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ASSESSMENT OF ANTIBACTERIAL EFFICACY OF LEAF EXTRACTS OF INDIAN MEDICINAL TREES, *AZADIRACHTA INDICA A.JUSS.*AND *VITEX NEGUNDO LINN.*

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ABSTRACT

In India, the burden of infectious diseases is enormous. Though significant improvements in socio-economic status have been done since independence, infectious diseases still contribute to 30% of disease burden in India.Many number of medicinal plants, traditionally used for over 1000 years are present in herbal preparations of Indian traditional health care systems. *Azardichta indica A. Juss.* And *Vitex negundo Linn.* are medicinal trees native to the Indian subcontinent credited with the ability to cure infection. The antibacterial efficacy of the crude leaf extracts of the two medicinal trees were studied with various pathogenic bacteria. The extracts which showed the highest activity were analysed and the minimum inhibitory concentration was determined. The leaf extracts of *Azardichta indica* exhibited maximum inhibition when compared to *Vitex negundo* extracts at a concentration of 100mg/ml.Thus, the leaf extracts of these medicinal trees can be effectively used for the treatment of infectious diseases.

KEYWORDS: Infectious diseases, pathogens, antibacterial.

INTRODUCTION

The incidence of infectious diseases in humans has increased in the recent past and the emergence of newer infectious microorganisms threatens the existence of mankind in the near future. Developing countries such as India with a huge population bear the brunt of infectious

diseases.[1] Antibiotic resistance in bacteria has been called as the world's most pressing public health problems. Drug resistance is an especially difficult problem for hospitals because they harbour critically ill patients who are more vulnerable to infections than the general population and therefore require more antibiotics.[2]

The use of plant based products for health purposes have been used for many centuries. Literature regarding medicinal uses of plants dates back to the Vedic period in India. Herbs and spices are generally considered safe and proved to be effective against certain ailments.[3] Great interest in the use and in the importance of Indian Medicinal Plants has been developed to establish the relationship between chemical, biological and therapeutic activities of folklore medicine. Plants have a limitless ability to synthesize secondary metabolites, most of which are phenols or phenolic compounds which serve as plant defence mechanisms against predation by microorganisms, insects and herbivores.[4] called as neem is a valuable medicinal tree, indigenous to India and every part of the tree is commercially exploitable. It is now considered as a valuable source of unique natural products for the development of medicines against various diseases and also for the development of industrial products.[5] *Vitex negundo Linn* commonly called as the Chaste tree is indigenous to South East Asia and is credited with innumerable medicinal properties validated by modern science and used since ancient times.[6]

MATERIALS AND METHODS

i) Preparation of plant powder[7]

The leaves of *Azardichta indica* and *Vitex negundo* were obtained from the medicinal farm of Arignar Anna Government Siddha College and Hospital, Arumbakkam, Chennai. The leaves were separated and washed twice with double distilled water and then surface sterilised using 70% ethanol.The leaves were shade dried for 1-2 weeks. The leaves were then ground into a coarse powder form using a mixer.

ii) Preparation of crude extract[8]

The crude extracts were prepared by hot and cold method of extraction. In the hot method of extraction, about 1 gram of the powdered plant material was mixed with 10 ml of the solvent,incubated in a shaker at 37ºC for 4 hours at 250 rpm after which it was placed in a water bath at 60ºC for 2 hours.The supernatant was filtered and dried in air at room temperature. For the cold method of extraction, about 1 gram of the of the powdered plant material was mixed with 10 ml of the solvent, incubated in a shaker at 37ºC for 4 hours at

250 rpm.The supernatant filtered and then dried in air at room temperature. The solvents used were water, ether, ethanol, methanol, chloroform, acetone and dichloromethane. The residue obtained after drying was dissolved in the appropriate solvent and used for antibacterial screening.

iii) Microbial cultures used

The test organisms used for screening were *Staphylococcus aureus, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, E.coli, Klebsiella pneumoniae, Proteus mirabilis and Proteus vulgaris.* All the strains were confirmed laboratory isolates of the Department of Microbiology, J.B.A.S college for Women, Chennai.

iv) Preliminary Screening using Disc Diffusion Assay[9]

Discs(6mm) prepared from Whatmann No.1 filter paper was sterilised and impregnated with 20µl of various crude solvent extracts (concentration:100mg/ml). Broth cultures of the microorganisms were prepared by transferring 2-3 isolated colonies to Nutrient Broth and incubating the culture at 37 ^{0}C for 4 hours in the incubator. The culture was checked for turbidity by comparing with the McFarland Standard (0.5).A lawn culture of the organisms to be tested was made on the Mueller Hinton agar media. The prepared discs were placed on the plate in a way such that each disc was at least 20mm from one another. The plates were then incubated at 37°C for 24 hours. The inhibition zone around each disc both in the experiment and the control were measured. Standard antibiotics as per the organisms tested were included as positive control and respective solvents without the plant extracts were used as the negative control.

v) Determination of Minimum Inhibition Concentration/Minimum Bactericidal Concentration[10]

The Microbroth dilution was performed on a microtitre plate. Doubling dilutions of the crude extract were prepared in Mueller Hinton broth. Bacterial cultures of 10^{6} cfu/ml dilution were prepared with McFarland standard (0.5) and 10µl were added to each well of the microtitre plate and mixed well. The microtitre plates were incubated at 37ºC overnight and a loopful of the culture was streaked on to nutrient agar plates.The plates were incubated at 37ºC overnight.The growth/no growth pattern of the organisms corresponded to the MIC /MBC of the crude extract.

RESULTS

Table I: Preliminary Screening Of Crude Solvent Extracts Of *Azadirachta indica A.Juss.*

Organisms Tested	Inhibition Zone Diameter in mm													
	Ethanol		Methanol		Ether		Aqueous		chloroform		Dichloro methanol		Acetone	
	H	C	H	C	H	C	H	C	H	C	H	C	H	C
Staphylococcus aureus	18	10	14	11	10	10	-	-	10	12	-	-	-	-
E.coli	12	8	-	8	11	10	-	-	9	9	-	-	-	-
Klebsiella pneumoniae	10	13	10	8	-	-	-	-	10	14	-	-	-	-
Salmonella typhi	10	16	10	-	9	9	-	-	9	9	-	-	-	-
Salmonella paratyphi A	16	17	-	10	8	7	-	-	11	10	-	-	-	-
Salmonella paratyphi B	18	12	8	9	-	10	-	-	10	12	-	-	-	-
Shigelladysenteriae	10	10	10	12	11	12	-	-	10	9	-	-	-	-
Shigellaboydii	14	12	-	-	9	9	-	-	10	9	-	-	-	-
Shigella flexneri	14	14	10	10	10	10	-	-	10	9	-	-	-	-
Shigellasonnei	15	13	11	11	11	11	-	-	9	8	-	-	-	-
Proteus mirabilis	16	10	9	9	-	10			11	10	-	-	-	-
Proteus vulgaris	13	12	9	9	-	10			11	11	-	-	-	-

H- Hot method, C-cold method

Disc Diffusion assay using the ethanolic crude extract of *Azadirachta indica*, showed inhibitory zones for all the strains followed by chloroform ,methanol and ether extracts whereas no inhibition zones were observed for acetone, dichloro methanol and aqueous crude extracts.

Table II: Preliminary Screening of Crude Solvent Extracts of *Vitex negundo L.*

Organisms Tested	Inhibition Zone Diameter in mm													
	Ethanol		Methanol		ether		aqueous		chloroform		Dichloro Methanol		acetone	
	H	C	H	C	H	C	H	C	H	C	H	C	H	C
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E.coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	12	-	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella typhi	14	-	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella paratyphi A	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella paratyphi B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shigelladysenteriae	-	-	-	-	-	-	-	-	10	12	-	-	-	-
Shigellaboydii	12	12	-	-	-	-	-	-	9	10	-	-	-	-
Shigella flexneri	12	12	-	-	-	-	-	-	15	14	-	-	-	-
Shigellasonnei	12	12	-	-	-	-	-	-	10	9	-	-	-	-
Proteus mirabilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris	-	-	-	-	-	-	-	-	-	-	-	-	-	-

-Denotes no inhibition zone

Disc diffusion assay using the ethanolic crude extract of *Vitex negundo* showed inhibition zones for some of the bacterial strains followed by chloroform extracts whereas no inhibition zones were observed by methanol, ether, acetone and aqueous extracts.

Table III: Determination of Minimum inhibitory concentration/Minimum bactericidal concentration

		Concentration of the extracts in mg/ml						
ORGANISMS TESTED	**CRUDE EXTRACTS**	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.56**
Staphylococcus aureus	NHE	+	+	+	+	+	+	-
	NHM	+	+	+	+	-	-	-
	NCC	+	+	+	-	-	-	-
Escherichia coli	NHE	+	+	+	+	-	-	-
Klebsiella pneumonaiae	NCE	+	+	+	+	+	+	-
	NCC	+	+	+	+	+	-	-
Salmonella typhi	NCE	+	+	+	+	+	+	-
	VHE	+	+	+	+	+	-	-
Salmonella paratyphi A	NHE	+	+	+	+	+	+	-
	NCE	+	+	+	+	+	+	-
Salmonella paratyphi B	NCE	+	+	+	+	-	-	-
	NHE	+	+	+	+	+	+	-
	NCC	+	+	+	+	-	-	-
Shigelladysenteriae	NCM	+	+	+	+	-	-	-
	NCET	+	+	+	+	-	-	-
Shigellaboydii	NHE	+	+	+	+	+	+	-
	NCE	+	+	+	+	+	-	-
	VHE	+	+	+	+	+	-	-
	VCE	+	+	+	+	+	-	-
Shigella flexneri	NHE	+	+	+	+	+	-	-
	NCE	+	+	+	+	+	-	-
	VHE	+	+	+	+	+	-	-
	VCE	+	+	+	+	-	-	-
	VHC	+	+	+	+	+	+	-
	VCC	+	+	+	+	+	-	-
Shigellasonnei	NHE	+	+	+	+	+	+	-
	NCE	+	+	+	+	+	-	-
	VHE	+	+	+	+	+	-	-
	VCE	+	+	+	+	+	-	-
Proteus mirabilis	NHE	+	+	+	+	-	-	-
Proteus vulgaris	NHE	+	+	+	+	+	-	-
	NCE	+	+	+	+	-	-	-

+-presence of bacterial growth; - absence of bacterial growth,

NHE-neem hot ethanolic extract;NHM-neem hot methanolicextract;NCC-neem cold chloroform extract;NCE-neem cold ethanolicextract;NCM-neem cold methanolicextract;NCET-neem cold ether extract,VCE-Vitex cold ethanolic extract; VHE-Vitex hot ethanolicextract;VHC-vitex hot chloroform extract; VCC-Vitex cold chloroform extract.

The neem hot ethanolic extract showed an MIC value of 3.125 mg/ml for most of the strains which is the lowest, followed by the neem cold ethanolic extract which showed an MIC value of 6.25mg/ml.

DISCUSSION

Infectious diseases are an economic burden to the progress of a developing country like India leading to increased mortality and morbidity. At present, there is an emergence of bacterial strains that are resistant to multiple antibiotics which is a threat for human survival. This condition provides a compulsion for seeking alternative source of treatment for such infectious agents. Indian medicinal plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well being.[11]

The present study revealed the potent antibacterial potentials of the leaf extracts of the medicinal trees against *Staphylococcus aureus* and enteropathogens that are the major causative agents of diarrhoea and dysentery, a problem that is highly prevailing in developing and underdeveloped countries especially in infants and children. Many plants that are conveniently available in India have been reported by early investigators to be effective against diarrhoea and dysentery as they are used by locals as traditional folklore medicine. These plants have been given special attention.[12]

One of the most important and fundamental considerations in designing a phytochemical screening procedure is the selection of a proper solvent. Ethanol extracts of the medicinal trees, exhibited a well-marked antibacterial activity whereas aqueous extracts represented no activity. The present study confirms the previous reports, indicating that the aqueous extracts were the least active extracts.[13] Several methods for preparing an initial extract of the plant material have been reported and methanol/ethanol appears to be the most useful solvents.[14] The hot method was more effective for antibacterial activity. Similar observation was found in our study where good antibacterial efficacy was demonstrated with ethanol and methanol.

In our study, the crude leaf extracts of *Azadirachta indica* exhibited a wide spectrum of activity against most of the pathogens tested thereby confirming previous reports.[15, 16] In the study of antibacterial activity of *Vitex negundo*, Zaidan *et al.*,[17] reported no antibacterial activity against five strains of bacteria species, MRSA, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *E.coli*, using standard protocol of disc diffusion method. This is contrary to our study where ethanolic and chloroform extracts had shown activity against

most of the pathogens tested. Prasanth *et al.*,[18] has reported the leaves of *Vitex negundo* to show antibacterial activity against *Staphylococcus aureus, E.coli, Klebsiella pneumonia* and a wide range of bacterial pathogens at a concentration of 1000 to 500 g/ml with a mixture of dichloromethane and methanol(1:1) as the extraction solvent. This is contrary to our study, where the methanol and dichloromethanol extracts of *Vitex negundo* has not shown any antibacterial activity at a concentration of 100mg/ml. Amongst the pathogens tested, *Staphylococcus aureus*, *Shigella* species and *Proteus* species were the most susceptible organisms towards all the extracts tested.

CONCLUSION

To conclude, the present study supports the ethanobotanical use of the leaves of the medicinal trees for the cure of various infections caused by pathogenic bacteria. The ethanol crude leaf extracts of both the medicinal trees, indigenous to India can be used to control various infectious diseases caused by multi drug resistant bacterial strains.

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