ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN 0976-9595

Research Article

PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF *AZIMA TETRACANTHA L*

Thaha Thariq J.*, Prince L.

PG and Research Department of Microbiology, Marudupandiyar College (Affiliated to Bharathidasan University), Thirucirappalli, Thanjavur, Tamilnadu, India

**Corresponding author: yourstharik@gmail.com*

ABSTRACT

Azima tetracantha L is the most important Indian medicinal plant used in the treatment of various diseases. The phytochemical studies on the plate (leaf) extract showed that the components present in the ethyl acetate and ethanol extracts were found to be carbohydrates, tannins and phenolic compounds, flavonoids and lignin. The antimicrobial activity of ethanol, diethyl ether, petroleum benzene and ethyl acetate of *Azima tetracantha L* leaves were tested on two gram positive organisms *(Streptococcus mutans, Staphylococcus epidermidis*), three gram negative organisms; (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi*) and three fungal species (*Aspergillus flavus, Aspergillus niger* and *Candida sp.*). The zones of inhibition ranged between 6mm to 19mm for bacterial pathogens and 9mm to 20mm for fungal pathogens. Extracts made in ethanol and ethyl acetate was found to be effective against most or the organisms. For interest, the four solvent extract of leaves of *Azima tetracantha L* were mixed or combined together and tested against eight microorganisms at varying concentration.

Keywords: *Azima tetracantha L,* Phytochemical, Antimicrobial activity

1. INTRODUCTION

Plants have been an essential part of human society since the start of civilization. During the vedic period, great importance was given to plants. *Rig Veda* and *Atharva Veda* describes around 250 medicinal plants. It is estimated and approximately fifty six percent of low income world's population use herbal medicine and supplementation for their primary health care. Respiratory infections, diarrhea, fungal or bacterial infections, diabetes and malaria are among the common health problems in rural communities in tropical developing countries. The rural population in the world is more disposed to traditional ways of treatment because of the easy availability and cheaper cost [1]. Numerous tropical medicinal plants are used traditionally which are remedial against these diseases [2]. Medicinal and aromatic plants and their essence, rich in antibacterial and antifungal compounds, could be an alternate way to combat against bacterial and fungal diseases [3].

Azima tetracantha L is the most important Indian medicinal plant used in the treatment of various diseases. Although some chemical constituents of its plant have been isolated earlier and their chemical structures were elucidated, detailed investigation to identify the chemical constituents responsible for the curative action of these crude drugs has not yet been reported. Therefore, the present investigation was undertaken with its primary motive to isolate and identify the chemical constituents of the above mentioned plant. The aim of this study is to screen the antimicrobial activity of the leaves of *Azima tetracantha L* using four different organic solvents such as ethanol, diethyl ether, petroleum benzene and ethyl acetate.

2. MATERIAL AND METHODS

2.1. Collection and identification of plant

The fresh plant materials were collected with sterilized knife in clean polyethylene bags separately and brought to the laboratory. Plant parts were washed and botanically identified, cut into small pieces and then dried in shade for about two weeks. The dried plant material was coarsely powdered and passed through sieve and used for the extraction by using the solvents of increasing polarity.

2.2. Continuous hot extraction-using soxhlet apparatus

The coarse powder was packed in the soxhlet apparatus and extracted with two solvents namely ethanol and

ethyl acetate separately by continuous hot percolation method. After the completion, each extract was filtered and the solvents were removed by distillation under reduced pressure, a colored residue was attained [4].

2.3. Phytochemical test

The extract was analyzed for alkaloids, carbohydrate, phytosterols, fixed oil and fats, tannins and phenolic compounds, proteins and amino acids, gums and mucilage, flavonoids, lignin and saponins carried out by standard [5].

2.4. Microorganisms

The microbial species were obtained from MTCC-(Microbial Type Culture Collection) IMTECH, Chandigarh. The plant extracts were tested for antimicrobial activity in the disc diffusion assay using six important test strains of bacteria viz. *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus epidermidis* and *Streptococcus mutans*. Bacterial cultures were maintained on nutrient agar at 4°C. Slants 0.1 ml of bacterial cultures (18 hrs) was spread over appropriate sterile Muller Hinton agar plates. The fungal strains, *Aspergillus niger* and *Aspergillus flavus* were maintained on Czapek Dox Agar (CDA) and the cultures were sub-cultured at regular intervals of one month. The *Candida* sp. was maintained and sub cultured in the Sauboraud's Dextrose Agar.

2.5. Inoculum preparation

Bacterial cultures were sub-cultured in nutrient broth at 37°C for 18 hours and used for the experiments. Fungal cultures were sub-cultured in Czapek Dox Agar and Sauboraud's Dextrose Agar (*Candida sp.*) plates at 25°C for 24 hours and used for the subsequent tests.

2.6. Disc preparation

Different concentrations of *Azima tetracantha L* with different solvents used extract were 50 mg, 60 mg; 90 mg and 100 mg were taken. 6 mm in diameter of whatman No.1 filter paper discs were imbibed in the extracts solutions and were allowed to dry. Each dried disc contained different concentrations of each extract such as 6.25 mg, 7.5 mg, 8.75 mg, 10 mg, 11.25 mg, 12.5 mg/disc.

2.7. Assay of antimicrobial activity

Antimicrobial activity was carried out using disc diffusion method [6]. Different concentration impregnated discs were placed on the selected bacterial, fungal and *Candida* sp. swabbed plates. Each plate contains 6 different concentrated discs. Bacterial and *Candida* sp. plates were incubated at 37°C for 24 hrs and fungal plates were incubated at 28°C for 72 hrs. After incubation results were observed.

3. RESULTS AND DISCUSSION

The present study has screened the different organic solvents (Ethanol, Diethyl acetate and Petroleum benzene used extracts of the leaves of *Azima tetracantha L* against grams positive organisms (*Streptococcus mutans, Staphylococcus epidermidis*), gram negative organism; (*Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi*) and fungal species (*Aspergillus flavus*, *Aspergillus niger* and *Candida* sp.).

The phytochemical studies on the plate (leaf) extract showed that, the components present in the ethyl acetate and ethanol extracts were found to be carbohydrates, tannins and phenolic compounds, flavonoids and lignin. However, alkaloids, phytosterols, fixed oil and fats, proteins and aminoacids, gums and mucilage and saponins were not detected in the materials used (Table 1). Hence, we can conclude that the antibacterial and antifungal activity of the ethanol and ethyl acetate extracts may be due to the above phytochemical components. The antimicrobial activity may be due to the presence of carbohydrates, tannins and phenolic compounds, flavonoids and lignin etc. the same report is also given by Chattopadhyay *et al.,* [7] for the leaves of *Alstonia macrophylla*. Highly susceptible organisms namely *Streptococcus mutans* and *Escherichia coli* were selected to analyse minimal inhibitory concentration of the test plant extract on that bacterial strains.

The effect of ethanolic extract of leaves of *Azima tetracantha L* was tabulated (Table 2). The ethanol extract was found highly effective against all the test organisms at a concentration of 12.5 mg/disc. When the zone formation for each organism was compared to that of concentration there was an increase in zone formation for every increased concentration and the zone of inhibition is 11 mm as the highest, against *Streptococcus mutans*. The negative control (Disc having only the solvent ethanol) had no effect on both the Gram negative and Gram positive organisms used in this study.

Satdive *et al.,* [8] reported about the ethanolic extract of *Gymnema sylvestre* leaves exhibited antimicrobial activity against *Pseudomonas aeruginosa* and inactivity against *Escherichia coli*. Similarly ethanolic extracts appeared to

exert more inhibitory action against the bacteria (*Escherichia coli*). This was reported by Irobi and Daramola, [9] in the same way, the ethanolic extracts of leaves of *Azima tetracantha L* were also exhibited good inhibitory activity on the *Escherichia coli*.

The results tabulated in Table 3 showed that the diethyl ether extract had good effect on Ampicillin resistant *Escherichia coli* (12mm) than *Staphylococcus epidermidis* (5 mm). They had no activity against *Streptococcus mutans*, *Salmonella typhi* and *Pseudomonas aeruginosa*. The variation in the zone formation for every increased concentration is only by 10 mm for Ampicillin resistant *Escherichia coli*. The negative control (Disc containing the solvent diethyl ether alone) showed no activity against all the organisms.

Table 1: Phytochemical screening of *Azima tetracantha L*

S. No.	Phytoconstituents	Ethyl acetate Extract	Ethanol Extract
1	Alkaloids	-	-
2	Carbohydrate	+	+
3	Phytosterols	-	-
4	Fixed oil and fats	-	-
5	Tannins and Phenolic compounds	+	+
6	Proteins and amino acids	-	-
7	Gums and mucilage	-	-
8	Flavonoids	+	+
9	Lignin	+	+
10	Saponins	-	-

Table 2: Antibacterial activity of ethanolic extract of *Azima tetracantha L* leaves

S. No.	Concentration of plant extract and control (µl) solvent ethanol	Zone of inhibition (mm in diameter)				
		Name of the organisms				
		Sm	*Se*	*Pa*	*Ec*	*St*
1	Negative control	-	-	-	-	-
2	6.25 mg/disc	7±0.20	5±0.10	-	15±0.20	-
3	7.5 mg/disc	7±0.10	5±0.40	5±0.20	16±0.50	5±0.20
4	8.75 mg/disc	8±0.20	5±0.30	6±0.40	18±0.40	6±0.40
5	10 mg/disc	8±0.15	5±0.20	7±0.30	17±0.50	7±0.20
6	11.25 mg/disc	9±0.10	4±0.20	8±0.20	18±0.70	5±0.20
7	12.5 mg/disc	11±0.20	5±0.40	8±0.20	19±0.50	5±0.20

Values are expressed Mean ± Standard Deviation; n=3

Sm-Staphylococcus epidermidis; Sm-Streptococcus mutans; Ec-Escherichia coli; Pa-Pseudomonas aeruginosa; St-Salmonella typhi

Table 3: Antibacterial activity of Diethyl ether extract of *Azima tetracantha L* leaves

S. No.	Concentration of plant extract and control (µl) solvent Diethyl ether	Zone of inhibition (mm in diameter)				
		Name of the organisms				
		Sm	*Se*	*Pa*	*Ec*	*St*
1	Negative control	-	-	-	-	-
2	6.25 mg/disc	-	-	-	08±0.24	-
3	7.5 mg/disc	-	-	-	08±0.30	-
4	8.75 mg/disc	-	-	-	09±0.40	-
5	10 mg/disc	-	-	-	10±0.50	-
6	11.25 mg/disc	-	-	-	10±0.50	-
7	12.5 mg/disc	-	-	-	12±0.20	-

Values are expressed Mean ± Standard Deviation; n=3

Sm-Staphylococcus epidermidis; Sm-Streptococcus mutans; Ec-Escherichia coli; Pa-Pseudomonas aeruginosa; St-Salmonella typhi

The ethanolic, diethyl ether and ethyl acetate extract of leaves of *P. reticulates* had good inhibitory effect on *Streptococcus mutans* growth [8]. But, the ethanolic and petroleum benzene extract of leaves of *Azima tetracantha L* also exhibited the same effect on *Streptococcus mutans*. A similar work was done by Nicole Didry *et al.,* [10] on aerial parts of *Drosera peltata* who showed that the ethanol, diethyl ether, ethyl acetate and petroleum ether exhibited antimicrobial activity against *Streptococcus mutans*.

In table 4 the effect of petroleum benzene extract of *Azima tetracantha L* against five organisms are indicated. Petroleum benzene was active only against *Streptococcus mutans* as 7 mm and no activity was observed for other tested organisms. The negative control (disc containing the solvent petroleum benzene alone) had no effect on all the tested organisms.

3.1. Ethyl acetate - Leaf extract

From table 5, it was seen that ethyl acetate extracts exhibited high activity against *Escherichia coli* (8 mm). The Ethyl acetate extract showed activity against *Staphylococcus epidermidis* (6 mm) only at high concentration. They did not exhibit any activity against *Streptococcus mutans*, *Pseudomonas aeruginosa* and *Salmonella typhi*. When the zone formation for *Escherichia coli* was compared to that of concentration, there was an increase of 1.0 mm for every concentration increased. The negative control (disc contained the solvent ethyl acetate) has no activity on bacterial cultures used in the study.

The ethyl acetate extract of *Euphorbia thymifolia* inhibited the growth of *Escherichia coli*; Ethyl acetate extract appeared to be more potent. This was given by Khan *et al.,* [11], But, the ethyl acetate extracts of *Azima tetracantha L* leaves has no effect on *Escherichia coli* and diethyl ether extract and ethanolic used extracts found to be more potent than ethyl acetate. Mensah *et al.,* [12] reported the alkaloid fraction of LWF from the leaves of *P. discoideus* inhibited the growth of *Escherichia coli* and *Pseudomonas aeruginosa*. The same results were obtained in the *Azima tetracantha L* leaf extract.

Table 4: Antibacterial activity of Petroleum benzene extract of *Azima tetracantha L* leaves

S. No.	Concentration of plant extract and control (µl) solvent petroleum benzene	Zone of inhibition (mm in diameter)				
		Name of the organisms				
		Sm	*Se*	*Pa*	*Ec*	*St*
1	Negative control	-	-	-	-	-
2	6.25 mg/disc	6±0.20	-	-	-	-
3	7.5 mg/disc	6±0.40	-	-	-	-
4	8.75 mg/disc	6±0.20	-	-	-	-
5	10 mg/disc	6±0.40	-	-	-	-
6	11.25 mg/disc	7±0.50	-	-	-	-
7	12.5 mg/disc	7±0.40	-	-	-	-

Values are expressed Mean ± Standard Deviation; n=3

Sm-Staphylococcus epidermidis; Sm-Streptococcus mutans; Ec-Escherichia coli; Pa-Pseudomonas aeruginosa; St-Salmonella typhi

Table 5: Antibacterial activity of ethyl acetate extract of *Azima tetracantha L* leaves

S. No.	Concentration of plant extract and control (µl) solvent ethyl acetate	Zone of inhibition (mm in diameter)				
		Name of the organisms				
		Sm	*Se*	*Pa*	*Ec*	*St*
1	Negative control	-	-	-	-	-
2	6.25 mg/disc	-	-	-	6±0.30	-
3	7.5 mg/disc	-	-	-	7±0.20	-
4	8.75 mg/disc	-	-	-	7±0.50	-
5	10 mg/disc	-	-	-	8±0.20	-
6	11.25 mg/disc	-	6±0.30	-	8±0.30	-
7	12.5 mg/disc	-	6±0.40	-	8±0.40	-

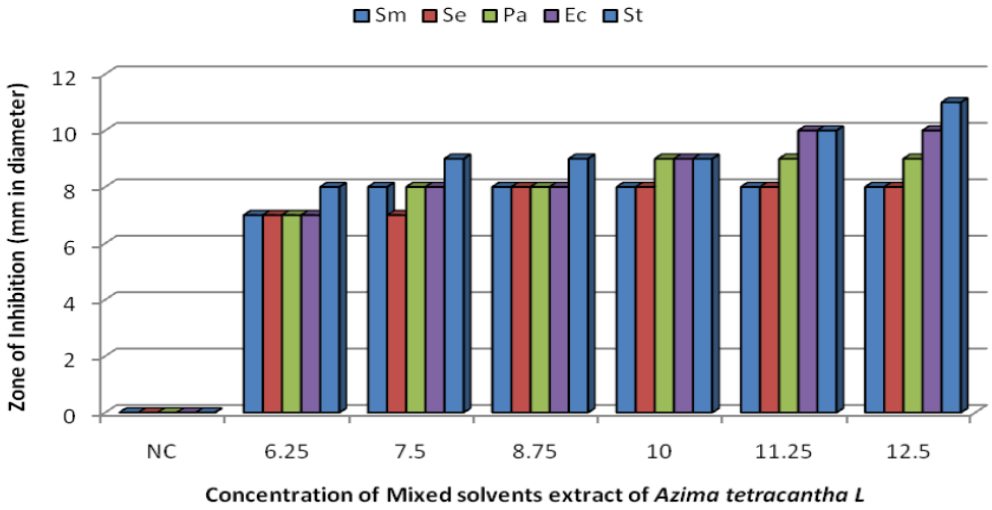
Values are expressed Mean ± Standard Deviation; n=3

Sm-Staphylococcus epidermidis; Sm-Streptococcus mutans; Ec-Escherichia coli; Pa-Pseudomonas aeruginosa; St-Salmonella typhi

From the table 2, 3, 4 and 5, it was understood that the extracts made in ethanol and ethyl acetate was found to be effective against most or the organisms. For interest, the four solvent extract of leaves of *Azima tetracantha L* were mixed or combined together and tested against eight microorganisms at varying concentration.

Fig. 1 indicated that the mixed solvent extract of *Azima tetracantha L* leaves had good effect on *Streptococcus mutans*, *Staphylococcus epidermidis* and *Escherichia coli*. They exhibited less activity as 5 mm against *Pseudomonas aeruginosa* and no effect on rest on the other organisms used in the study. As far as susceptibility of the organisms concerned, *Staphylococcus epidermidis* and *Escherichia coli* was found to be the most susceptible and *Salmonella typhi* and *Pseudomonas aeruginosa* were the most resistant towards the four solvent extracts.

The analysis was done with the fungus *Candida* sp. and the results were recorded in Fig. 2. From the current study results, the ethanol extract of *Azima tetracantha L* have antifungal properties. The growth inhibition effect is revealed by the zone of inhibition. The zone ranges from 7 to 9 mm and 11 to 20 mm in diameter as related to their concentration. Whereas the inhibition of *Candida* species to the *Phyllanthis reticulates* extract is a side spectrum and the results indicates a strong antifungal activity with the inhibition zone of 16-20 mm in diameter as related to concentration of lower to higher level.



Sm-Staphylococcus epidermidis; Sm-Streptococcus mutans; Ec-Escherichia coli; Pa-Pseudomonas aeruginosa; St-Salmonella typhi

Fig. 1: Antibacterial activity of mixed solvent extract of *Azima tetracantha L*

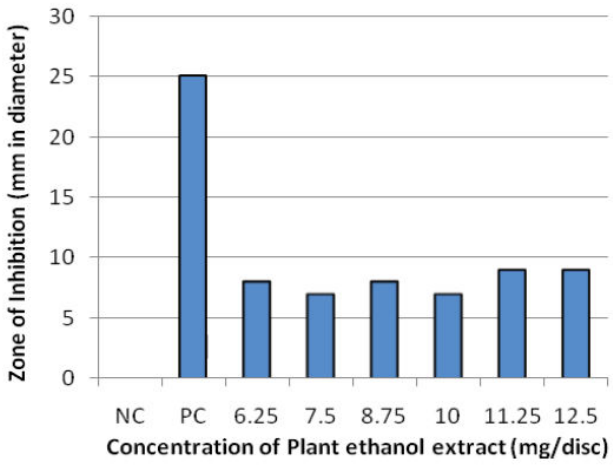


Fig. 2: Antifungal activity of *Candida sp.*

The selected fungi *Aspergillus niger*, *Aspergillus flavus* revealed growth in both extracts added plates. When compared with the control culture of *Aspergillus niger* and *Aspergillus flavus* has shown white colour cottony mycelium without spore formation after 3rd day of incubation at 25 mg and 50 mg/25ml concentration plates. In the controlled plate, the fungi produced all characters with spore formation after 2nd day of incubation. In the higher concentration of *Azima tetracantha L* (50 mg/25ml) plate, *Aspergillus niger* and *Aspergillus flavus* growth was observed. But their growth characters are mostly nil except white aerial mycelia growth up to 5 days of incubation. The growth diameter of *Aspergillus niger* and *Aspergillus flavus* the range are 17 mm, 20 mm and 19 mm, 18 mm in extract added plates at the concentration of 25mg/25ml. like this, 15mm,

18mm and 17mm, 13 mm was observed in *Aspergillus flavus* and *Aspergillus niger* plates at the concentration of 50mmg/20 ml. The efficiency was compared with Ketoconazole (100 µg/25 ml) compound (Fig. 3). The test plant extract did not completely inhibit the growth at both concentration (25mg and 50 mg/25ml). No fungal growth was observed at the concentration of 100 µg/25 ml in Ketaconazole incorporated plate.

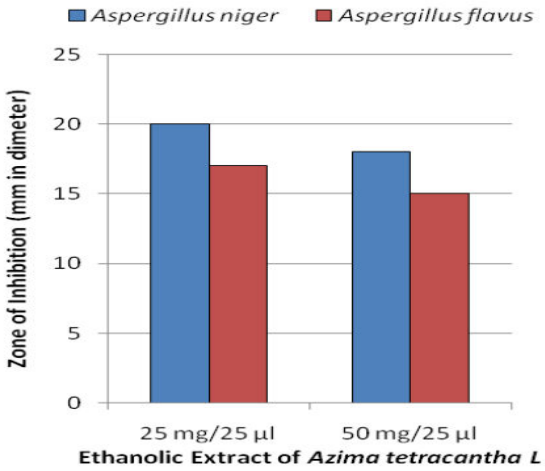


Fig. 3: Antifungal activity of ethanolic extract of *Azima tetracantha L* leaves

4. CONCLUSION

The results of antibacterial and antifungal properties of the plant extracts were compared with known standard broad spectrum antibacterial and antifungal agents namely Gentamycin, Trimethoprime for bacteria and Erythromycin for *Candida sp.* and Ketogonazole for fungi. While comparing, the inhibitory activity of both plant extracts to the antibiotics Gentamycin, *Azima tetracantha L* showed good level of inhibitory activity on *Streptococcus mutans* at 12.5 mg/disc; and showed activity on *Escherichia coli* and *Staphylococcus epidermidis*. But Trimethoprime normally did not suppress the bacterial growth except *Pseudomonas aeruginosa* and *Salmonella typhi*.

5. ACKNOWLEDGMENTS

The authors are thankful to Specialty Lab & Research, Thanjavur for offering facilities to carry out this study.

6. REFERENCES

1. Subbarayappa BV. *Journal of Bioscience,* 2001; **26**:134-144.
2. Somchit MN, Mutalib AR, Ahmad Z, Sulaiman MR, Norli S. *Jr. Trop. Med. Plants,* 2004; **5**:15-19.
3. Ramasamy S, Charles Monoharan A. *Asian Jr. of Microbial. Biotech. Env. Sci,* 2004; **6(2)**:209-210.
4. Kokate CK, Practical Pharmacogonsy, Vallabh Prakashan, New Delhi, 2005; 110.
5. Harborne JB, Phytochemical methods, Chapman and Hall, London, 1998; 60.
6. Bauer AW, Sherris TM, Kirbay WMM, Turk M. *Amer. J. Clinical Pathol,* 1966; **45**:493-496.
7. Chattopadhyay D, Maithi K, Kundu AP, Chakraborty MS, Bhadra R, Mandal SC, Mandal AB. *Journal of Ethanopharmacology,* 2001; **77**:49-55.
8. Satdive RK, Abhilash P, Fulzela DP. *Fitoterapia,* 2003; **74(7-8):**699-701.
9. Irobi ON, Daramola SO, *Jr. of Ethanopharmacology,* 1994; **42**:39-43.
10. Nicole Didry, Luc Dubraeuil, Francis Trotil, Madeleine Pinkas, *Journal of Ethanopharmacology,* 1998; **60**:91-96.
11. Khan NH, Rahman M, Nur-e-Kamal MSA, *India J. Med. Res,* 1988; **87**:395-397.
12. Mensah JL, Lagarde I, Ceschin C, Michel G, Gleye J, Fouraste I. *Journal of Ethanopharmacology,* 1990; **281**:129-133.