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Antimicrobial Activity of Leaves of *Acacia Arabica* against Pathogenic Organisms Compared with Control Drug

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A B S T R A C T

The crude extract from the leaves of Acacia Arabica traditionally used in Indian system of medicines were screened against *Escherichia coli* NCIM 2931, *Salmonella typhi* MTCC 734, *Salmonella typhimurium* MTCC 98, *Klebsiella pneumoniae* MTCC432, *Proteus vulgaris* NCIM2857, Proteus mirabilis MTCC425, *Pseudomonas aeruginosa* NCIM5029, *Staphylococcus aureus* MTCC 96, *Staphylococcus epidermis* MTCC 435, Bacillus cereus NCIM2155, *Bacillus subtilis* NCIM 2063 and *Bacillus megaterium* NCIM 2087 by using agar well diffusion method. *Acacia arabica* crude extract showed significant activity against Gram negative organisms. Zone of inhibition of the extract compared with the standard antibiotics.

Introduction

Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines (Stuffness and Douros, 1982). Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health care since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural product play a vital role in modern drug development in the pharmaceutical industry (Baker *et al.*, 1995). Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extract on bacteria have been studied by a very large number of researches in different parts of the world (Ates and Erdogrul, 2003). Much work has been done on ethnomedicinal plants in India (Negi *et al.*, 1993). Interest in a large number of traditional natural products has increased (Taylor *et al.*, 1996). It has been suggested that aqueous and Ethanolic extract from plants used in allopathic medicine are potential sources of antiviral, Anti tumural and antimicrobial agents (Chung *et al.*, 1995). The selection of the crude plants extract for screening programmes has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products (Kusumoto *et al.*, 1995).

There is continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of actions because there has been alarming increase in the incidence of new and re-emerging infectious diseases.

Materials and Methods

Selection of medicinal plant for this study:

Acacia Arabica
Family: *Leguminosae*
Parts used: Leaf

Traditional uses: *A*cacia *species* are commonly known as 'Babool' in India and traditionally used for its medicinal properties for the treatment of skin, sexual, stomach and tooth problems

Identification and Preservation of Plant materials:

Fresh plant leaves were collected from the Nagpur area of India. The taxonomic identities of this plant were determined by the expertise of the Post Graduate Department of Botany of Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. Specimen was labeled, numbered and noted with date of collection, the locality, their medicinal uses and their approximate dosages of administration were recorded. Plant leaves were washed with 70% alcohol and then rinsed with sterilized distilled water, air dried and stored in airtight bottles at 4^{o}C for further use.

Preparation of crude extracts (Fresh juice)

Acacia arabica plant leaves were collected from around Nagpur region in the month of August-September. Leaves were cleaned under running potable water and cut into pieces and grounded in pestle and mortar (made up of dolerite stone) till homogenized mass was obtained. Homogenized mass was squeezed in 400 mesh nylon cloth (pore size 37 micron) to obtain crude extract. Crude extract was kept in sterilized glass bottle. All crude extract were prepared fresh and used within 2 hours for further testing.

Crude extraction

Aqueous extraction: Ten grams of dried powder was extracted in 100 ml distilled water for 6 h at slow heat. Every 2 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000g for 15 min. The supernatant was collected. This process was repeated twice and after 6 h, the supernatant was concentrated to make the final volume one-fourth of the original volume (Shahidi Bonjar, 2004). It was then autoclaved at 121ºC and 15 lbs pressure and then stored at 4ºC.

Solvent extraction

Ten grams of dried powder was extracted with 100 ml of each solvent (acetone, chloroform, methanol and petroleum ether) and flasks were kept on a rotary shaker at 190-220 rpm for 24h. Thereafter, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (Shahidi Bonjar, 2004). It was stored at 4^{o}C in airtight bottles for further studies.

Bacterial cultures

The microbial strains are identified strains and were procured from the National Chemical Laboratory (NCL), Pune, India. The studied bacterial strains were *Bacillus cereus* NCIM2155, *Bacillus subtilis* NCIM2063, *Bacillus megaterium* NCIM2087, *Escherichia coli* NCIM2931, *Proteus vulgaris* NCIM2857 and *Pseudomonas aeruginosa* NCIM5029. *Staphylococcus aureus* MTCC96,

Staphylococcus epidermis MTCC 435, *Salmonella typhi* MTCC 734, *Salmonella typhimurium* MTCC 98, *Klebsiella pneumoniae* MTCC432, *Proteus mirabilis* MTCC425, these strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. They were sub-cultured on nutrient agar for every 15 days and maintained on nutrient agar slants at 4^0C, fresh inoculums were taken for test.

Media

Hi -Sensitivity test broth (M 486) and Hi-sensitivity test agar (M 485) were procured from Hi-media Mumbai, India. The media were prepared according to the instructions given.

Screening for the antimicrobial potential of the plant leaves extracts

The antimicrobial activity of different solvent extracts was evaluated by agar well diffusion (Perez C, *et al.*, 1990, Nair and Chanda, 2005 and Parekh, J. *et al.*, 2007) using Hi-sensitivity test agar (M 485).

Preparation of inoculum – A loopful of culture was inoculated from the stock slant culture in 5 ml of Hi-sensitivity test broth and broth was incubated at 35±0.5^{0}C in incubator for 18-20 hrs. After incubation a loopful of actively growing culture was inoculated into 10 ml of Hi-sensitivity broth. Broth was incubated at 35±0.5^{0}C for 6-8 hours. This culture was used for the inoculation of Hi-sensitivity test agar plates.

Preparation of Hi-sensitivity test agar medium

Hi-sensitivity test agar medium was prepared as per instructions of manufacturer. Required amount of agar medium was melted and 25 ml of molten medium was distributed in test tubes (25x150 mm). Medium was autoclaved at 15 lb. for 20 min. After autoclaving, medium was maintained at 45-50^{0}C in constant temperature water bath.

Inoculation of medium with test organism

0.5 ml of 6-8 hours old test organism is transferred to petridish of 100mm size (Sterilized in oven at 180^{0}C for 1 hr.) using sterile micropipette. Hi-sensitivity test agar medium maintained at 45-50^{0}C was poured and mixed properly to ensure uniform distribution of organism with medium. Seeded plates are allowed to set at room temperature.

Preparation of agar well for fresh leaves juice

10 mm borer was used to prepare wells in agar. Four wells per plate at four equidistant corners were made. A 100 µl crude extract (fresh leaves juice) was transferred by micropipette per well.

Plates were immediately kept at 4^0C in refrigerator for 1 hr. for the good diffusion of extract and then shifted to 35±0.5^{0}C in incubator (Venkatesan *et al.*, 2009). Zone of inhibition was measured after 24 hrs of incubation by zone scale.

Preparation of agar wells for different solvent extracts

5 mm borer was used to prepare wells in agar. Four wells per plate at four equidistant corners were made.

A 50 ul solvent extract was transferred by micropipette per well. Plates were immediately kept at 4^0C in refrigerator for 1 hr. and then shifted to 35^{0}C+0.5^{0}C in incubator. Zone of inhibition was measured after 24 h of incubation.

Antibacterial activity of different solvent extracts of leaves of *Acacia arabica (AA)*, zone of inhibition in millimetre (mm)

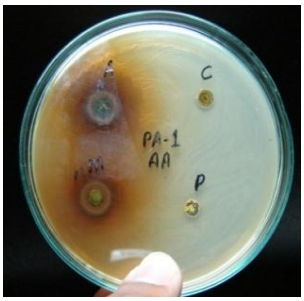

Figure-- 1
Activity against *Pseudomonas aeruginosa*
Acetone extract (A)
Chloroform extract (C)
Methanol extract (M) -12 mm
Petroleum ether extract (P)

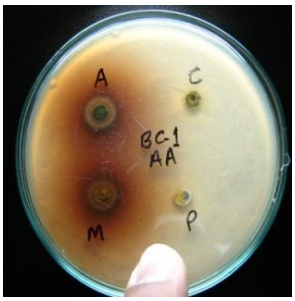

Figure--2
Activity against *Bacillus cereus*
Acetone extract (A)-13 mm
Chloroform extract (C)
Methanol extract (M)-13 mm
Petroleum ether extract (P)

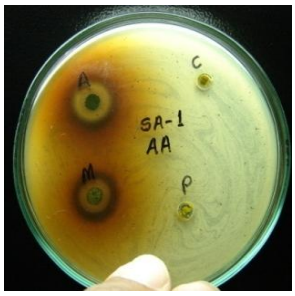

Figure-3
Activity against *Staphylococcus aureus*
Acetone extract (A)-20 mm
Chloroform extract (C)
Methanol extract (M)-18 mm
Petroleum ether extract (P)

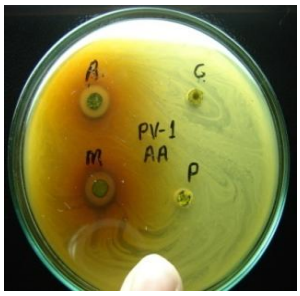

Figure--4
Activity against *Proteus vulgaris*
Acetone extract (A)-12 mm
Chloroform extract (C)
Methanol extract (M)-13 mm
Petroleum ether extract (P)

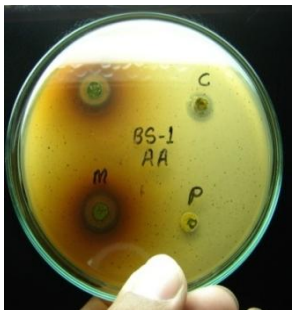

Figure-5
Activity against *Bacillus subtilis*
Acetone extract (A)-16mm
Chloroform extract (C)-11 mm
Methanol extract (M)-14 mm
Petroleum ether extract (P)

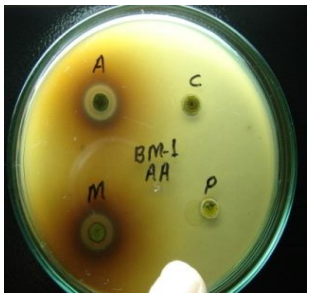

Figure--6
Activity against *Bacillus megaterium*
Acetone extract (A)-13 mm
Chloroform extract (C)
Methanol extract (M)-13 mm
Petroleum ether extract (P)

Table.1 Results of antimicrobial activities of fresh leaves juice and solvent extracts of *Acacia arabica* leaves and compared with standard antibiotics

Sr.No.	Microorganisms	Zone of inhibition in millimeter										
		Leaves extracts						Standard antibiotics				
		FJ	WE	AE	CE	ME	PE	Am^{30}	Cf^{30}	Co^{25}	G^{50}	T^{30}
1.	*Escherichia coli*	---	---	---	---	---	---	32	29	24	17	22
2.	*Proteus vulgaris*	12	---	12	---	13	---	---	23	31	20	24
3.	*Pseudomonas aureginosa*	---	---	---	---	12	---	14	36	---	34	22
4.	*Staphylococcus aureus*	20	12	20	---	18	---	31	23	20	16	17
5.	*Bacillus cereus*	23	---	13	---	13	---	15	27	---	23	24
6.	*Bacillus subtilis*	14	---	16	11	14	---	31	50	36	40	32
7.	*Bacillus megaterium*	13	---	13	---	13	---	29	46	24	23	33

Key: FJ—Fresh juice of leaves; WE—Water extract; AE—Acetone extract; ME—Methanol extract; CE-Chloroform Extract; PE—Petroleumether Extract; Am^{30}--Amoxycilin; Cf^{30} –Ciprofloxacin; Co^{25} –Cotrimaxazole; G^{50} --Gentamicin; Tetracycline--T^{30}; --- Negative.

For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract.

The control zones were subtracted from the test zones and the resulting zone diameter is obtained.

Results and Discussion

The extracts prepared from *Acacia arabica* leaves using different solvents showed varying degree of antimicrobial activity against organisms selected for the study. Among the extracts prepared using different solvents, methanol extract was found to be effective against all the organisms except *Escherichia coli*, While acetone extract and fresh juice was found to be effective against all the selected organisms except *Pseudomonas aeruginosa* and *Escherichia coli*, while Water extract and Chloroform Extract showed inhabitance only against *Staphylococcus aureus* and *Bacillus subtili*s respectively, petroleum ether was found ineffective against selected strains (Table 1).

Other workers also found similar results in the above discussed extracts (Deen and Sadiq, 2002; Kavitha *et al.,* 2013) (Rubina *et al.,* 2015).

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