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RESEARCH ARTICLE

IN VITRO ANTIMICROBIAL AND ANTIOXIDANT STUDIES ON LEAVES OF *AZIMA TETRACANTHA* LAM. (SALVADORACEAE)

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ABSTRACT

Azima tetracantha Lam., (Salvadoraceae), 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. The present study evaluated the antimicrobial and antioxidant activity of *A. tetracantha* leaves. Methanolic extract showed greater antimicrobial activity than the chloroform extract. The extracts showed both the radical scavenging activity and reducing capability to fight against free radicals. The results from antimicrobial and radical scavenging assays of *A.tetracantha* leaves showed significant medicinal properties.

INTRODUCTION

There is a renewed interest in traditional medicine and an increasing demand for more drugs from plant resources. The interest in plant derived drugs is mainly due to the widespread belief that herbal medicines are safe and more dependable than the costly synthetic drugs, many of which have adverse side effects. Many of the plants used today were known to the people of ancient cultures throughout the world and they were valued for their preservative and medicinal powers. *Azima tetracantha* (Salvadoraceae) is known as 'Esanku' in Malayalam, 'Mulsangu' in Tamil and 'Kundali' in Sanskrit, respectively. Its root, root bark and leaves are used with food as a remedy for rheumatism (Kritikar and Basu, 1984). It is a powerful diuretic given in rheumatism, dropsy, dyspepsia and chronic diarrhoea and as a stimulant tonic after confinement (Nadkarni, 1976). *Azima tetracantha* as efficient acute phase anti-inflammatory drug is traditionally used by Indian medical practitioners (Ismail *et al.,* 1997). The plant is used to treat cough, phthisis, asthma, small pox and diarrhea. The decoction of the stem bark is considered astringent, expectorant and antiperiodic (Chelladurai, 1983, Kapoor and Kapoor, 1980, Reddy *et al.,* 1991). Its leaves were found to possess azimine, azecarpin, carpine and isorhamnitine-3-O-rutinoside (Rail *et al.,* 1967, Williams and Nagarajan, 1988). Friedelin, lupeol, glutinol and βsitosterol have been isolated from the leaves of *A. tetracantha* (Rao and Rao, 1978). Recently, some novel fatty acids were isolated from seeds of this plant (Daulatabad *et al.,* 1991). Plant extracts and essential oils show antifungal activity against wide range of fungi (Kurita *et al*., 1981). In the present study, the *in vitro* static and microbicidal activity of chloroform and methanolic extract of leaves of *Azima tetracantha* were assessed against two Gram positive, three Gram negative bacterial and five fungal strains. The extracts were also validated for their antioxidant potential.

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MATERIALS AND METHODS

Plant material and preparation of extracts

Leaves of *Azima tetracantha* were collected from Vembayam, Thiruvananthapuram. The leaves were cleaned, air dried and powdered. The powdered samples were successively extracted with solvents, chloroform (CE) and methanol (ME) based on order of polarity using Soxhlet apparatus. The extracts obtained were subsequently concentrated under reduced pressure to get their corresponding residues. The extractive value was calculated and the residues were used for further studies.

Microbial strains and Standard drugs

The organisms studied include five bacterial and five fungal strains. The gram-positive strains used were *Staphylococcus aureus* (MTCC 740) and *Bacillus subtilis* (MTCC 47), while the gram negative strains used were *Proteus vulgaris* (MTCC 426), *Klebsiella pneumoniae,* and *Eschericia coli* (MTCC 43). The fungal strains used for the study were*, Aspergillus flavus, Candida albicans, Cryptococcus neoformanes, Penicillium notatum* and *Mucor* species. Streptomycin was used as the standard drug for bacteria and Flucanazole for fungi. The bacterial strains were cultured and maintained in Nutrient broth and Nutrient agar while the fungal strains were grown and maintained in Sabouraud's broth and agar respectively.

Antimicrobial Activity

1. *Disc Diffusion Method:* The antibacterial and antifungal assay was performed by Agar Disc Diffusion Method (Bauer *et al.,* 1966). Different concentrations of CE and ME were prepared and the test microorganisms, bacteria and fungi -were spread on sterile petriplates loaded with nutrient agar. Streptomycin (10mcg/disc) and Flucanazole (10mcg/disc) were used as the standard drugs (controls) against bacteria and fungi respectively.
2. *Susceptibility Test:* The micro-broth-dilution method was used to determine the susceptibility of the organisms to the ME and CE of *Azima tetracantha* (Rios *et al.,* 1988). The stock solution was prepared by dissolving a known quantity of CE and ME in 10% DMSO. The two-fold dilution method was adopted and eleven such concentrations ranging from10mg ml^{-1} to 0.0097mg ml^{-1} were prepared for the study. Streptomycin and Flucanazole were used as the positive controls against bacteria and fungi respectively.

Determination of Total Phenol Content

Total phenolic content was determined using Folin Ciocalteau (FC) reagent (Mayr *et al.,* 1995). The sample was added with 0.5ml Folin Ciocalteau reagent and 1.5ml of 20% sodium carbonate solution. The mixtures were incubated at room temperature and the absorbance was read at 765 nm.

Antioxidant Activity

The preliminary studies of antioxidant activity were made by quantifying the hydrogen donating ability and reductive potential of the extracts.

DPPH scavenging assay

The hydrogen donating or radical scavenging ability was measured using 1,1 diphenyl-2-picryl hydrazine (DPPH) as described by Blois (1958) and modified according to Wong *et al.* (2005). In this assay, a known volume of extract solution was added to methanolic DPPH and incubated for 15 min. The decrease in DPPH absorption at 517 nm was correlated with scavenging action of the sample. The DPPH solution without sample was considered as the control and ascorbic acid was used as the positive control. The radical scavenging ability is expressed as inhibition percentage.

Reducing power assay

The Fe^{3+} reducing power of the leaves were determined by the method described by Oyaizu (1986). The increase in absorbance is interpreted as the increase in reducing activity and the results were compared with ascorbic acid (positive control).

RESULTS

The leaf powder was successively extracted with chloroform and methanol. The extractive values for chloroform and methanolic extracts are 0.814 % and 7.8 % respectively.

Table1: Zone of inhibition of ME and CE of *Azima tetracantha* against bacterial strain compared with Streptomycin

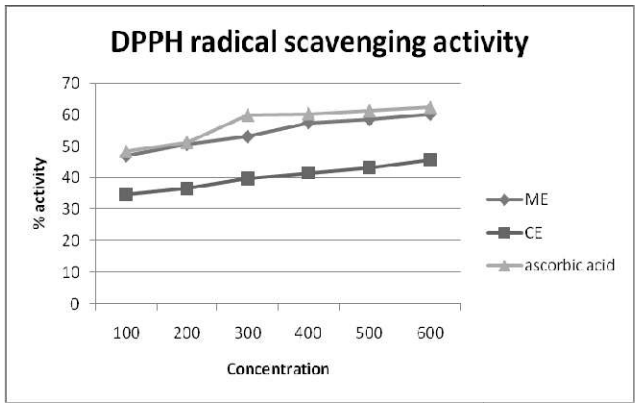
Bacterial Strain		Zone of Inhibition(mm)					
		Methanolic extract			Chloroform extract		
		1mg ml^{-1}	2mg ml^{-1}	Streptomycin (10mcg/disc)	1mg ml^{-1}	2mg ml^{-1}	Streptomycin (10mcg/disc)
1.	*S.aureus*	10	11	22	10	11	22
2.	*E.coli*	10	12	23	11	12	21
3.	*P.vulgaris*	---	---	12	---	---	11
4.	*B.subtilis*	10	11	25	10	11	21
5.	*K.pneumoniae*	10	11	19	08	10	16

Table 2: MIC and MBC of ME and CE against bacterial strains in comparison with Streptomycin

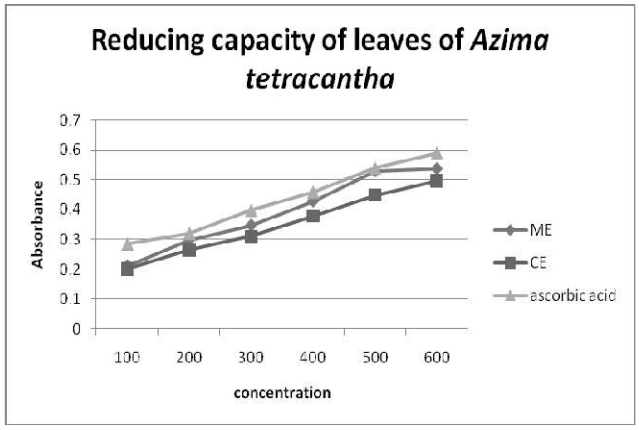
Bacterial strain		MIC (mg ml^{-1})			MBC (mg ml^{-1})		
		ME	CE	Streptomycin	ME	CE	Streptomycin
1.	*S.aureus*	1.25	0.625	0.195	10	10	0.1562
2.	*E.coli*	1.25	5	0.195	10	5	0.0781
3.	*P.vulgaris*	2.5	5	1.25	2.5	5	5
4.	*B.subtilis*	0.195	5	0.195	10	5	0.3125
5.	*K.pneumoniae*	1.25	5	0.195	2.5	5	0.625

Table 3: MIC of ME and CE against fungal strains in comparison with Flucanazole

Fungal strain		MIC (mg ml^{-1})			MFC(mg ml^{-1})		
		ME	CE	Flucanazole	ME	CE	Flucanazole
1.	*C.neoformanes*	5	0.625	0.1562	5	1.25	10
2.	*A.flavus*	0.1562	0.1562	0.3125	0.625	10	0.0097
3.	*C.albicans*	>10	>10	0.0781	>10	>10	0.3125
4.	*P.notatum*	5	5	10	5	5	0.3125
5.	*Mucor sp.*	5	5	0.0781	5	10	1.25



Graph 1.DPPH radical scavenging activity of *A. tetracantha* leaves



Graph 2. Reducing power of *A. tetracantha* leaves

Antimicrobial activity

Antibiogram of chloroform and methanolic extracts of leaves of *Azima tetracantha* is described below

a) Antibacterial Assay

1. Disc Diffusion Assay

Both CE and ME possess antibacterial activity against all the strains tested except *P.vulgaris*. The effect of the extracts was found to be lower than the reference drug, Streptomycin, at concentrations studied (Table 1).

2. Susceptibility Test

All strains could be inhibited at very low concentrations by ME, but higher concentrations of CE were required for the same, except *S. aureus*. *P. vulgaris* was resistant even to Streptomycin (Table 2). It is evident from the Table 1 that ME is more efficient in inhibiting the bacterial strains, than CE. MBC for methanolic and chloroform extracts were also determined (Tabel 2).

b) Antifungal Activity

1. Disc Diffusion Assay

All the fungal strains were found to be resistant against 1mg ml^{-1} and 2mg ml^{-1} of methanolic and chloroform extracts as no prominent zone of inhibition were recorded for the five fungal strains.

2. Susceptibility Test

Methanolic and chloroform extracts showed MIC at a concentration of 0.1562 mg ml^{-1} against *A. flavus*, while both the extracts were required at higher concentration than 10 mg ml^{-1} to inhibit the growth of *C.albicans*. Minimum fungicidal concentrations were also determined for both the methanolic and chloroform extracts, against the five fungal strains (Table 3).

Antioxidant activity

The total phenolic content in the leaves of *A. tetracantha* is 8.21±0.65mg g^{-1} on fresh weight basis. Methanolic extract in all the concentrations investigated for DPPH radical scavenging assay showed nearly similar activity to the standard, ascorbic acid (Graph 1). The IC_{50} value of the ME was 100µg ml^{-1} and that of CE is 600 µg ml^{-1}. In this study, the reducing capacity of the extracts increased with increasing concentration (Graph 2). It was noticed that both the methanolic and chloroform extracts showed lower reducing power potential than that of the natural antioxidant, ascorbic acid.

DISCUSSION

Chloroform and methanolic extracts were used for the micro broth dilution assay. The antibacterial activity was considered significant if MIC≤ 200mg ml^{-1} (Suffredini *et al*., 2006). The current study revealed that ME possess greater antimicrobial activity than CE. The test extracts showed percent inhibition in a concentration dependent manner against the test organisms and was comparable with the standard drugs.

Phenolic compounds are typical active oxygen scavengers in plants and are known to contribute directly to antioxidant action. The results indicate a high concentration of polyphenols in the leaves of *A. tetracantha.* The hydroxyl groups of the phenolic compounds confer the scavenging ability of the plant (Yildrim *et al,* 2000). The decrease in absorbance of DPPH radical is due to its reduction by different antioxidants, which in turn indicates the free radical scavenging property of the leaves of *A. tetracantha*. Absorbance decreases as a result of the colour change from purple to yellow, through the donation of hydrogen to form stable DPPH-H. Antioxidant activity of the leaves may also attribute to unidentified substances or to the synergistic interactions (Qian and Nihorimbere, 2004). The studies conducted by Siriwardhana *et al.* (2003) reported a high correlation between DPPH radical scavenging potential and total phenolic content. The reducing capacity of a compound may also serve as a significant indicator of its potential antioxidant activity (Sreekanth *et al,* 2003, Leskovar *et al.,* 2004). In this study, the reducing capacity increased with increasing concentration. This shows that the antioxidant compounds can react with free radical to convert them to more stable products and thereby terminate radical chain reactions.

The present study throws light in understanding the antimicrobial and antioxidant potential of the leaves of *A.*

tetracantha. Previous studies have reported the presence of alkaloids, steroids, terpenoids, flavonoids and coumarins in the methanolic leaf extract of *Azima tetracantha*. Thus this study reveals the efficacy of ME over CE, against all the bacteria and fungi included in the study. The activity of the extracts was comparable with the standard drugs. Hence, elucidation of antimicrobial principles from *Azima tetracantha* necessitates the isolation for the development of new antimicrobial agents. The results show that the leaves of *A. tetracantha* have antioxidant activity and free radical scavenging activity. This property may be one of the mechanisms by which this plant is useful as a rejuvenating drug in the traditional medicinal practices. Thus, this plant might be helpful in preventing the progress of various oxidative stresses and therefore it demands for the further investigations to isolate and identify the antioxidant compounds present in the leaves of *A. tetracantha*.

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