

Screening of Phytochemical and Antimicrobial Activities of *Azima tetracantha* Lam. Leaf Extracts

Vinoth B., M. Gomathinayagam, *Rengarajan Manivasagaperumal

Department of Botany, Annamalai University, Chidambaram, Tamil Nadu, India.

ABSTRACT

The medicinal plant *Azima tetracantha* was analysed for screening of phytochemical and antimicrobial activity. The *In-vitro* antimicrobial activity was studied against human pathogens gram positive strains *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*, and gram negative strains *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and three fungal strains: *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus* using disc diffusion method. Phytochemical analysis of *Azima tetracantha* leaf extracts result reveals that the hexane extracts showed the presence of alkaloids, phenol, protein and flavonoids, chloroform extracts showed the presence of alkaloids, glycosides, phenol, protein, flavonoids and tannins, ethyl acetate and methanol extracts showed the presence of alkaloids, glycosides, saponins, steroids, phenol, flavonoids, tannins and terpenoids. The *In-vitro* antibacterial activity of *Azima tetracantha* leaf showed the maximum zone of inhibition in methanol and ethyl acetate extract against gram positive bacteria *S. aureus* at 1000µg concentration. *In-vitro* antifungal activity of *Azima tetracantha* leaf showed the maximum zone of inhibition in methanol and ethyl acetate extracts against fungi *A. fumigatus* at 1000µg concentration. The *In-vitro* antibacterial activity of *Azima tetracantha* leaf showed the maximum zone of inhibition in chloroform and hexane extracts against gram positive bacteria *B. cereus* at 1000µg concentration. *In-vitro* antifungal activity of *Azima tetracantha* leaf showed the maximum zone of inhibition was found in chloroform and hexane extracts against fungi *A. fumigatus* at 1000µg concentration. This result justifies the presence of secondary metabolites and their activity.

Keywords: *Azima tetracantha*, antibacterial and antifungal activity, phytochemical analysis

Received 19 August 2014

Received in revised form 10 Sept 2014

Accepted 12 Sept 2014

*Address for correspondence:

Rengarajan Manivasagaperumal,

Department of Botany, Annamalai University, Chidambaram, Tamil Nadu, India.

E-mail: rmvperumal@gmail.com, vbvinobio@gmail.com

INTRODUCTION

Medicinal plants are still 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 [1].

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, seeds, etc, that may contain active components [2]. About 50% of modern drugs are natural products, which play an important role in pharmaceutical industry [3].

Scientists from divergent fields are investigating plants for their antimicrobial usefulness as an alternative source to existing drugs. Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agents with general as well as specific activity [4]. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world [5].

Azima tetraacantha belongs to Salvadoraceae consisting of three genera (*Azima* L., *Dobera* Juss., and *Salvadora* L.) with 12 species. It comprises trees, shrubs, and scramblers distributed in dry, hot regions of Africa, Madagascar, and Asia. Two genera and three species are represented in the Indian Subcontinent. *Azima* has two species of erect, thorny shrubs distributed in Myanmar and India. Only one species, *Azima tetraacantha* Lam., occurs in India [6].

Azima tetraacantha Lam. locally known as "Mulsangu", is a rambling spinous shrub flowering throughout the year found in peninsular India, West Bengal, Orissa, African countries and extending through Arabia to tropical Asia. The juices of the leaves are used to relieve the cough phthisis and asthma. In western India, juices of the

leaves are applied as eardrops against earache and crushed leaves are placed on painful teeth. In India and Sri Lanka the root, root bark and leaves were administered with food as a remedy for rheumatism [7]. This plant is considered as a powerful diuretic and is also used to treat rheumatism, dropsy, dyspepsia, chronic diarrhea and as a stimulant tonic for woman after confinement [8]. It is also used as food for various herbal medicines in Africa, India and Madagascar [9]. Locally, the traditional healers from Tirunelveli district of Tamilnadu use the root bark (Paste with butter milk) as potent remedy for jaundice. This plant has been reported to possess different biological activities like anti-inflammatory, wound-healing, diuretic and analgesic activities [10].



Morphological features of *Azima tetraacantha* Lam.

There are few reports on phytochemical composition of the plants like presence of dimeric piperidine, alkaloids azimine, azacarpaine and carpaine [11] Triterpenoids [12] isorhamnetin 3-rutinoside [13] and novel fatty acid [14]. Presence of neoscorbinogen and glucosinolates [15] has also been reported. The present study is aimed at Screening of Phytochemical and Antimicrobial activities of *Azima tetraacantha* leaves.

MATERIALS AND METHODS:

Plant collection

The fresh plants were collected from Athamangalam Village, Sirkazhi Taluk Nagapattinam District of TamilNadu, India. The taxonomic identity of the plant was confirmed by the Botanists of Annamalai University, TamilNadu. The plant materials were

washed under tap water, shade dried and it was grounded with the mechanical blender into fine coarse powder, packed in a zip lock cover and labeled.

Extract preparation

One hundred grams of each powdered leaves were extracted with hexane, chloroform, ethyl acetate and methanol by 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 [16-18].

1. 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.

2. 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.

3. 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.

4. Test for Flavonoids:

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

5. 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.

6. 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.

7. Test for Glycosides:

To 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.

8. 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 reagent. Appearance of pink or violet colour indicated the presence of carbohydrates. The filtrate 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.

9. 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.

10. 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**

Seven bacterial strains and three fungal strains were used in the present study. The clinical isolates were obtained from the Department of Clinical Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar.

The seven bacterial species used in this study were, the gram positive strains: *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus* and gram negative strains: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and three fungal strains: *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus*. They were identified according to standard phenotype tests.

Antibacterial and Antifungal assays**Disc diffusion method**

The agar diffusion method [19] was followed for antibacterial susceptibility test. Petri plates were prepared by pouring 20 ml of Muller Hinton Agar and Sabouraud Dextrose Broth allowed solidifying for the use in susceptibility test against bacteria and fungi. Plates were dried and uniformly spread. The excess inoculum was drained and the plates were allowed to dry for 5 min. After drying, the discs with extract were placed on the surface of the plate with sterile forceps and gently pressed to ensure the contact with the incubated agar surface. Ciprofloxacin (5µg/disc) for bacteria and Ketoconazole (10µg/disc) was used as positive control. 5 percent Dimethyl sulphoxide was used as blind

control in these assays. The inoculated plates were incubated at 37°C for 24h (bacteria) and 28°C for 24 – 72 h (fungi). The zone of inhibition was observed and measured in millimeters. Each assay in this experiment was repeated for three times.

RESULTS AND DISCUSSION

In the present study, the phytochemical screening, antimicrobial activities were studied with hexane, chloroform, ethyl acetate and methanol 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 Qualitative Phytochemical analysis of Leaf extracts of *Azima tetracantha* Lam.

Sr. No.	Name of the Test	Leaf			
		H	C	EA	M
1	Alkaloids	+	+	+	+
2	Carbohydrates	-	-	-	-
3	Glycosides	-	+	+	+
4	Saponins	-	-	+	+
5	Protein	+	+	-	-
6	Steroids	-	-	+	+
7	Phenol	+	+	+	+
8	Flavonoids	+	+	+	+
9	Tannins	-	+	+	+
10	Terpenoids	-	-	+	+

(+Present; - Absent), H – Hexane, C – Chloroform, EA– Ethyl acetate and M – Methanol

Antimicrobial activity of the leaves of *Azima tetracantha*

The antimicrobial activity of different concentration of hexane, chloroform, ethyl acetate and methanol extract of the leaves of *Azima tetracantha* against bacterial and fungal pathogens are presented in Figure 1, 2, 3 and 4. The mean zone of inhibition for methanol ranged from methanol extract showed the maximum zone of inhibition ranging from 12.8 to 22.7 mm against bacteria such as 22.7 mm against *S. aureus*, 19.1 mm against *B. cereus*, 18.2 mm against *B. subtilis*, 16.9 mm against *P. aeruginosa*, 16.2 mm *S. typhi* mild inhibitory effect on 13.9 mm against *K. pneumonia* and 12.8 mm against *E. coli* at 1000µg concentration and methanol

extract showed the maximum zone of inhibition ranging from 9.5 to 11.8mm against fungi such as 11.8mm against *A. fumigatus*, 10.2mm against *A. flavus* but mild inhibitory effect on 9.5mm against *A. niger* 1000µg concentration.

The mean zone of inhibition for ethyl acetate extract ranging from 11.3 to 18.3 mm against bacteria such as 18.3 mm against *S. aureus*, 16.4 mm against *B. cereus*, 15.3 mm against *S. typhi*, 14.3 mm against *P. aeruginosa*, 13.8 mm against *B. subtilis* mild inhibitory effect on 12.7 mm against *K. pneumonia* and 11.3 mm against *E. coli* at 1000µg concentration and ethyl acetate extract showed the maximum zone of inhibition ranging from 9.1 to 10.3 mm against fungi such as 10.3 mm against *A.*

fumigatus, mild inhibitory effect on 9.7 mm against *A. flavus* and 9.1 mm against *A. niger* 1000 μ g concentration.

The mean zone of inhibition for chloroform extract ranging from 9.2 to 14.6 mm against bacteria such as 14.6 mm against *B. cereus*, 13.1 mm against *P. aeruginosa*, 13.0 mm against *S. aureus*, 14.3 mm against 12.6 mm against *S. typhi*, 12.3 mm against *K.*

pneumonia, 11.3 mm against *B. subtilis* mild inhibitory effect on 9.2 mm against *E. coli* at 1000 μ g concentration and chloroform extract showed the maximum zone of inhibition ranging from 8.0 to 10.1 mm against fungi such as 10.1 mm against *A. fumigatus*, mild inhibitory effect on 8.6 mm against *A. flavus* and 8.0 mm against *A. niger* 1000 μ g concentration.

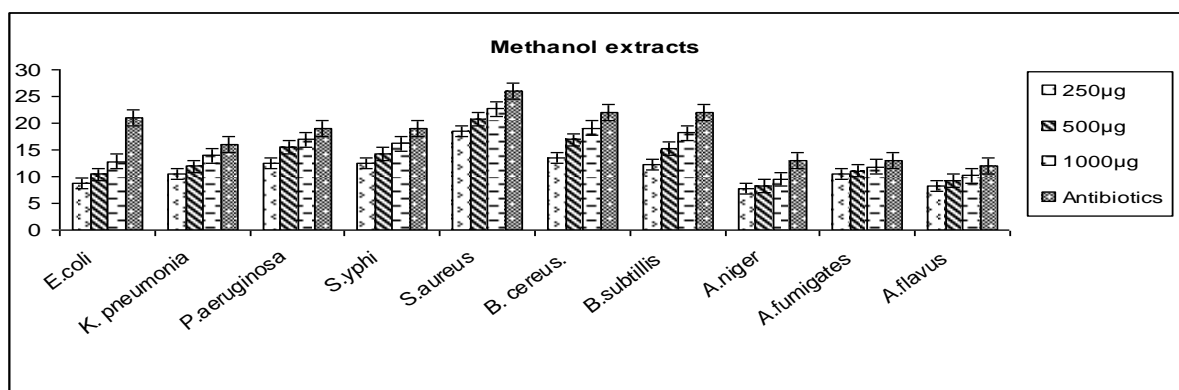


Figure 1: Antimicrobial activity of the Methanol extracts of *Azima tetraacantha* leaves Lam.

Figure given are mean of three assays; \pm standard deviation; Ciprofloxacin (5 μ g/disc) – antibacterial drug; Ketoconazole (10 μ g/disc) – antifungal.

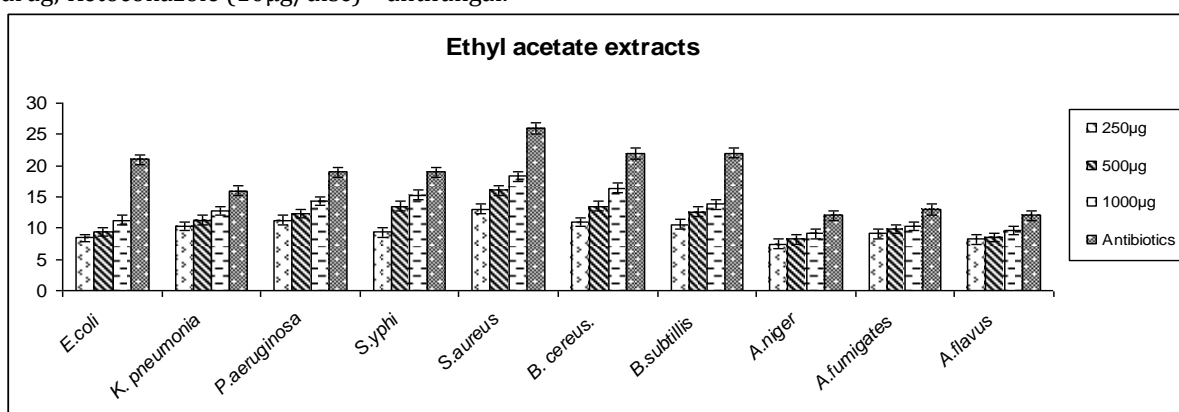


Figure 2: Antimicrobial activity of the Ethyl acetate extracts of *Azima tetraacantha* leaves Lam.

Figure given are mean of three assays; \pm standard deviation; Ciprofloxacin (5 μ g/disc) – antibacterial drug; Ketoconazole (10 μ g/disc) – antifungal.

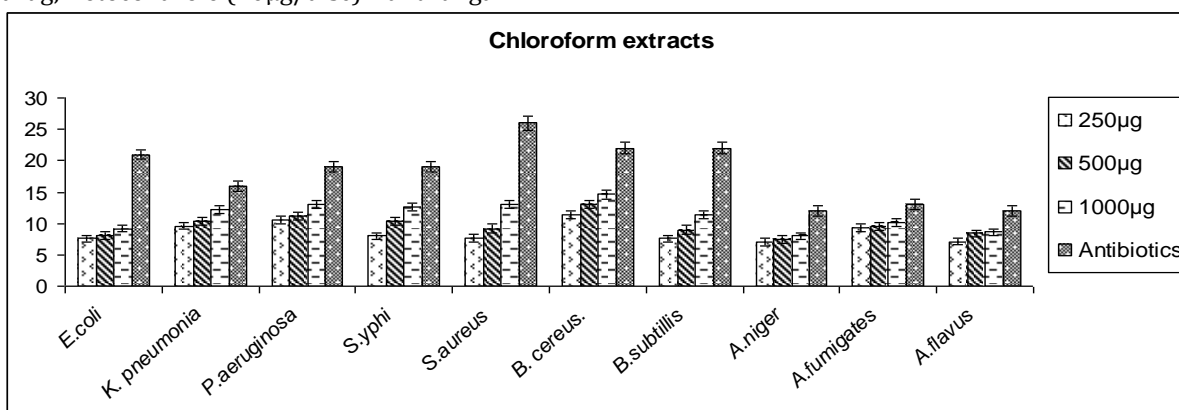


Figure 3: Antimicrobial activity of the Chloroform extracts of *Azima tetraacantha* leaves Lam.

Figure given are mean of three assays; \pm standard deviation; Ciprofloxacin (5 μ g/disc) – antibacterial drug; Ketoconazole (10 μ g/disc) – antifungal

The mean zone of inhibition for hexane extract ranging from 7.6 to 10.1 mm against bacteria such as 10.1 mm against *B. cereus*, 9.6 mm against *S. aureus*, 9.0 mm against *B. subtilis* mild inhibitory effect on 8.8 mm against *K. pneumonia* and *S. typhi*, 7.6 mm against *P. aeruginosa* and

no inhibitory effect on *E. coli* at 1000µg concentration and hexane extract showed the maximum zone of inhibition ranging from 7.2 to 10.6 mm against fungi such as 10.6 mm against *A. fumigatus* mild inhibition effect on 8.1 mm against *A. flavus* and 7.2 mm against *A. niger* 1000µg concentration.

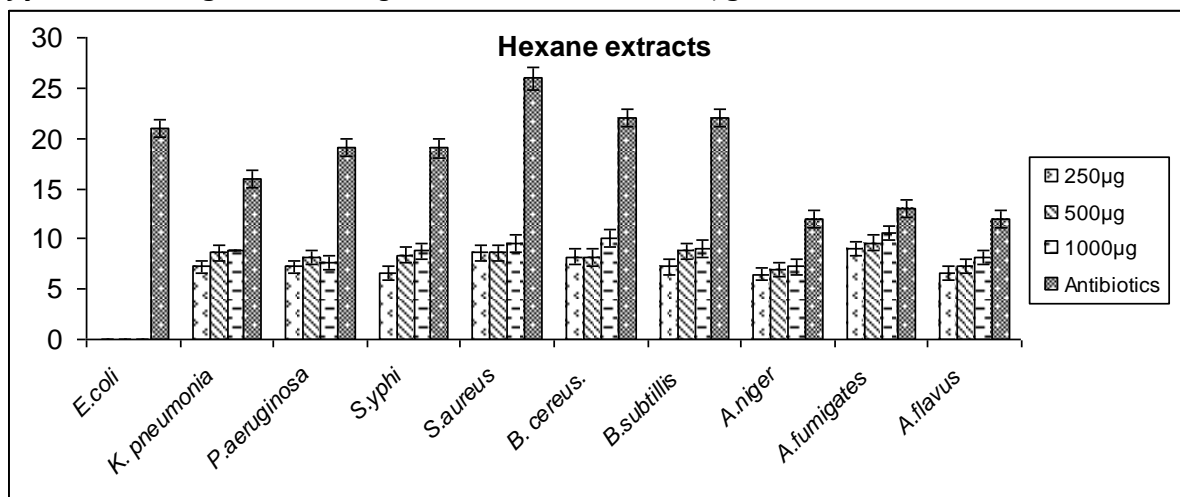


Figure 4: Antimicrobial activity of the Hexane extracts of *Azima tetraacantha* leaves Lam.

Figure given are mean of three assays; \pm standard deviation; Ciprofloxacin (5µg/disc) – antibacterial drug; Ketoconazole (10µg/disc) – antifungal

These results are in correlation with the results of several medicinal plants such as *Erythrina senegalensis*, *Vitex negundo* and *Terminalia glaucescens* [20-22].

The evaluation of antimicrobial potential by disc diffusion method indicated that all the bacterial tested organisms showed growth inhibition towards the plant extract, with differing sensitivity. Among the bacterial pathogens, *S. aureus* is more sensitive when compared to other bacteria. Gram-positive bacteria were exhibited more sensitiveness to plant extracts when compared to Gram-negative bacteria [23]. The methanol extract of pomegranate peels were more active when compared to water extract against *E. coli*, *S. aureus* and *B. subtilis* [24].

The methanol extract showed higher antifungal activity than that of aqueous extract. This may be due to the solvent to extract the different constituents having antifungal activity. The crude aqueous seed extract of *Syzygium jambolanum* showed antifungal activity against *A. flavus*, *A. fumigatus* and *A. niger* [25]. In addition, these results confirmed the evidences in previous studies reported that methanol as a better solvent for more consistent

extraction of antimicrobial substances from medicinal plant compared to other solvents, such as water, ethanol and hexane [26-28].

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 extracts prove the usage of the plant as a traditional medicine. Our future work is to isolate and identify the active compounds present in this extract against various diseases.

REFERENCES

1. Sukanya SL, Sudisha J, Hariprasad P, Niranjana SR, Prakash HS, Fthima SK. "Antimicrobial activity of leaf extracts of Indian medicinal plant against clinical and phytopathogenic bacteria", African Journal of Biotechnology, 2009; 8(23): 6677-6682.
2. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. "Phytochemical screening and extraction", A review. IPS., 2011; 1(1): 98-106.
3. Manjuri SA, Kanti CC, sarojini N, Sriti K. "Correlation between phytochemical screening and *In-vitro* antibacterial study of *Rosa indica* Linn. Leaves", IJRAP, 2011; (5): 1595-1597.
4. Nair R, Chanda S. "*In-vitro* antimicrobial activity of *Psidium guajava* L. Leaf extracts

- against clinically important pathogenic microbial strains", Braz J Micro., 2007; 38: 452-458.
5. Nair R, Kalariya T, Sumitra C. "Antibacterial activity of some selected Indian medicinal flora", Turk J Bio., 2005; 29: 41-47.
 6. Purkayastha SK. "Indian woods—their identification, properties and uses", vol. 5. Forest Research Institute, Dehradun, Uttarakhand, India. 1985.
 7. Hebbar SS, Harsha VH, Shripathi V, Hegde GR. "Ethnomedicine of Dharwad District in Karnataka, India: Plant used in oral health care", J. Ethnopharmacol., 2004; 94(2-3): 261-266.
 8. Nadkarni KM. "Indian Materia Medica", 3rd ed. Vol. 1, Popular Prakhasan, Bombay. 1976; 45-52.
 9. Bennett RN, Mellon FA, Rosa EA, Perkins L, Kroon PA. "Profiling glucosinolates, flavonoids, alkaloids, and other secondary metabolites in tissues of *Azima tetraacantha* L. (Salvadoraceae)", Journal of Agriculture and Food Chemistry., 2004; 52(19): 5856-5862.
 10. Nandgude TD, Bhojwani AP, Kinage Krishna. "Analgesic activity of various extract of leaves of *Azima tetraacantha* Lam", Intl. J. Green. Pharm., 2007; 1(1): 37-38.
 11. Rall GJH, Smalberger TM, de Waal HL, Arndt RR. "Dimeric piperidine alkaloids from *Azima tetraacantha* Tetrahedron Letters", 1967; 36: 3465-66.
 12. Venkatrao E, Prasad Rao PRS. "Occurrence of triterpenoids in *Azima tetraacantha*", Current Science., 1978; 47(22): 857.
 13. Vasikaran Williams U, Nagarajan S. "Isorhamnetin 3- rutinoside from leaves of *Azima tetraacantha* Lam", Indian Journal of Chemistry., 1987; 27B: 387.
 14. Daulatabad CD, Desai VA, Hosmani, K M, Jamkhandi. Journal of Am. Opl Chem. Soc., 1991; 68(12): 978-979.
 15. Bennett RN, Mellon FA, Rosa EA, Perkins L, Kroon PA. "Profiling Glucosinolates, Flavonoids, Alkaloids and other Secondary Metabotities in tissues of *Azima tetraacantha* L. (Salvadraceae)", Journal of Agricult. Food Chemistry. 2004; 52(19): 5856-5862.
 16. Kokate CK, Khandelwal KR, Pawar AP, Gohale SB. "Practical Pharmacognosy", 3rd ed. Nirali Prakashan, Punc. 1994; 137-139.
 17. Jigna P, Sumitra VC, "In-vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plant", Turk. J. Biol., 2007; 31: 53- 58.
 18. Makut MD. "Phytochemical screening and antimicrobial activity of the Ethyl acetate and methanol extracts of the leaf and bark of *Khaya senegalensi*", Afr. J. Biotechnol., 2008; 7(9): 1216-1219.
 19. Bauer AW, Kirbg KM, Sherris JC, Truck M. "Antibiotic susceptibility testing by a standardized single disk method", Am. J. Clin. Pathol., 1966; 45: 493 – 496.
 20. Bako SS, Madhu PC. "Phytochemical and antibacterial investigation of crude extracts of the leaves of *Erythrina senegalensi*", Ind. J. Bot. Res., 2007; 3(1): 17-22.
 21. Panda SK, Thatoi HN, Dutta. "Antibacterial activity and phytochemical screening of leaf and bark extracts of *Vitex negundo*". J. Med. Plant. Res., 2009; 3(4): 294-300.
 22. Adebayo EA, Ishola OR. "Phytochemical and antimicrobial screening of crude extracts of *Terminalia glaucescens*", Afr. J. Pharm. Pharmacol., 2009; 3(5): 217-221.
 23. Mirzaeil A, Toori MK, Mirzaei N, Shirazi RG. "Antioxidant, Antimicrobial and Antimutogenic Potential of 4 Iranian Medicinal Plants", L. Sci. J., 2013; 10(7s): 108-1091.
 24. Prashanth D, Asha M, Amit A. "Antimicrobial activity of *Punica granatum*", Fitoterapia., 2001; 72: 171-173.
 25. Chandarasekaran M, Venkatesalu V. "Antibacterial and antifungal activity of *Syzygium jambolanum* Seeds", J. Ethnopharmacol., 2004; 91: 105-108.
 26. Ahmad I, Mehmood Z, Mohammad F. "Screening of some Indian Medicinal plants for their antimicrobial properties", J. Ethanopharmacol., 1998; 62: 183-193.
 27. Eloff JN. "Which extract should be used for the screening and isolation of antimicrobial compounds from plants?", J. Ethnopharmacol., 1998; 60: 1-8.
 28. Lin J, Opoku AR, Keller MG, Hutching AD, Terblanche SE, Jager AK, Staden JV. "Preliminary screening of some traditional Zulu medicinal plant anti-inflammatory and antimicrobial activities", J. Ethanopharmacol., 1999; 68: 267-274.