IJBPAS, May, 2025, 14(5): 3004-3013

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICAL AND ANTIBACTERIAL SCREENING OF DIFFERENT SOLVENT EXTRACTS OF *AZIMA TETRACANTHA*

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Received 16th Jan. 2024; Revised 20th Feb. 2024; Accepted 24th July 2024; Available online 1st May 2025

https://doi.org/10.31032/IJBPAS/2025/14.5.9063

ABSTRACT

The aim of this research work is to evaluate the pharmacological potentials of the *Azima tetracantha*. The agar diffusion method was used to examine the antibacterial activity of different solvent extracts of *Azima tetracantha* tested against *Escherichia coli*, *Pseudomonas aeruginosa*, Bacillus subtilis and *Staphylococcus aureus*. For qualitative phytochemical investigation, several solvent extracts of the complete *A. tetracantha* plant were investigated. The minimum inhibitory concentration (MIC) of *M. spicata* ethanol extract had significant antimicrobial effectiveness against *Escherichia coli* (480 µg/ml), and *Pseudomonas aeruginosa* (430 µg/ml). The ethanolic extract showed (425 µg/ml and 395 µg/ml) antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus* respectively. The lowest antibacterial activity was found in acetone extract with MIC (206µg/ml) of *A. tetracantha*. Various parts of the plant have the presence of variety of phytoconstituents such as flavonoids, saponins, tannins, terpenes, steroids, amino acids, essential oil, polysaccharides and pectin.

Keywords: *Azima tetracantha*, antimicrobial activity, agar diffusion method, Phytochemical analysis

INTRODUCTION

Due to increasing consumers concerns regarding processed and ready-to-eat foods containing antibiotics, pesticides, hormones, and synthetic additives and also increasing demand to replace artificial antimicrobial agents with natural alternatives, the usage of natural and organic foods has been experiencing explosive market growth **[1-3]**. However, the untreated products and natural foods may be more susceptible to growth of food-borne pathogens than the conventional food

version **[4]**. The most important food-borne pathogenic bacteria that have survived and grow in these products include *Staphylococcus aureus, Bacillus sp p., Listeria monocytogenes, Salmonella spp. Escherichia coli, Yersinia spp.,* and *Clostridium* spp. **[5-6]**. These bacteria cause a great proportion of food-borne outbreaks in different foods such as dairy products, vegetables, and meat and fish products **[7]**. In this context, plant essential oils are attracting interest as natural food preservatives in order to ensure the safety of food **[8]**.

Azima tetracantha is widely used in the alternative systems of medicine for the treatment various disease conditions including rheumatoid arthritis, cough, cold, fever, body pain, bronchitis, asthma, dropsy, etc. **[9]**. The roots of the plant are used in the herbal formulation called Pilavaikkalimbu, which is used topically in the treatment of tumors. The root of this plant is one of the components of Parangichakkai Choornam, polyherbal Siddha formulation which is indicated in the treatment of various diseases of pitha and kabha origin **[10]**. There are studies that have corroborated pharmacological actions of leaf extract of this plant. However, there is little information about phytochemical composition and pharmacological actions of root extracts.

Azima tetracantha Lam., (Salvadoraceae) commonly known as "mulluchangu" is a glabrous, rigid, rambling, thorny shrub commonly called "Bee sting bush" found in Africa, India and Madagascar. Several medicinal properties are attributed to this plant in the Indian systems of medicine and included in the check list of traded medicinal plants. The ethno-botanical survey reveals the usage of this plant as a unique folk medicine by the adivasis (tribal) **[11-13]**.

The root, root bark and leaves are administered with food as a remedy for rheumatism **[14-15]**. It is a powerful diuretic given for rheumatism, dropsy, dyspepsia and chronic diarrhoea and as a stimulant tonic after confinement **[16]**. Efficient acute phase anti-inflammatory drug is traditionally used by Indian medical practitioners **[17]**. The leaves are found to contain azimine, azcarpine, carpine and isorhamnitine-3-O-rutinoside etc., **[18-20]**, which are used to treat cough, phthisis, asthma, small pox and diarrhoea. The decoction of the stem bark is considered as astringent, expectorant and antipatriotic **[21]**.

The aqueous extract of the roots of this plant has traditionally been used for treatment of various liver disorders and jaundice **[22]**, is considered diuretic and is also used to treat dropsy, dyspepsia, chronic diarrhoea and as a stimulant tonic. In

western India, the leaf juice is applied as eardrops against earache and crushed leaves are placed on painful teeth. The fruit is edible and used by livestock. It is planted as live fence in Bangalore (India). In Malaysia pickled leaves are used as an appetizer and against colds. The plant is promoted as ornamental in the United States and in East Africa. The pounded roots 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 are used similarly. The Bajun people of the Kenyan coast use the root decoction to treat stomach disorders. In Madagascar an infusion of the leaves is used to treat venereal diseases. In order to find new antimicrobial chemicals, researchers were motivated to investigate in other sources, particularly herbal resources.

MATERIALS AND METHODS

Collection and Identification of plant material: For the study, the *Azima tetracantha* belongs to Salvadoraceae family was collected Thanjavur, Tamil Nadu, South India. The whole plant was identified taxonomically and authenticated according to various literatures, Flora of Madras Presidency and Wealth of India including other pertinent taxonomic literature.

Preparation of plant materials and extract: The whole plants were carefully cleaned, shade dried and powdered. The powdered material was stored in a closed air-tight plastic container at low temperature. The powdered plant material (50 g) was extracted with 300 mL of each solvent ethanol by maceration (3×24 h) at room temperature. The collected solvents were concentrated by rotary vacuum evaporator at 45°C and then dried using a freeze dryer. All extracts and acyclovir (extracted from commercial tablet) were dissolved in dimethyl sulphoxide (DMSO). The final concentration of DMSO was 0.1% v/v in cell culture environment.

Phytochemical Analysis: The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The resultant extracts were evaporated to dryness under vacuum. These extracts were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc. Chemical tests were identifying the phytochemicals as described **[23-24]**. Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Test microorganisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* were used as test organisms in the current study. For the current experiment, the obtained cultures were repeatedly subcultured.

Antimicrobial activity by agar diffusion method: The antibacterial efficacy of several *Azima tetracantha solvent* extracts was assessed using the agar diffusion method. For spreading agar media, a subcultured microbial suspension (100 µl) was prepared. Various concentrated varied extracts were used to measure antimicrobial activity **[25]**. The plates were filled with the sample and then left to allow for an hour to enable the extract to disperse. The plates were maintained in an incubator for 24 hours at 37°C, and the inhibitory zone was measured in millimeters (mm). Results are compared with those of conventional antibacterial drugs.

RESULTS AND DISCUSSION

Phytochemical studies of *Azima tetracantha* showed that it contains a number of phenolic and polyphenolic compounds, saponin, essential oil, polysaccharides and pectin. The main bioactive constituents found are bioflavonoid, amentoflavone with minute amount of cupressoflavone **[26]**. All of the extracts from the *Azima tetracantha* contained saponin, phenols, tannins, glycosides, terpenoids, flavonoids, alkaloids and coumarins, according to a preliminary phytochemical examination. With the exception of the chloroform extract's lack of saponins, glycosides, and coumarins and the extract from ethyl acetate's absence of saponin. The results of the phytochemical analysis are displayed in **Table 1**. A higher degree of biological activity derives from the presence of a high concentration of phytochemicals in the plant.

These plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized **[27]**. In the present study, it was clearly understood that the ethanolic extracted maximum amount of the different type of metabolites present in the *Azima tetracantha*. Boominathan and Ramamurthy **[28]** reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

For instance, the presence of flavonoids suggest that the plant have been reported to exert multiple biological effects including, anti-allergic, anti-inflammatory, anti- microbial antioxidant, anti- cancer activity **[29]**. It also suggests that the plant might have diuretic properties **[30]**. The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and anti-bacterial activities and can relief in wound healing

and burns **[31]**. Saponins and glycoside are also very important classes of secondary metabolites as some are cardio-active and used in treatment of heart conditions **[32]**. Some researchers have also investigated that some saponins have anti-cancer and immune modulatory properties **[33]**. Volatile oils are used in the industries for various purposes, both as a pharmaceutical/ cosmetic raw material for production of emollients and active ingredient for the respiratory tract infections.

The antibacterial properties of various solvent-based extracts of *Azima tetracantha* are displayed in **Table 2**. The ethanol extract of *A. tetracantha* had the highest antimicrobial activity with MIC (480 µg/ml) against the *Escherichia coli*, (430 µg/ml) against the *Pseudomonas aeruginosa*, (425 µg/ml) against the *Bacillus subtilis* and (495 µg/ml) against the *Staphylococcus aureus*. The various extracts of *A. tetracantha* tested against *Escherichia coli* and showed considerable MIC results in water extract (385 µg/ml), chloroform extract (317 µg/ml), ethyl acetate extract (302 µg/ml), acetone extract (220 µg/ml), hexane extract (247 µg/ml). The results were compared with standard Cephalosporins as reference compounds with MIC (50µg/ml). The different extracts of *A. tetracantha* were checked against the *Pseudomonas aeruginosa* and exhibited significant MIC values in water extract (376 µg/ml), chloroform extract (277 µg/ml), ethyl acetate extract (292 µg/ml), acetone extract (217 µg/ml), hexane extract (253 µg/ml). The obtained results were compared with Cephalosporins with MIC (44 µg/ml).

The individual extract of *A. tetracantha* was checked against *Bacillus subtilis* and found impressive MIC values in water extract (375 µg/ml), chloroform extract (284 µg/ml), ethyl acetate extract (264 µg/ml), acetone extract (199 µg/ml), hexane extract (232 µg/ml). The different solvent extract of *A. tetracantha* was evaluated against *Staphylococcus aureus* and found impressive MIC values in water extract (326 µg/ml), chloroform extract (248 µg/ml), ethyl acetate extract (245 µg/ml), acetone extract (206 µg/ml), hexane extract (214 µg/ml). The gentamicin (30 µg/ml) was used as a standard compound.

A considerable inhibitory zone may also be caused by the variety of phytochemicals present in the extract. The presence of different flavonoids, alkaloids, terpenoids, phenols, saponins, and coumarins has bactericidal properties **[34]**. According to various scientific studies, high concentrations of phytochemicals and bioactive compounds are thought to have a stronger potential for treating a variety of pathogenic bacteria. Numerous plants and their various portions of them have historically been used to treat a variety of chronic illnesses, such as gastrointestinal

problems, urinary tract infections, skin conditions, and various respiratory issues, etc. **[35]**. Several chronic illnesses caused by various bacteria may be prevented and managed with the use of plant-based remedies. Many societies still employ ethnomedicines to treat illnesses and overcome obstacles without creating negative side effects. The inclusion of several phytoconstituents, including alkaloids, flavonoids, coumarins, saponins, polyphenols, tannins, and terpenoids, is what gives herbal preparations their therapeutic effects **[36]**. The presence of secondary metabolites prevents the growth of harmful microorganisms causing serious diseases. The microorganisms are resistant to many antibiotics that is very harmful to humans. The researchers are finding an alternative to commercial antibiotics to prevent harmful infections against a variety of microorganisms using plant-based medicines **[37]**. The higher concentration of crude extracts sometimes may cause cytotoxicity in humans hence the dose-dependent values are determined using in vitro cell cytotoxicity assay **[38]**. As compared to commercial antibiotics, plant-based medicines have very small side effects if they are consumed in excess quantity **[39]**. In the world, 80 % of different pharmaceuticals are prepared from plant-based medicines and which are effective to cure any chronic disease.

Table 1: Qualitative Phytochemical screening on extracts of *Azima tetracantha*

S. No	Name of Test	Test applied / Reagent used	Ethanol	Water	Chloroform	Hexane	Acetone	Ethyl acetate
1	Alkaloids	A] Mayer's B] Wagner's C] Hagner's D]Dragndorff's test	+++ +++ +++ ++	++ ++ ++ ++	++ ++ ++ ++	++ ++ +++ ++	+++ +++ +++ ++	++ ++ ++ +
2	Flavonoids	HCl and magnesium turnings	+++	++	+	++	+	++
3	Carbohydrate	Molisch's test	+	+	+	+	+	+
4	Tannins & Phenols	A] 10% Lead acetate B] $Fecl_3$	+++ +++	+ +	++ ++	++ ++	++ ++	++ ++
5	Test for Steroids	A]Salkowski's Test B] Libermann-Burchard's Test	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++
6	Gums & Mucilages	Alcoholic Precipitation	-	-	-	-	-	-
7	Fixed oil & Fats	Spot test	+	-	+	+	-	-
8	Saponins	Foam test	+	+	+	+	+	+

9	**Phytosterols**	**LB test**	+	+	+	+	+	+
10	**Volatile oils**	**Hydro distillation method**	+	+	+	+	+	+
11	**Protein & free amino acids.**	**A] Biuret test** **B] Ninhydrin test** **C]Xanthoprotein test**	++ +++ +++	++ ++ ++	++ ++ ++	++ ++ ++	++ ++ ++	++ ++ ++

Table 2: Antimicrobial activity of the plant extracts *Azima tetracantha*

Microorganism	**Minimum inhibitory concentration (MIC)**							
	Plant extract of *Azima tetracantha* (µg/ml)							
	Ethanol	**Water**	**Chloroform**	**Hexane**	**Acetone**	**Ethyl acetate**	**Gentamicin (µg/ml)**	**Cephalosporins (µg/ml)**
P.aeruginosa	**430**	**376**	**277**	**253**	**217**	**292**	**ND**	**44**
B. subtilis	**425**	**375**	**284**	**232**	**199**	**264**	**ND**	**47**
E. coli	**480**	**385**	**317**	**247**	**220**	**302**	**ND**	**45**
S. aureus	**395**	**326**	**248**	**214**	**206**	**245**	**30**	**ND**

CONCLUSION

The present study demonstrated that *Azima tetracantha* extracts a rich source of secondary metabolites. The antimicrobial property of the *Azima tetracantha* extracts showed a correlation with the anticancer property. *Azima tetracantha* used for its wide therapeutic potential of antimicrobial agents. According to the studies, ethanol extract has the most potential, which may be because it includes the majority of the phytochemical compounds and bioactive compounds that have antibacterial activity. The complete plant extract of *Azima tetracantha* has to be further studied in order to identify and purify chemicals that might be used as natural medicinal alternatives to synthetic commercial ones. The future aspects of the plant can be anti microbial as it contains many of the phytochemicals and work has not been performed yet.

REFERENCES

[1] Lv F., Liang H., Yuan Q., Li C. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. Food Res. Inter., 44(9): (2011) 3057–3064.

[2] Moosavy M.-H., Shavisi N. Determination of antimicrobial effects of nisin and Mentha spicata essential oil against *Escherichia coli* O157:H7 under various conditions (pH, temperature and NaCl concentration). J. Faculty of Pharmacy, 19(2): (2013) 61–67.

[3] Sepahvand R., Delfan B., Ghanbarzadeh S., Rashidipour M., Veiskarami G. H., Ghasemian-Yadegari J. Chemical composition, antioxidant activity and antibacterial effect of essential oil of the aerial parts of Salvia sclareoides. Asian Pacific Journal of

Tropical Medicine, 7(1): (2014) S491–S496.

[4] Jay J. M., Loessner M. J., Golden D. A Modern Food Microbiology. 7th. New York, NY, USA: Springer Science+Business Media; (2005).

[5] Oroojalian F., Kasra-Kermanshahi R., Azizi M., Bassami M. R. Phytochemical composition of the essential oils from three Apiaceae species and their antibacterial effects on food-borne pathogens. Food Chemistry, 120(3): (2010) 765–770.

[6] Friedman M., Henika P. R., Mandrell R.E. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes* and *Salmonella enterica*. J. Food Protection. 65(10): (2002) 1545–1560.

[7] Warriner K and Namvar A. What is the hysteria with Listeria? Trends in Food Science & Technology. 20(6-7): (2009) 245–254.

[8] Burt S. Essential oils: their antibacterial properties and potential applications in foods: a review. Inter. J. Food Micro.,94(3): (2004) 223–253.

[9] Kekuda, P.T. and Raghavendra, H.L. Phytochemistry, traditional uses and pharmacological activities of *Azima atetracantha* Lam. (Salvadoraceae)-An updated review. International Journal of Green Pharmacy. 2017; 10: 217-229.

[10] Kumarasamy, S., Kumarasamy, M. A conspectus on siddha polyherbal formulation: Parangichakkai choornam. International Journal of Research in Ayurveda and Pharmacy. 2014; 5(2): 209-218.

[11] Hebbar, S.S., V.H. Harsha, V. Shripathi and V.R. Hegde. Ethnomedicine of Dharwad district in Karnataka, India—plants used in oral health care. J. Ethnopharmacol., 2004; 94: 261– 266.

[12] Mohamed, A.F., M. Wurster, G. Schroder and U. Lindequist. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J. Ethnopharmacol., 2007; 111: 657–666.

[13] Ignacimuthu, S.M., A.K. Ayyanar and Sankarasivaraman. Ethnobotanical study of medicinal plants used by Paliyar tribals in Theni district of Tamil Nadu, India. Fitoterapia., 2008; 79: 562–568.

[14] Chopra, R.N., S.L. Nayar and C. Chopra, Glossary of lndian Medicinal Plants. CSIR, New Delhi, 1956; pp32, 218.

[15] Kirtikar, K.R., B.D. Basu and I.C.S. An. Ind. Med. Plants. 1 & 2, 2nd Edn. Bishen Singh Mahendra Pal Singh, Dehra Dun. 1984; pp: 582, 1541.

[16] Nadkarni, K.M. Indian Meteria Medica, 3rd Edn. Popular Prakhasan, Bombay, 1976; 1: 165.

[17] Ismail, T.S., S. Gopalakrishnan and V.H. Begum. Anti-inflammatory activity of Salacia oblonga Wall. and

Azima tetracantha Lam. J. Ethnopharmacol., 1997; 56: 145-152.

[18] Rall, G.J.H., T.M. Smalberger, H.L. De Waal and R.R. Arndt. Dimeric piperidine alkaloids from Azima tetracantha. Tetrahedron Letts., 1967; 3465-3469.

[19] Williams, U.V. and S. Nagarajan. Isorhamnetin-3-Orutinoside from leaves of Azima tetracantha Lam. Ind. J. Chem., 1988; 27: 387.

[20] Bennett, R.N., E.A. Rosa, L. Perkins and P.A. Kroon, Profiling glucosinolates, flavonoids, alkaloids, and other secondary metabolites in tissues of Azima tetracantha L (Salvadoraceae). J. Agri. Food Chem., 2004; 52(19): 5856-5862.

[21] Duraipandiyan, V., M. Gnanasekar and S. Ignacimuthu, Antifungal activity of triterpenoid isolated from Azima tetracantha leaves. Folia. Histochem. Cytobiol., 2010; 48(2): 311-313.

[22] IMPCOPS Publisher. Formulary of Siddha medicines, Chennai. 1989, pp. 332.

[23] Trease, GE and Evans, WC. Text book of Pharmacognosy.12th ed. Balliere, Tindall, London, Pg No: 57-59 (1983).

[24] Harborne J B. Phytochemical Methods, A Guide to modern Techniques of Plant Analysis. Chapman and Hall, London, PP. 33-41 (1973).

[25] Magaldi S, Mata-Essayag S, Hartung DE, Capriles C, Perez C, Colella MT, Olaizola C, Ontiveros Y. Well diffusion for antimicrobial susceptibility testing. Inter. J. Infectious Diseases, 8(1): (2004) 39-45.

[26] Abinash, C., Bharati and Alakh, N., Sahu. Ethnobotany, phytochemistry and pharmacology of *Biophytum sensitivum* DC. Pharmacogn Rev., 6(11): [2012] 68–73.

[27] Sofowora EA. Medicinal Plants and Traditional Medicine in African, John Wiley and Sons Ltd, Nigeria, Pg No: 1-3 (1993).

[28] Boominathan, M and Ramamurthy, V. Antimicrobial activity of *Heliotropium indicum* and *Coldenia procumbens*. J. Ecobiol., 24 (1): (2009) 11 – 15.

[29] Kunle O.F.and Egharevba H.O. Preliminary studies on Vernonia ambigua: phytochemistry and antimicrobial screening of whole plant. Ethnobotanical Leaflets, 13: (2009) 1216-1221.

[30] Jayvir, A., Minoo, P., Gauri, B and Ripal, K. Nature Heals. A glossary of selected indigenous medicinal plant of India. 2nd Ed., SRIST Innovations, India, 22 (2002).

[31] Haslem, E.Plant polyphenols: Vegetable tannins revisited – chemistry and pharmacology of natural products. Cambridge University Press, Cambridge, 169 (1989).

[32] Oloyode, O.I. Chemical profile of unripe pulp of Carica papaya. Pakistan Journal of Nutrition, 4(6): (2005) 379-381.

[33] Evans, W.C. Trease and Evans Pharmacognosy, 15th Ed., W.B. Sanders, London, 2002; 183-184 and 191-393.

[34] Kalidindi N, Thimmaiah NV, Jagadeesh NV, Nandeep R, Swetha S, Kalidindi B. Antimicrobial and antioxidant activities oforganic and aqueous extracts of *Biophytum sensitivum* Linn. Leaves. Journal of Food and Drug Analysis, 23: (2015) 795-802.

[35] Alzoreky NS, Nakahara K. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. Int. J. Food Microbiology. 80, (2003) 223-230.

[36] Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities from leaf extract of *Cassia fistula* an ethnomedicinal plant. J. Adv. Pharm. Technol. Res. 2, (2011) 104-109.

[37] Mathew George, Lincy Joesph and Umesh Kumar. *Biophytum sensitivum* Chemical Constituents and Medicinal Properties: A Review. Int. J. Curr. Res. Aca. Rev. 4(7): (2016) 57-67.

[38] Khan UA, Rahman H, Niaz Z, Qasim M, Khan J, Tayyaba. Antibacterial activity of some medicinal plants against selected human pathogenic bacteria. Eur. J. Microbiol. Immunol. 3, (2013) 272-274.

[39] Talib WH, Mahasneh AM. Antimicrobial, cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine. Molecule 15, (2010) 1811-1824.