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Phytochemical screening and antimicrobial activity of *Azima Tertacantha*

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Abstract
The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China and the Near east, but it is doubtless an art as old as mankind. Nowadays the plants are widely used for medicinal purposes. Many microorganisms induce variety of diseases. So the medicinal herb is the only way to treat diseases because microorganisms are becoming resistant to the commercial drugs. In the present study chloroform, acetone, ethanol and aqueous extracts of *Azima tetracantha* were tested for the presence of phytochemicals and potential antimicrobial efficacy against a few bacterial pathogens such as *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Enterococcus sp and klebsiella sp* by using agar well diffusion method. Ethanol extract of *Azima tetracantha* showed considerably moderate activity against all the microorganisms tested.

Keywords: antimicrobial, commercial drugs and medicinal herbs etc

Introduction
The traditional medicinal method, especially the use of medicinal plants, still plays a vital role to cover the basic health needs in the developing countries. Therefore, it is of great interest to carry out a screening of the plants in order to validate their use in folk medicine [1]. Plants produce a multitude of organic compounds that have antimicrobial activity. The compounds are found in various plant parts such as stems, roots, leaves, bark, flowers or fruits and seeds and include alliin/allicins, isothiocyanates, plant pigments, hydrolytic enzymes, proteins, essential oils and phytoalexins or phenolic compounds [2]. Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases [3]. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [4]. *Azimatetracantha* (Salvadoraceae) is known as ‘Esanku’ inMalayalam, 'Mulsangu' in Tamil and 'Kundali' in Sanskrit, respectively. Its root, root bark and leaves are used with food as a remedy for rheumatism [5]. It is a powerful diuretic given in rheumatism, dropsy, dyspepsia and chronic diarrhoea and as a stimulant tonic after confinement [6]. It is widely used in folklore herbal medicine practices in the villages of southern Kerala. The plant is claimed to have anti-inflammatory, antiperiodic, analgesic and wound healing properties [7].

The objective of the current study is to check for the presence of phytochemicals and antimicrobial activity of chloroform, Acetone Ethanol and aqueous extract of *Azima tetracantha* based on the polarity of the solvents.

Materials and Methods
Selection and screening of plants
The leaves of *Azima tetracantha* was collected in and around Erode District, Tamil Nadu. The plant materials were taxonomically identified. The leaves of the selected plants were removed from the plants and then washed under running tap water to remove dust. The plant samples were then shadow dried for about 15 days and the leaves were crushed into powder and stored in airtight container for further extraction and analysis.

Preparation of extract
20 grams powdered leaves of *Azima tetracantha* were mixed with 100ml of chloroform and kept over shaker for about 3 days followed by filtration of the extract. The filtrates obtained were allowed to evaporate the solvent to get the crude extract. The residue left after the filtration were dried and subsequently extracted with acetone, ethanol and distilled water respectively in the same manner by maceration method. The solvents were selected in the order of increasing polarity [8 & 9].

Phytochemical Screening
The phytochemicals such as alkaloids, sterols, steroids, phenols, flavonoids, tannins, saponins, oils and fats were screened according to the standard methods [10, 11, 12, 13].

Alkaloids
The plants extract were added with 2 ml HCL and the acetified filtrate was mixed with amyl alcohol at room temperature. Pink alcoholic layer indicates the presence of alkaloids**.**

Sterols and steroids
Salkowski's test
Small aliquots of extract were dissolved in 1-2 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of tube. The upper red layer will reveal the presence of steroid presence.

Flavonoids
1 ml of the extract was treated with magnesium turnings and few drops of concentrated sulphuric acid. Pink or red colour will indicate the presence of flavanoid.

Tannins and phenols
1 ml of plant extract was treated with few drops of 5% ferric chloride. Bluish colour indicates tannins and phenolics.
1ml of plant extract was mixed with 1ml of gelatin solution to give white precipitate.

Saponins
1 ml of plant extract was diluted with 20 ml distilled water and was shaken vigoursly to get foam of about 1 cm indicating the presence of saponins.

Oils and fats
A small quantity of extract was processed between two filter papers to get oil stains between them.

Terpenoids
1 ml of plant extract was mixed with 3 ml of acetic acid and few drops of sulphuric acid to give red to blue colour range.

Carbohydrates
Fehling's test
1 ml of plant extract was mixed with 5 ml of Fehling's reagent and kept in water bath to give red, yellow or brown precipitate.

Benedict's test
1ml of plan t extract was mixed with 5 ml of Benedict's reagent and kept in water bath to give red, yellow or precipitate.

Coumarine
1ml of aqueous extract was added with 1ml of 40% sodium hydroxide. Presence of yellow colour indicate coumarine.

Quinone
1ml of aqueous extract was added with 1ml of concentrated sulphuric acid. Red colour precipitation shows the presence of quinine.

Glycosides
2 drops of ninhydine was added with 1ml of aqueous extract. Violet colour indicate presence of glycosides.

Phlobatannins
Add about 3ml of aqueous extract was added to 2ml of 1% HCl and the extract was boiled. Deposition of a red precipitate was taken as an evidence for the presence of Phylobatannins.

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 activity
The extracts of Chloroform, Acetone and Ethanol were weighed and dissolved in an adequate amount of dimethyl sulfoxide respectively. Liquid nutrient agar media and the Petri plates were sterilized by autoclaving at 120º C for 30 minutes. Under aseptic conditions in the laminar airflow chamber, about 20ml of the agar medium was dispensed into each Petri plate to yield a uniform depth of 4mm. After solidification of the media, the bacterial strains such as *E. coli, Bacillus cereus, Staphylococcus sp, Klebsiella sp and Enterococcus sp* obtained from MTCC, Chandigarh were swabbed on the surface of the agar plates. Well was prepared by using cork borer and 20 µl,40 µl,60 µl of extracts were added to distinct well with DMSO as negative control, Tetracycline as positive control and incubated the plates at 37°c for 24 hours to observe the zone of inhibition[14].

Results
The phytochemical screening had shown for the presence of Sterols, Steroids, Flavanoids, Coumarin and Phlobatannin etc., are mentioned in the table1.The presence or absence of phytochemicals may vary with reference to the location, season, age and even with the parts of the plant. The data reported in Table 2 represents the antimicrobial activity of the organic extracts of *Azima tetracantha*. The results indicate that the extracts from the medicinal plant studied had shown inhibition of growth of some of the tested microorganisms. Ethanol extract had shown moderate action against all the microorganisms tested whereas no considerable results were found for aqueous extracts. Chloroform extract also have shown a considerable action over *E.coli, Bacillus cereus and Enterococcus sp.*

Table 1: Phytochemical Screening of *Azima tetracantha*

S.No	Test	Acetone	Ethanol	Distilled water
1	Alkaloids	-	-	-
2	Sterols and Steroids	+	+	+
3	Flavanoids	+	+	+

4	Tannins and Phenolics	-	-	-
5	Carbohydrates	-	-	-
	Fehlings			
	Benedicts	-	+	-
6	Saponins	-	-	+
7	Terpenoids	-	-	-
8	Oils and fats	-	-	-
9	Coumarine	-	-	+
10	Quinine	-	-	-
11	Protein	-	+	-
12	Phlobatannins	-	+	-
13	Glycosides	-	-	-

Note: + indicate Presence and – indicates Absence

Table 2: Antimicrobial activity of *Azima tetracantha*

S.No	Microorganism	Zone of inhibition (Diameter) in mm												Std. Antibiotic (Tetracycline) 10mcg/ disc
		Chloroform			Acetone			Ethanol			Distilled water			
		20 µl	40 µl	60 µl	20 µl	40 µl	60 µl	20 µl	40 µl	60 µl	20 µl	40 µl	60 µl	
	Klebsiella sp	11	-	-	18	16	-	12	12	13	-	-	-	24.4
2	*Staphylococcus sp*	12	-	-	-	-	-	18	17	16	-	-	-	19.4
3	*Escherichia coli*	15	14	-	-	-	-	15	12	15	-	-	-	16.6
4	*Bacillus cereus*	17	15	-	17	14	-	12	18	12	-	-	-	19.2
5	*Enterococcus sp*	12	11	10	23	11	-	11`	12	12	-	-	-	22.2

Discussion

From the results, it is crystal clear that plant extracts have great potential as antimicrobial compounds against microorganisms and can be used in the treatment of infectious diseases caused by resistant microorganisms. Earlier, similar studies of this plant on antifungal activity [15], which made an effort to check the antibacterial efficacy. As this plant had shown the maximum antibacterial activity, it must be used to discover bioactive natural products which may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

Conclusion

India has the plant resource with appreciable medicinal properties. In this aspect, the current study was carried out and had shown the effective property. Still more the research has to be made in view of isolating the valuable compound.

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References

1. NA Awadh Ali, WD. Ju¨ lich, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. Journal of Ethnopharmacology, 2001; 74:173-179
2. Borchardt JR, Wyse DL Sheaffer *et al*., Antioxidant and Antimicrobial Activity of Seed from Plants of the Mississippi River Basin. Journal of Medicinal Plants Research, 2009; 3:707-718.
3. Verpoorte R. Chemodiversity and the Biological Role of Secondary metabolites some thoughts for selecting plant material for drug development. *Proc Phytochem Soc Europe*, Kluwer Publishers, 1998; 43:11-24.
4. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complementary Altern. Med, 2006, 6:35-41.
5. Kirtikar KR, Basu BD. An ICS. Indian Medicinal Plants, Vol.1 and 2, 2nd Edn, 1984, 582-1541.
6. Nadkarni KM. Indian Meteria Medica, Vol. 1, 3rd Edn.Popular Prakhasan, Bombay, 1976, 165-1089.
7. Gayathri G, Bindu R, Nair Babu. *In Vitro* Antimicrobial and Antioxidant Studies on Leaves of *Azima tetracantha Lam.* (salvadoraceae). International Journal of Current Research, 2011; 3:087-090.
8. Amita Pandey, Shalini Tripathi. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry. 2014; 2(5):115-119.
9. Sasidharan S, Chen Y, Saravanan D, Sundaram KM, Yoga Latha L. Extraction, Isolation and characterization of bioactive compounds from extracts, Afr. J Tradit Complement Altern. Med. 2011; 8(1):1-10.
10. Harborne JB. Phytochemical Methods, Chapman and Hall, Ltd., London, 1973, 49-188.
11. Harborne JB. Phytochemical methods, 2nd edition, Chapman and Hall publications, London, New York, 1984, 288.
12. Trease GE, Evans WC. Pharmacognosy, 13th edition, Balliere Tindall, London, 1989, 176-80.
13. Sofowora A. Screening Plants for Bioactive Agents. Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria, 1993, 134-156.
14. Dattatraya BG, Sasidharan S, Deepak M,

Hanumantharaju N, Rajendra CE, Mehaboob AN. Antimicrobial Activities of the Petroleum Ether, Chloroform, Methanol and Water Extracts of *Trigonella foenum-graecum* L. Seeds.www.ijcrrmds.info, 1993.
15. Duraipandiyan V, Gnanasekar M, Ignacimuthu S. Antifungal activity of triterpenoid isolated from *Azima tetracantha* leaves Folia Histochemica ET Cytobiologica. 2010; 48(2):311-313.