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EVALUATION OF ANTIMICROBIAL POTENTIALITY OF AQUEOUS ETHANOLIC LEAF EXTRACT OF *ACACIA ARABICA* EXTRACT

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ABSTRACT

The present study is the continuation of a program aimed at investigation of antimicrobial properties of *Acacia arabica* extract to justify the traditional claim endowed upon this herbal drug as a *rasayana* in Ayurveda. The antimicrobial activity of *Acacia arabica* was evaluated according to the disk diffusion method by using Gram positive; *B. subtilis*, *S. aureus* and *S. epidermidis* and Gram Negative; *E. coli*, *S. flexneri*, *P. aeruginosa* bacteria. This study show that methanolic fruits extract of *Acacia arabica* Linn inhibits the growth of microorganism dose dependently. These results confirm the antibacterial activity of *Acacia arabica* leaves and support the traditional use of the plant in therapy of bacterial infection. This finding suggested the presence of antibacterial activity in the tested plant material, exhibited by its bioactive compounds, and serving them as an alternative antimicrobial agent.

KEYWORDS

Acacia arabica, Antimicrobial activity, Disk diffusion and Inhibition zone.

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INTRODUCTON

Scientific communities using many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines. Systematic screening of herbal plant may result in the discovery of novel effective compounds. They using the multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to

antibiotics raises the specter of untreatable fungal and bacterial infections.

The antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases *Acacia* is the important significant genus belong to family: Leguminosae, first of all described by Linnaeus in 1773. Maslin, 2003¹ and Orchard, 2003² reported that it is assumed that there are roughly 1380 species of *Acacia* worldwide, about two-third of them native to Australia and rest of spread around tropical and subtropical regions of the world, Gamble, (1918) have also reported more than 40 species of this genus in India in his 'Flora of Madras Presidency'. *Acacia arabica* are commonly known as 'Babool' in India and ethno medicinally have long been used for the treatment of skin, sexual, stomach and tooth problems. It is also known as kikar or Indian gum Arabic tree has been recognized worldwide as a multipurpose tree. It is distributed throughout arid and semi-arid zones of the world. *Acacia arabica* has been proved as effective medicine in treatment of malaria; sore throat (aerial part) and toothache (bark) (Chopra *et al*, 1956³, Jain *et al*, 2005⁴, Jain *et al*, 1987⁵, Joshi, 2007⁶, Kubmarawa *et al*, 2007⁷, Chowdhury *et al*, 1983)⁸ have tested the anti-fertility activity of *A. arabica* pods and nuts. The methanolic extracts of *A. arabica* pods have been claimed against HIV-PR (Hussein *et al*, 1999⁹, Bessong and Obi, 2006)¹⁰. The researchers has tested the antiplasmodial activity of *A. nilotica* ethyl acetate extract against different chloroquine resistant and sensitive strains of *Plasmodium falciparum* (EI-Tahir *et al*, 1999)¹¹. The fresh plant parts of this species have been reported to be most active against Hepatitis C virus (Hussein *et al*, 2000)¹². It is an important multipurpose tree that has been used extensively for the treatment of various diseases, e.g. colds, bronchitis, diarrhoea, dysentery, biliousness bleeding piles and leucoderma (Rahaman, 2010)¹³. Therefore, we have planned to carry out antimicrobial potentiality of aqueous ethanolic leaf extract of *acacia arabica* extract using different bacteria's.

MATERIAL AND METHODS

Extract preparation

Acacia arabica (100g) was defatted with petroleum ether (1000ml) and the residue was extracted in 50% methanol with the help of soxhlet extraction unit. The sample was collected and concentrated in water bath at 40-50°C and dried in hot air oven at 40°C. Final powder was kept in air tied box for further studies.

Microorganism

The test organism included the gram negative bacteria; *Acetobactor*, *Citrobactor* and *E. coli* and gram positive bacteria; *Bacillus subtilis*, *S. aureus* and *Enterobacter*. All the bacterial strain was obtained from National Chemical Laboratory (NCL), Pune, India. The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slant at 4°C.

Antibacterial Assay

The Antibacterial test was done by prepared bacterial suspension followed by the disc diffusion test. Antibacterial activity of *Acacia arabica* extract was determined by agar disk diffusion method at four different concentrations i.e., 100, 75, 50 and 25mg/ml. Muller Hinton agar was prepared according to the manufacturer's instruction and the plates were seeded with appropriate microorganism (Gram negative bacteria; *Acetobactor*, *Citrobactor* and *E. coli* and gram positive bacteria; *Bacillus subtilis*, *S. aureus* and *Enterobacter*). Discs of 6 mm diameter were prepared from Whatmann filter paper No.1 and sterilized. The discs were than impregnated with the extracts and solvent DMSO. Antibiotics for Gram positive (TE- Tetracycline, OF- Ofloxacin, AZ- Azithromycin and PC- Pipracillin) and Gram negative (Fu- Nitrofurantoin, GM- Gentamicin, CX- Cefotaxime and NF- Norfloxacin, 5µl/disc) bacteria were used as standard. The plates were incubated at 37°C for 24 hrs and the zone of inhibition was measured with measuring scale (Nair, *et al*, 2005)¹⁴. The above experiment was carried out in triplicate for their confirmation.

RESULTS AND DISCUSSION

Antibacterial activity

The result for microbial growth was considered as zero hour and further accordingly reading was

taken. Our present study shows that antibacterial activity of 50 % methanolic extract of *Acacia arabica* against *S. aureus* is best in 100 % concentration after 12 hrs. (12.66 mm zone of inhibition). Although 75% concentration is having mild effect as 11.00 mm zone of inhibition. In *Citrobacter* 100% concentration of extract is having good antibacterial activity at maximum zone of inhibition 12.00 mm. On the other hand 75 % is showing static activity from, with zone of inhibition of 11.00 mm. For *E. coli* 100% concentration of extract show maximum zone of inhibition 10.00 mm. Although the same effect of 75% concentration of extract is also revealing as showing zone of inhibition 10.00 mm. In the case of *Acetobacter* 75% and 100% concentration of extract show good activity with zone of inhibition of 10.66 mm and 10.33 mm respectively, and *Citrobacter* shows the inhibition 11.00 mm and 12.00 mm for 75% and 100 % concentration respectively. The above observations suggest that different concentration (50 %, 75 % and 100 %) were having good antibacterial activity against *Acetobacter*, *Citrobacter*, *E. coli*, *B. subtilis*, *S. aureus* and *Enterococcus*. Thus the extract is showing varying activity against all microorganisms. On comparing the zone of inhibition of extract to that of standard antibiotics extract showed better activity than Norfloxacin and Ofloxacin. In the case of erythromycin and amoxicillin the extract is not potent in these conditions.

Herbs have long been used as the basis of traditional Chinese herbal medicine, with us aged dating as far back as the first century CE and far before. The Ayurveda medicinal system is based on herbs and natural products. The herbs use as medicinal purpose in western cultures has its roots in the Hippocratic (Greek) elements healing system, based on a quaternary elements healing metaphor. The natural products and herbal drugs are use for the prevention and treatment of various health ailments has been in practice from time immemorial. The *Acacia arabica* leaf extract has been reported to be effective against a variety of disease including diabetes, skin disease and most concerning with cancer. The parts of *Acacia arabica* is considered as astringent, demulcent, aphrodisiac, anthelmintic, antimicrobial,

antidiarrhoeal, with good nutritional value in Indian traditional medicine system. This article briefly reviews the ethano botanical as well as medicinal uses of *Acacia arabica* with plant description. This data has been useful on different aspect of *Acacia arabica* and its potential use. Some studies are also required for the verify this hypothesis.

The antimicrobial activity was considered as zero hour and further accordingly reading was taken. Our present study shows that antibacterial activity of 50 % methanolic extract of *Acacia arabica* against *S. aureus* is best in 100 % concentration after 12 hrs. (12.66 mm zone of inhibition). Although 75% concentration is having mild effect as 11.00 mm zone of inhibition. In *Citrobacter* 100% concentration of extract is having good antibacterial activity at maximum zone of inhibition 12.00 mm. On the other hand 75 % is showing static activity from, with zone of inhibition of 11.00 mm. For *E. coli* 100% concentration of extract show maximum zone of inhibition 10.00 mm. Although the same effect of 75% concentration of extract is also revealing as showing zone of inhibition 10.00 mm. In the case of *Acetobacter* 75% and 100% concentration of extract show good activity with zone of inhibition of 10.66 mm and 10.33 mm respectively, and *Citrobacter* shows the inhibition 11.00 mm and 12.00 mm for 75% and 100 % concentration respectively. The above observations suggest that different concentration (50 %, 75 % and 100 %) were having good antibacterial activity against *Acetobacter*, *Citrobacter*, *E. coli*, *B. subtilis*, *S. aureus* and *Enterococcus*. Thus the extract is showing varying activity against all microorganisms. On comparing the zone of inhibition of extract to that of standard antibiotics extract showed better activity than Norfloxacin and Ofloxacin.

The antibacterial study was also done which shows a better antibacterial activity against all the six test gram-positive and gram-negative bacteria species used and shown antibacterial susceptibility to *Acacia arabica* seed extracts with clear zone of inhibition. So, in future it can be used as an alternate to antibiotics. This work provides an insight to understanding some molecular basis of therapeutic properties of *Acacia arabica* in traditional medicine. Furthermore, detailed studies

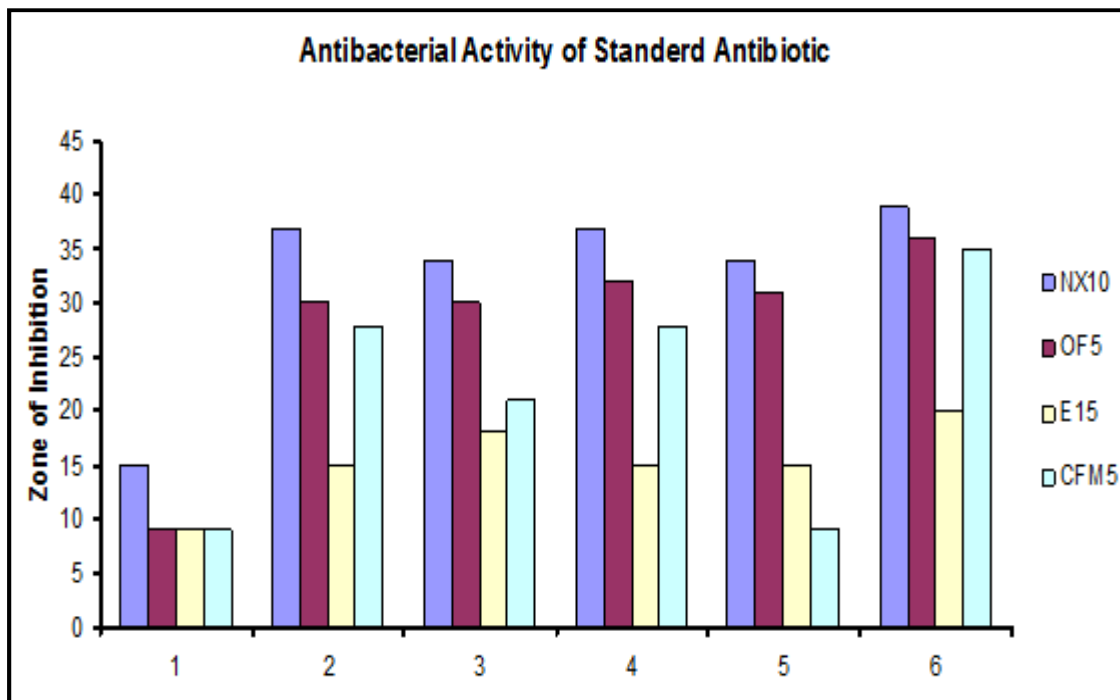
on the isolation and characterization of the plant extract as well as *in vivo* assays will be necessary in discovering new biological antibiotic agent.

Table No.1: The study of anti-bacterial activities of standard antibiotics using disk diffusion method

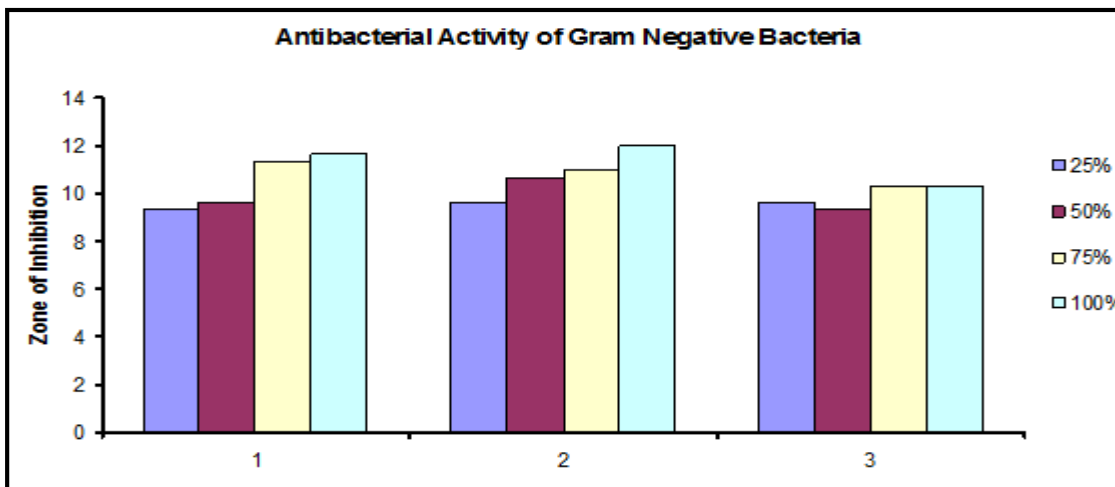
S.No	Name of Bacteria	Zone of Inhibition (In MM)			
		NX10	OF5	E15	CFM5
1	Gram Negative (-)				
	<i>Acetobacter</i>	15.00	09.00	09.00	09.00
	<i>Citrobacter</i>	37.00	30.00	15.00	28.00
	<i>E. coli</i>	34.00	30.00	18.00	21.00
2	Gram Positive (+)				
	<i>Bacillus subtilis</i>	37.00	32.00	15.00	28.00
	<i>S. aureus</i>	34.00	31.00	15.00	09.00
	<i>Enterococcus</i>	39.00	36.00	20.00	35.00

Table No.2: The study of anti-bacterial activities of *Acacia arabica* extracts using Disk Diffusion method (Mean ± SE)

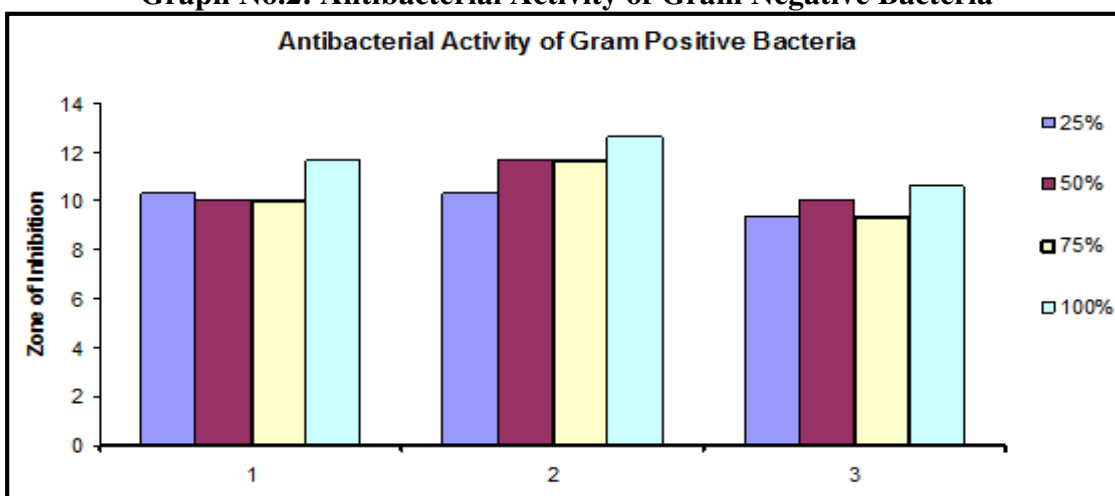
S.No	Bacterial Stain	Bacteria Use	Zone of Inhibition (In MM)			
			25%	50%	75%	100%
1	Gram Negative (-)	<i>Acetobacter</i>	09.33 ± 0.57	09.66 ± 0.57	11.33 ± 0.57	11.66 ± 0.57
		<i>Citrobacter</i>	09.66 ± 1.54	10.66 ± 2.08	11.00 ± 1.73	12.0 ± 1.75
		<i>E. coli</i>	09.66 ± 0.57	09.33 ± 0.57	10.33 ± 0.57	10.33 ± 0.57
2	Gram Positive (+)	<i>B. subtilis</i>	10.33 ± 1.15	10.00 ± 1.00	10.00 ± 0.00	11.66 ± 0.57
		<i>S. aureus</i>	10.42 ± 0.57	11.66 ± 0.57	11.66 ± 1.15	12.66 ± 0.57
		<i>Enterococcus</i>	09.33 ± 0.57	10.00 ± 1.00	09.33 ± 0.57	10.66 ± 0.57



Graph No.1: Antibacterial Activity of Standard Antibiotic



Graph No.2: Antibacterial Activity of Gram Negative Bacteria



Graph No.3: Antibacterial Activities of Gram Positive Bacteria

CONCLUSION

Traditional medicine has been practiced in India for decades and is still widely practiced even today. The knowledge of medicinal plants is passed on based on indigenous knowledge system and orally by the traditional herbal practitioners from one generation to the next. The medicinal plants are extracted from trees and shrubs. The common practice is the use of the bark, roots and sometimes both. Medicinal plants have a wide range of pharmaceutical use in disease diagnosis etc.

The antibacterial study was also done which shows a better antibacterial activity against all the six test gram-positive and gram-negative bacteria species used and shown antibacterial susceptibility to *Acacia arabica* extracts with clear zone of inhibition. So, in future it can be used as an alternate to synthetic antibiotics. Much effort has

needed to increase *Acacia arabica* plant as herbal medicine so as to resist the human pathogenic bacterial disease.

It showed most promising antibacterial effect against some human pathogenic bacteria. This study presents valuable data on antimicrobial property of *Acacia arabica* leaf extract, which should be very useful for clinical study of this plant extract. Thus, it shows that *Acacia arabica* has a great potential as an effective antimicrobial agent for medicinal purposes.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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