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Qualitative analysis of phytoconstituents from *Azima tetracantha* L. and evaluation of its antimicrobial activity

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Abstract

Azima tetracantha L., a medicinal plant from the Salvadoraceae family, is traditionally recognized for its diverse therapeutic properties, including antimicrobial, anti-inflammatory, hepatoprotective, and hypoglycemic effects. This study aimed to screen the phytochemical constituents and evaluate the antimicrobial potential of its leaf extracts. The phytochemical analysis revealed the presence of key bioactive compounds such as tannins, flavonoids, phenolic compounds, lignin, and terpenoids in ethyl acetate and ethanol extracts. The antimicrobial activity was assessed using the disc diffusion method against various bacterial (*Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Salmonella typhi*, and *Staphylococcus epidermidis*) and fungal (*Aspergillus niger*, *Aspergillus flavus*, and *Candida* sp.) strains. The ethanol extract demonstrated the highest inhibition against *Escherichia coli* (11 mm), *Streptococcus mutans* (11 mm), and *Staphylococcus epidermidis* (12 mm). The ethyl acetate extract exhibited notable inhibition against *Escherichia coli* (8 mm) and *Staphylococcus epidermidis* (6 mm). The diethyl ether extract showed strong inhibition against *Escherichia coli* (12 mm) but was less effective against other bacterial strains. Petroleum benzene extract exhibited limited activity, with inhibition observed only against *Streptococcus mutans* (7 mm). Mixed solvent extracts displayed the highest inhibition against *Escherichia coli* (20 mm), *Streptococcus mutans* (20 mm), *Staphylococcus epidermidis* (15 mm), and *Candida* sp. (16–20 mm). GC-MS analysis identified several bioactive metabolites, further highlighting the pharmaceutical potential of *Azima tetracantha* L. The findings support its traditional medicinal use and suggest its potential role in developing novel antimicrobial agents.

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Introduction

Since ancient times, medicinal plants have been valued for their potent abilities to treat and manage a wide range of health issues (Begum *et al.*, 2009). The World Health Organization reports that 80% of the world's population relies on medicinal plants, which are incorporated into over 30% of pharmaceutical products. Medicinal plants owe their diverse range of effects to the many phytochemicals they contain, including alkaloids, anthraquinones, flavonoids, glycosides, phenols, saponins, steroids, sterols, tannins, terpenoids, triterpenoids, phytosterols, hydrocarbons, mono- and sesquiterpenes, phlobatannins, and other secondary metabolites (Bennett *et al.*, 2004).

Various studies, including *in vitro* and *in vivo* experiments, have investigated the antioxidant properties of spices, their role in promoting digestion, reducing lipid levels, and their possible antibacterial, anti-inflammatory, antiviral, and anticancer effects. These plant components or their extracts are usually taken orally, either alone or in combination with various ingredients like water, honey, milk, juices, or black pepper (Nandgude *et al.*, 2007; Rall *et al.*, 1967; Rao and Prasada Rao, 1978). In modern times, plant-derived secondary metabolites are employed in numerous sectors, including food production (for flavor enhancement, nutritional improvement, and spoilage prevention), medicine (for the prevention and treatment of diseases, chemoprevention, and as natural antimicrobials), as well as in pharmacology and cosmetology (e.g., cosmetics and dietary supplements without preservatives). Ayurveda, Siddha, and Unani are traditional forms of medicine that have been practiced for hundreds of years. Drugs developed from Ayurvedic practices to treat current health conditions are now accessible in the market (Williams and Nagarajan, 1988). The World Health Organization reports that about 80% of individuals in developing countries use traditional medicine as their main source of primary health care. *Azima tetracantha* L. (belonging to the Salvadoraceae family) is valued for its significant medicinal properties (Nandagude *et al.*, 2007). Its roots, leaves, fruits, and stems have been traditionally used to address various health conditions and are noted for their stimulant, expectorant, antispasmodic, analgesic, anti-inflammatory, anti-ulcer, anti-diarrheal, antimicrobial, hepatoprotective, nephroprotective, hypoglycemic, and hyperlipidemic effects (Bennett *et al.*, 2004; Kekuda and Raghavendra, 2017). The plant *Azima tetracantha* L. is recognized by a variety of synonyms, such as *Monetia barlerioides* L'Herit., *Azima nova* J. F. Gmel., *Kandena spinosa* Rafin., *Monetia angustifolia* Boj. Ex A. DC., and *Monetia tetracantha* (Lam.) Salisb. The plant is called "Kundali" in Ayurvedic medicine, whereas in Siddha, it goes by the name "Mulchangan." *Azima tetracantha* is a perennial shrub that grows to about 3 meters in height, often thriving in hot, dry riverine scrub regions, especially in soils that are alluvial or saline (Tiwari *et al.*, 2011). The leaves are noted for their rigid texture and elliptical shape, along with a pale green color. These unisexual flowers, which are small and can be greenish-white or yellow, cluster together in the axils. The fruits of this plant are spherical and shiny white, containing seeds that are flat and circular in shape (Oman *et al.*, 2013; Prabu *et al.*, 2013; Saxena *et al.*, 2013; Mottaleb and Sarker, 2012). *A. tetracantha* L. is recognized for its diuretic properties, which help manage rheumatism, dropsy, dyspepsia, and chronic diarrhea, and it is additionally used as a tonic stimulant post-childbirth. Beneficial for addressing cough, phthisis, asthma, smallpox, and diarrhea, this plant's leaves, root, and root bark are particularly noted for their effectiveness in treating rheumatism. The root has been traditionally used as a diuretic and is applied in Siddha medicine to treat dropsy and rheumatism. In herbal practices, the leaves serve as a stimulant, expectorant, and antispasmodic. Moreover, the bark is utilized for its antiperiodic, astringent, and expectorant properties, particularly in managing cough and asthma (Raaman, 2006). In this study we screened and evaluate the phytoconstuents from *Azima tetracantha* L. plant extract.

Materials and methods

Gathering and classification of plants

The garden-fresh plant materials were collected by using sterilized knife into clean polyethylene bags and finally it brought to the laboratory. Leaf of the plant was washed and identified by botanically, then cut into tiny pieces

and allowed to dry for approximately two weeks in shade dry. The shaded dried plant leaf was ground into a coarse powder put through a filter and at last extracted by using various solvents of increased polarity.

Preparation of phyto-extraction by using soxhlet

The grainy powder was filled into the soxhlet apparatus and passed through two exacting solvents known as ethyl acetate and ethanol distinctly by continuous hot percolation method. After collecting the filtered extraction the solvents were eliminated through distillation under reduced pressure and to the end the coloured filtrate was attained (Rall *et al.*, 1967).

Phytoconstituent test

The phytochemical analysis of the plant leaf was examined for carbohydrate, fixed oil and lipids, amino acid as well as protein, alkaloids, tannins, phenolic compounds, lignin, saponins, flavonoids, phytosterols, gums and mucilage carried out by standard (Rao and Prasada Rao, 1978).

Microorganisms

The microbe species were collected from MTCC-(Microbial Type Culture Collection) IMTECH, Chandigarh. The plant leaf extracts were examined for activity of antimicrobial in disc diffusion method for six important strains of bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Salmonella typhi*, *Staphylococcus epidermidis* and bacterial cultures were maintained at 4 °C nutrient agar. Slants 0.1 ml of bacterial cultures (18 hrs) was spread over suitable 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.

Preparation of inoculum

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

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.

Antimicrobial assay

Disc diffusion method was used to examine the antimicrobial activity (Williams and Nagarajan, 1988). Various 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 ℃ for 24 hrs and fungal plates were incubated at 28 ℃ for 72 hrs. The results were observed after incubation.

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 phytoconstituent study of the plant 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). Therefore, we can concluded that the antifungal and antibacterial of the ethanol and ethyl acetate exacts might 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 (Nandagude *et al.*, 2007) 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.

Table 1. Phytochemical screening of *Azima tetracantha* L.

Phytochemical compounds	Observation			
	Control	Aqueous	Acetone	Methanol
Tannins	-	-	+	+
Flavonaids	-	+	+	+
Terpenoids	-	-	+	+
Saponins	-	+	+	+
Phlobatannins	-	-	-	+
Steroids	-	-	-	+
Carbohydrates	-	-	-	-
Glycosides	-	-	-	+
Cournarins	-	-	-	-
Proteins	-	-	-	-
Emodins	-	-	-	-
Anthraquinones	-	-	-	-
Anthocyanins	-	-	-	-
Leucoantho cyaninsturns	-	-	-	-

The effect of ethanolic extract of leaves of *Azima tetracantha* L. incomycin (15mcg) was tabulated. The ethanol extract was found highly effective against all the test organisms at a concentration of 12.5 mg/disc (Table 2). 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. Bennett *et al.* (2004) 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 Kekuda and Raghavendra (2017) 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 showed that the diethyl ether extract had good effect on Ampicillin resistant *Escherichia coli* (12mm) than *Staphylococcus epidermidis* (5 mm). They didn't have any 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.

The ethanolic, diethyl ether and ethyl acetate extract of leaves of *P. reticulates* had good inhibitory effect on *Streptococcus mutans* growth (Bennett *et al.*, 2004). 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 (Tiwari *et al.*, 2011) on aerial parts of *Drosera peltata* who showed that the ethanol, diethyl ether, ethyl acetate and petroleum ether exhibited antimicrobial activity against *Streptococcus mutans*.

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.

It was seen that ethyl acetate extracts showed high activity against *Escherichia coli* (8 mm). At higher concentration the ethyl acetate extract showed activity against *Staphylococcus epidermidis* (6 mm). 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 *Euphorbia thymifolia* ethyl acetate extract inhibited the *Escherichia coli* growth. Ethyl acetate extract appeared to be more effective. This was given by (Oman *et al.*, 2013), 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.

Table 2. Antimicrobial activity (Zone of inhibition)

Microorganisms	Ethanol (mm)	Ethyl acetate (mm)	Diethyl ether (mm)	Petroleum benzene (mm)	Mixed solvent (mm)
Escherichia coli	11	8	12	0	20
Pseudomonas aeruginosa	0	0	0	0	5
Streptococcus mutans	11	0	0	7	20
Salmonella typhi	0	0	0	0	0
Staphylococcus epidermidis	12	6	5	0	15
Aspergillus niger	-	-	-	-	-
Aspergillus flavus	-	-	-	-	-
Candida sp.	-	-	-	-	16-20

Table 3. GC-MS analysis of *Azima tetracantha* L. extract

Sl	RT	Compound name	Area %	Height %
1	4.117	N,N-DIMETHYL-1,3-BUTADIEN-1-AMINE	18.9	14.72
2	8.469	3-PYRIDINECARBOXYLIC ACID, 1,2,5,6-TETRAHYDRO-1-METHYL-, METHYL ESTER	0.75	1.08
3	10.656	Methanamine, N-[3-methyl-2-butenylidene]	0.85	0.86
4	10.954	Neophytadiene	0.74	1.12
5	11.18	1-Methyl-pyrrolidine-2-carboxylic acid	3.49	1.01
6	11.357	2-METHYL-2-NONENE	4.83	3.02
7	11.529	Dodecane	0.56	0.6
8	12.43	4,6,7-TRIMETHYL-2H-AZEPINE-2,5(6H)-DIONE	2.92	2.55
9	17.779	2-CYCLOPENTEN-1-OL, 5-(1,1,3-TRIMETHYL-2-BUTENYL)-	0.35	0.56
10	18.087	2-CYCLOPENTEN-1-OL, 5-(1,1,3-TRIMETHYL-2-BUTENYL)-	0.99	1.39
11	19.979	1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER	0.36	0.47
12	22.005	Oxalic acid, cyclohexylmethyl tridecyl ester	4.73	7.18
13	23.5	2(4H)-BENZOFURANONE, 5,6,7,7A-TETRAHYDRO-6-HYDROXY-4,4,7A-TRIMETHYL-, (6S-CIS)-	0.46	0.44
14	23.83	PLUCHIDIOL	1.29	1.01
15	24.534	Neophytadiene	1.09	1.51
16	24.629	2-PENTADECANON, 6,10,14-TRIMETHYL-	0.79	1.09
17	24.954	Phytol, acetate	0.5	0.69
18	25.17	9-Heptadecanone	0.29	0.48
19	25.271	Neophytadiene	0.39	0.6
20	25.807	5,9,13-PENTADECATRIEN-2-ONE, 6,10,14-TRIMETHYL-, (E,E)-	0.68	0.96
21	26.037	Hexadecanoic acid, methyl ester	1.91	2.5
22	26.113	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	1.04	1.51
23	26.693	DECANE, 5,6-BIS(2,2-DIMETHYLPROPYLIDENE)-, (E,Z)-	4.44	5.49
24	26.829	Sulfurous acid, cyclohexylmethyl hexyl ester	2.57	3.24
25	27.148	HEXADECANOIC ACID, ETHYL ESTER	0.54	0.71
26	27.434	Decane, 5,6-bis(2,2-dimethylpropylidene)-, (E,Z)-	7.39	10.99
27	28.714	9,12-OCTADECADIENOIC ACID (Z,Z)-, METHYL ESTER	0.24	0.43
28	28.811	8,11,14-Eicosatrienoic acid, methyl ester	0.71	0.86
29	28.983	Phytol	5.62	8.04
30	29.228	Methyl stearate	0.54	0.81
31	29.379	gamma-Sitosterol	0.46	0.49
32	29.899	beta-Sitosterol	0.49	0.55
33	31.818	Octadecanoic acid, 10-oxo-, methyl ester	1.42	1.4
34	32.451	4,8,12,16-Tetramethylheptadecan-4-olide	1.35	1.14
35	32.584	Friedelan-3-one	1.45	0.73
36	32.736	Octadecanoic acid, 10-oxo-, methyl ester	0.67	0.77
37	34.903	Tetradecanal	0.58	0.52
38	37.975	13-Docosenamide, (Z)-	21.56	16.59
39	38.957	alpha-Tocospiro A	0.95	0.67
40	40.047	delta-Tocopherol	1.09	1.24

Prabu *et al.* (2013) 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. incomycin (15mcg) leaf extract. It was found that the ethanol and ethyl acetate

extracts was found to be effective against most of the organisms. For interest, the four dissolvable concentrate of leaves of *Azima tetracantha* L. were combined or joined as one and tried against eight microorganisms at different fixation.

The results indicated that the mixed solvent extract of *Azima tetracantha* L. leaves had much more effect on *Escherichia coli*, *Streptococcus mutans*, and *Staphylococcus epidermidis*. They displayed less action as 5 mm against *Pseudomonas aeruginosa* and no impact on lay on different organisms utilized in the study. To the extent as susceptibility of the organisms concerned, *Staphylococcus epidermidis* and *Escherichia coli* was known to be the most susceptible and *Salmonella typhi* and *Pseudomonas aeruginosa* were the most resistant towards the four solvent extracts.

The examination was done with the fungus *Candida* sp. from the ongoing study results, the ethanol extract of *Azima tetracantha* L. have antifungal activities. The zone of inhibition denotes the growth inhibition effect. The zone ranges from 7 to 9 mm and 11 to 20 mm in diameter as connected with their concentration. Whereas the inhibition of *Candida* species to the Phyllan this reticulates extract is a side spectrum and the results denotes a strong antifungal activity with the inhibition zone of 16-20 mm in diameter as related to concentration of lower to higher level. Validation of the antimicrobial properties and GC-MS analysis of *Azima tetracantha* L. has been achieved (Table 3).

Conclusion

This study highlights the significant antimicrobial potential of *Azima tetracantha* L., confirming its traditional medicinal use. The phytochemical analysis revealed the presence of key bioactive compounds, including tannins, flavonoids, phenolic compounds, and lignin, which likely contribute to its antimicrobial properties. The antimicrobial activity assessment demonstrated that ethanol and ethyl acetate extracts exhibited strong inhibitory effects against *Escherichia coli*, *Streptococcus mutans*, and *Staphylococcus epidermidis*, with inhibition zones reaching up to 12 mm in ethanol extracts and 8 mm in ethyl acetate extracts. The mixed solvent extract exhibited the highest inhibition, particularly against *Escherichia coli* (20 mm), *Streptococcus mutans* (20 mm), *Staphylococcus epidermidis* (15 mm), and *Candida* sp. (16–20 mm). These findings suggest that *Azima tetracantha* L. possesses potent antimicrobial compounds that could be explored for pharmaceutical and therapeutic applications. Furthermore, GC-MS analysis identified multiple bioactive metabolites, reinforcing the plant's potential as a natural source for novel antimicrobial agents. Future research should focus on isolating and characterizing these bioactive compounds and conducting in vivo studies to validate their efficacy and safety for clinical use.

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