Popular Prakhasan, Bombay, 1,165.

Prusti, A. Mishra, SR. Sahoo, S.and Mishra, SK (2008). Antibacterial activity of some Indian medicinal plants. "*Ethnobotanical Leaflets".*; 12, 227-230.

Rall GJH, Smalberger TM, de Waal HL, and Arndt RR. (1967) "Dimeric piperdine alkaloids from *Azima tetracantha* Tetrahedron Letters", 36: 3465-66.

Rao VE, and Prasada Rao PRS (1978). Occurrence of triterpenoids in *Azima tetracantha*. *Curr Sci*.47:857.

Sukanya SL, Sudisha J, Hariprasad P, Niranjana SR, Prakash HS, and Fthima SK (2009)

"Antimicrobial activity of leaf extracts of Indian medicinal plant against clinical and phytopathogenic bacteria, "*African Journal of Biotechnology"* 8(23): 6677-6682.

Vasikaran Williams U, and Nagarajan S. (1987) "Isorhamnetin 3-rutinoside from leaves of *Azima tetracantha* Lam", "*Indian Journal of Chemistry*"., 27B: 387.

Venkatrao E, and Prasad Rao PRS. 1978 "Occrurrence of triterpenoids in *Azima tetracantha", Current Science*., 47(22): 857.

Williams UV, and Nagarajan S. (1988) Isorhamnetin-3-O-rutinoside from leaves of *Azima tetracantha* Lain. "*Indian Journal of Chemistry*".; 27B-387.

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