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Phytochemical analysis and antibacterial properties of leaf extract of *Azima tetracantha* (Lam.)

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ABSTRACT

Azima tetracantha (Lam.) belonging to family Salvadoraceae was collected from Orathanadu, Tahnjavur District. The phytocompounds were separated by using TLC (Thin Layer Chromatography). Phytocompound analysis of Azima tetracantha revealed the presence of alkaloids, flavonoids and sterols. The isolated phytocompounds were used for antibacterial activity performed by using agar well diffusion method. Antibacterial activity of phytocompounds separation from alkaloids, flavonoids and sterols were tested against Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Bacillus subtilis. The sterols compound exhibited maximum zone of inhibition against Bacillus subtilis (25mm), and Pseudomonas aeruginosa (24mm). The alkaloid compound showed minimum zone of inhibition was observed in Bacillus subtilis (12mm) and Pseudomonas aeruginosa (12mm). Maximum zone of inhibition was observed in sterols compound when compared with alkaloids and flavonoids.

INTRODUCTION

Medicinal plant are still major parts of traditional medicinal systems in developing countries many infections 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 from the backbone of traditional medicine have in the last few decades been the subject of very intense pharmacological studies. This has been brought about by the acknowledgement of the value of medicinal plant as potential source of new compounds of therapeutic value and as source of new compounds in drug development. 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 affective and loss toxic medicinal. Tribal medicine has not been studied extensively (Zakaria, 1989).

Infections disease is the number one among all causes of death, accounting approximately one-lady all deaths throughout the world. About 50-75% of hospital deaths are reported due to infections disease. These numbers are still increasing due to development of resistance in microorganisms to the existing first line drug (Akinpelu *et al.*, 2008).

Scientists from divergent fields are investigating plants with a new age for their antimicrobial usefulness and as an alternative source to existing drugs. Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agent with general as well as specific activity (Nair and Chanda, 2007). There is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. Several

screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the worlds (Nair *et al*., 2005).

Azima tetracantha (Lam.) is considered diuretic and is also used to treat dropsy, dyspepsia, chronic diarrhoea and as a stimulant tonic. In western India juice of the leaves is applied as eardrops against earache and Crushed leaves are placed on painful teeth. The fruit is edible. *Azima tetracantha* is browsed 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 an ornamental in the United States.

MATERIALS AND METHODS

Plant Collection

The leaves of *Azima tetracantha* (Lam.) were collected from Thanjavur (Dt) brought into the laboratory for further processes.

Preparation of Plant Extracts

Two grams of sterilized plant leaves were kept in the 10 ml organic solvents such as methanol and ethanol. Then the leaves are grind with the help of mortar and pestle. The grind plant material was filtered through Whatmann No.1 filter paper. The supernatant was collected and stored for further antibacterial screening purpose.

Selection of Microorganisms

Totally five pathogenic microorganisms were selected for the present investigation. They are *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherchia coli*, *Bacillus Subtilis* and *Pseudomonas aeruginosa*. The Organisms were originally obtained from Microbial Germ Plasm Culture Collection Unit (MGPCCU) Sri Gowri Biotech Research Academy, Thanjavur and used for present investigation.

Phytochemical Analysis

Quantitative phytochemical screening was done by TLC (Thin Layer Chromatography).

Detection of Phytochemical Compounds

Alkaloids (Adeloye *et al*., 2007)

One gram of the plant sample were extracted with 40% Calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extract twice with 10 ml portions of chloroform then these extracts were applied in thin layer plates. The alkaloids were separated by using chloroform, ethyl acetate (9:1) solvent mixture. The presence of alkaloids in the developed chromatograms was detected by spraying the freshly prepared Wagner's reagent A positive reaction in the chromatogram (orange brown) was confirmatory evidence that the alkaloid was present in the extract.

Sterols (Wagner and Bladt, 1996)

One gram of plant sample was extracted with 10 ml of methanol in boiling water bath. The condensed filtrate was used for thin layer chromatography. The sterols were separated by using chloroform, glacial acetic, methanol and water (64:34:12:8) solvent mixture. The presence of sterols in the developed chromatogram was detected by spraying the (folin phenol ciocaltu's reagent). After the plates were heated at 100^{o}C for 6 minutes, a positive reaction was formation of blue colour spot.

Flavonoids (Simong Loang Vicas *et al*., 2000)

One gram of plant sample was extracted with 10 ml of ethanol. Then the extract was heated for few minutes and 100 ml of extract was applied on the silica gel plates. The flavonoids were separated by using N. butanol, acetic acid, water (4:1:5) mixture. The presence of flavonoid was detected by the formation of colour in the plate a positive reaction was formation of yellow colour spot.

Rf Value

It is a ratio of distance travelled by the sample and distance travelled by the solvent.

$$\text{Rf Value} = \frac{\text{Distance travelled by the sample}}{\text{Distance travelled by the solvent}}$$

Purification of the Chemical Compounds

TLC using various solvent systems to separate phytochemical compounds in the ethanol and methanol extract into visible fractions with retention factor value. The fractions plus the origin were purified from the developed duplicate plate by collecting the silica placing it in to a polypropylene test tube and dissolving the fractions purified in chloroform: methanol (1:2) centrifuged to remove silica partials and the supernatant was collected used for further antibacterial activity.

Antibacterial Activity of Phytocompounds by Agar Well Diffusion Method

Sensitivity of five different bacterial strains to various phytochemical extracts was measured in terms of Zone of inhibition using agar well diffusion assay. The sterile plates containing nutrient agar medium were spread with fresh bacterial culture by using sterile cotton buds. Well (5 mm size) were made from agar plates by using sterile cork borer, the wells were loaded with 200ml of phytocompound extracts. The plates were incubated at 37^{o}C for 25 hours. After, inhibition the plates were observed for the presence of clean inhibition zone around the well. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis of *Azima tetracantha*

The phytocompounds were separated by TLC. The results recorded that the presences of phytocompound such as alkaloids, flavonoids and sterols. In leaf extract of *Azima tetracantha* the Rf values of phytocompounds alkaloids (0.7), flavonoids (0.6) and sterols (0.6) were given in table 1.

Antibacterial activity of phytocompounds

Antibacterial activity of phytocompound separation from alkaloids, flavonoids and sterol were tested against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *E.coli.*

Sukumar, (1987) reported that the phytocompounds like flavonoids and alkaloids may be responsible for antimicrobial activity. In the present study the alkaloids compounds showed maximum zone of inhibition against *Staphylococcus aureus* (15mm). The minimum zone of inhibition was observered in *Bacillus subtilis* (12mm) and *Pseudomonas aeruginosa* (12mm).

Millogo-kone *et al.,* (2006) reported that the stem (*Jatropha*) activity is due to steroids, terpenoids, flavonoids and alkaloids. The leaves extract were most susceptible to clinical isolates *Staphylococcus aureus* and *E.coli*. The flavonoids compound showed maximum zone of inhibition against *Bacillus subtilis* (16mm). The minimum zone of inhibition was observed in *Staphylococcus aureus* (13mm).

The sterols compounds showed moderate zone of inhibition against *Bacillus subtilis* (25mm). The minimum zone of inhibition was observed (20mm) in *Staphylococcus aureus* (Table 2).

Antibiotic sensitivity test of bacteria

The antibiotic sensitivity test using standard antibiotic Amphicillin, Tetracyclin and Kanamycin were tested against bacteria studied. The Kanamycin standard antibiotic showed maximum zone of inhibition of *E. coli, Klebsiella pneumoniae* and *Bacillus subtilis, Staphylococcus aureus* such as 22mm, 22mm, 22mm and 22mm (Table 3).

Table 1 Qualitative phytochemical analysis of *Azima tetracantha* (Lam.) by TLC

S. No	Compounds Test	Test Applied/Reagent used	Observation	Result
1.	Alkaloids	Wagner's reagent	Orange brown colour spot	+
2.	Flavonoids	Spot test	Yellow colour spot	+
3.	Sterol	Folin–ciocalteus reagent	Blue colour	+

Table 2 Rf value of phytocompounds in *Azima tetracantha* Lam.

S. No	Phytocompounds	Rf-value
1.	Alkaloids	0.7
2.	Flavonoids	0.6
3.	Sterol	0.6

Fig 1 Rf value of phytocompounds in *Azima tetracantha* Lam.

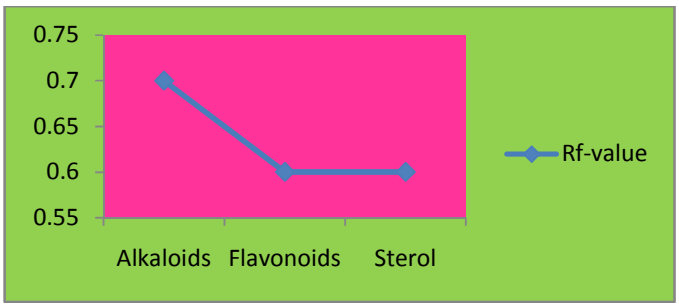


Table 3 Antibacterial activity of phytocompounds in *Azima tetracantha* Lam.

S. No	Bacterial Cultures	Phytocompounds test		
		Alkaloids	Flavonoids	Sterol
1.	*Staphylococcus aureus*	15	13	20
2.	*Klebsiella pneumoniae*	14	15	22
3.	*Escherichia coli*	13	15	20
4.	*Bacillus subtilis*	12	16	25
5.	*Pseudomonas aeruginosa*	12	15	24

Fig 2 Antibacterial activity of phytocompounds in *Azima tetracantha* Lam.

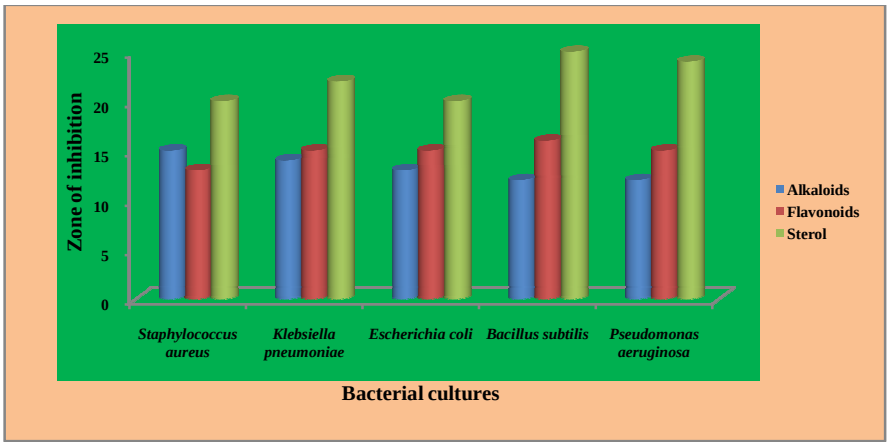
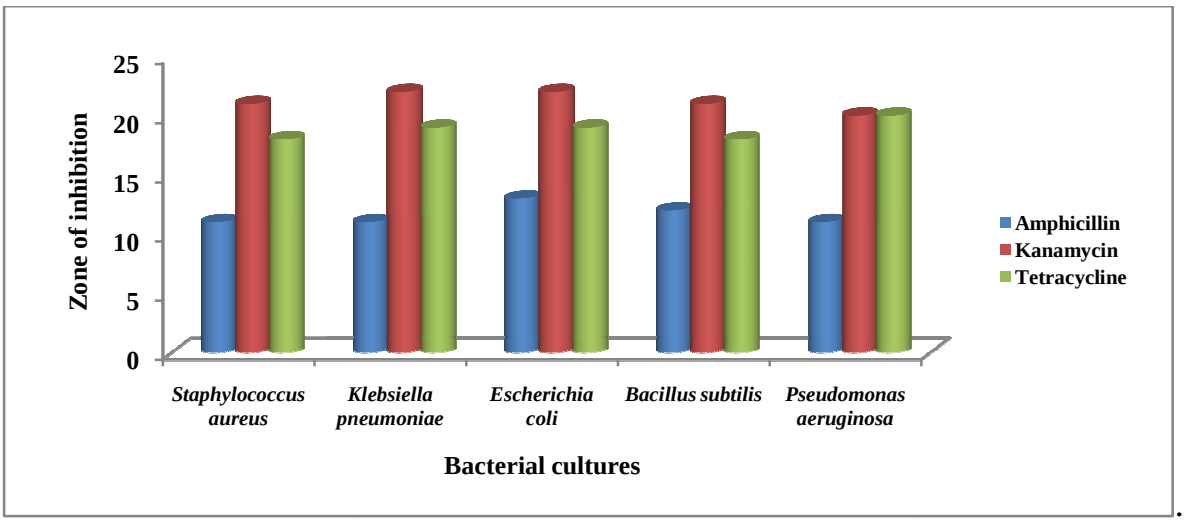


Table 3 Antibiotic sensitivity test (Positive control)

S. No	Bacterial Cultures	Standard Antibiotics Zone of inhibition (Diameter in mm)		
		Amphicillin	Kanamycin	Tetracycline
1.	*Staphylococcus aureus*	11	21	18
2.	*Klebsiella pneumoniae*	11	22	19
3.	*Escherichia coli*	13	22	19
4.	*Bacillus subtilis*	12	21	18
5.	*Pseudomonas aeruginosa*	11	20	20

Fig 3 Antibiotic sensitivity test (Positive control)



CONCLUSION

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. Given the alarming incidence of antibiotic resistances in bacteria of medicinal importance, there is a constant need for new and effective therapeutic agents.

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