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## Search for Antimicrobial Potentials from Certain Indian Medicinal Plants

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### ABSTRACT

Ethanol extracts of eight Indian Medicinal Plants *Arnebia nobilis*, *Garcinia indica*, *Boehavia diffusa*, *Solanum albicaule*, *Vitex negundu*, *Bunium persicum*, *Acacia concinna* and *Albizia lebbek* were examined for their anti-microbial potentials against selected bacteria and fungi. The purpose of screening is to justify, authenticate and validate the use of Indian Medicinal Plants in ethnomedicinal or folklore as traditional treasure to cure various ailments. In present investigations attempts were made to screen the Indian Medicinal Plants as antibiotics. The extracts were tested against selected test bacteria and fungi as antimicrobial assay through disc diffusion assay where standard tetracycline is used and solvent ethanol as control. Indian Medicinal Plants have a traditional background that they have potentials to use as antimicrobial agents. The results showed that all the extracts possess good antimicrobial activity against selected test bacteria and intermediate against fungus. The present results therefore offer a scientific basis for traditional use of ethanolic extracts *Arnebia nobilis*, *Garcinia indica*, *Boehavia diffusa*, *Solanum albicaule*, *Vitex negundu*, *Bunium persicum*, *Acacia concinna* and *Albizia lebbek*. These results explain that *G. indica* showed potential antimicrobial activity against *S. aureus* negative can be used as a very good treatment for acne if added to daily diet and *V. negundu* showed potentials against *S. aureus* positive. Further, *B. diffusa*, *A. concinna* and *A. lebbek* have also possessed antimicrobial potentials against all test bacteria and fungi which explains that their use in daily life will generate a resistant or immunity to fight against microorganisms. It is also noteworthy that screening of antimicrobial potentials of *S. albicaule* were performed for the first time.

**Key words:** *S. albicaule*, *Garcinia indica*, bioactivity, ethnomedicinal, traditional treasure

### INTRODUCTION

The plant kingdom has been the best source of remedies for curing a variety of disease and pain. Plant derived drugs remain an important resource, especially countries to combat serious diseases. Approximately 60-80% of the world's population still relies on traditional medicines for the treatment of common illnesses (WHO, 2002; Zhang, 2004). It is estimated that there are 250,000 to 500,000 species of plants on Earth (Borris, 1996). A relatively small percentage (1 to 10%) of these are used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes (Moerman, 1996). The problem of microbial resistance is growing and

the outlook for the use of antimicrobial drugs in the future is still uncertain. Hence, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. Natural products of higher plants are an important source of therapeutic agents, therefore, many research groups are currently screening the different biological activities of plants (Mothana *et al.*, 2008; Mulabagal *et al.*, 2007; Leu *et al.*, 2006). The use of phytochemicals as natural antimicrobial agents commonly called biocides is gaining popularity (Smid and Gorris, 1999).

There is growing interest in correlating phytochemical constituents of plant with its pharmacological activity. 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 them may result in the discovery of novel effective compounds (Nitta *et al.*, 2002; Sieradzki *et al.*, 1999b). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies.

Indian Medicinal plants throughout its long history, has accumulated a rich body of empirical knowledge of the use of medicinal plants for the treatment of various diseases. Chemical studies of Indian medicinal plants provide a valuable material base for the discovery and development of new drugs of natural origin. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu *et al.*, 1999).

Therefore, in present project attempts have been made to eight medicinal plants *Arnebia nobilis*, *Garcinia indica*, *Boehavia diffusa*, *Solanum albicaule*, *Vitex negundu*, *Bunium persicum*, *Acacia concinna* and *Albizia lebbeck* each belonging to different families were evaluated for antibacterial potentials. It is worth mentioning that *S. albicaule* was screened for antimicrobial potentials for the first time. Further, all the selected medicinal plants were used to justify and authenticate on scientific basis where antimicrobial characters will be aid as a markers to characterize these drugs from their adulterants. These biomarkers can be used further for formation of Indian Pharmacopoeia.

## **MATERIALS AND METHODS**

**Collection:** Plant samples (*Arnebia nobilis*, *Garcinia indica*, *Boehavia diffusa*, *Solanum albicaule*, *Vitex negundu*, *Bunium persicum*, *Acacia concinna* and *Albizia lebbeck*) were collected from various tribes living in tribal pockets of Mt. Abu, arid zone of Rajasthan. These plants were used by these tribes in their daily lives to cure various ailments and few from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of May, 2009.

**Identification:** All the samples were authenticated and were given identification number *Arnebia nobilis*, *Garcinia indica*, *Boehavia diffusa*, *Solanum albicaule*, *Vitex negundu*, *Bunium persicum*, *Acacia concinna* and *Albizia lebbeck*. These samples were authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

**Sources of test organisms:** Bacteria-Pure culture of all test organisms, namely *Pseudomonas aeruginosa*, *Staphylococcus aureus positive*, *Escherichia coli*, *Staphylococcus aureus negative* and

fungi *Candida albicans*, were obtained through the courtesy of Mahatma Gandhi Institute of applied Sciences(MGias), Jaipur, which were maintained on Nutrient broth media.

**Culture of test microbes:** For the cultivation of bacteria, Nutrient Agar Medium (NAM) was prepared by using 20 g Agar, 5 g Peptone, 3 g beef extract and 3 g NaCl in 1 L distilled water and sterilized at 15 lbs pressure and 121°C temperature for 25-30 min. Agar test plates were prepared by pouring approximately 15 mL of NAM into the Petri dishes (10 mm) under aseptic conditions. A saline solution was prepared (by mixing 0.8% NaCl) in distilled water, followed by autoclaving and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24-48 h.

To prepare the test plates, in bacteria, 10-15 mL of the respective medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant.

**Preparation of test extracts:** Crushed powder (50 g) of all the species were successively soxhlet extracted with ethanol. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were pooled individually. Each filtrate was concentrated to dryness *in vitro* and re dissolved in respective solvents, out of which 80 mg/10 disc i.e. 8 mg disc<sup>-1</sup> concentration were stored at 4°C in a refrigerator, until screened for antibacterial activity.

**Bactericidal assay:** For both, bactericidal *in vitro* Disc diffusion method was adopted (Gould and Bowie, 1952), because of reproducibility and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whattman No. 1 paper (6 mm in diameter), which were containing 8 mg of the text extracts, its control (of the respective solvent) and tetracycline as reference drugs (standard disk) separately. Such treated discs were air-dried at room temperature to remove any residual solvent, which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low temperature for 1 h so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 h in case of bacteria, after which the zones of inhibition could be easily observed. Five replicates of each test extract were examined and the mean values were then referred.

The Inhibition Zone (IZ) in each case were recorded and the Activity Index (AI) was calculated as compared with those of their respective standard reference drugs (AI = Inhibition Zone of test sample/Inhibition zone of standard).

## RESULTS

The profile of eight medicinal plants used in present investigation is shown in Table 1. The results of antimicrobial activity of the crude extracts of Selected Indian Medicinal Plants (*A. nobilils*, *G. indica*, *B. diffusa*, *S. albicaule*, *V. nigundu*, *B. persicum*, *A. concinna* and *A. lebbeck*) showed good antimicrobial activity against selected test bacteria and intermediate against fungi (Table 2). Overall, these ethanolic extract showed appreciable activity against selected test bacteria and fungi and hence, it justify their use in our traditional system of medicine to cure various diseases (Fig. 1).

Table 1: List of the certain Indian medicinal plants and their uses

Botanical name	Common name	Family	Part	Uses <sup>(Glossary of Indian Medicinal Plants, 1992)</sup>
<i>Arnebia nobilis</i>	Ratanjot	Boraginaceae	Roots	Used as Anthelmintic, in disease of eye, bronchitis, abdominal pain, itch, fever, wounds and eruptions.
<i>Garcinia indica</i>	Kokum	Clusiaceae	Fruit aril	Anthelmintic, cardiogenic, useful in piles, dysentery, tumor, pains and heart complaints
<i>Boerhavia diffusa</i>	Punarnava	Nyctaginaceae	Whole plant	Used for oedema and ascites resulting from cirrhosis of liver and chronic peritonitis., relieve asthma, abdominal tumor and cancer, anti-inflammatory,
<i>Solanum albicaule</i>	Naharkanta	Solanaceae	Whole plant	
<i>Vitex negundu</i>	Nirgundi	Lamiaceae	Leaves	Possess discutient properties and applied to rheumatic swelling of joints and in sprains
<i>Bunium persicum</i>	Kalajeera	Apiaceae	Fruit	Paste applied over snake bite
<i>Acacia concinna</i>	Shikakai	Fabaceae	Fruit	Used as Natural shampoos or hair powders, fruits used for saponins-hormonal effect, leading to its use for contraceptive purposes
<i>Albizia lebbek</i>	Sherish chal	Fabaceae	Bark	Used as astringent, treat boils, cough, eye flu, lung problem, tonic, abdominal tumors and anti inflammatory

Table 2: Antimicrobial efficacy in terms of inhibition zone and activity index of certain Indian Medicinal Plants against selected test bacteria and fungi where tetracycline is used as standard

Ethanol extract	Measures	<i>Pseudomonas</i>	<i>Staphylococcus</i>	<i>Escherichia</i>	<i>Staphylococcus</i>	<i>Candida</i>
		<i>aeruginosa</i>	<i>aureus</i> +ve	<i>coli</i>	<i>aureus</i> -ve	<i>albicans</i>
	Standard IZ	27.00	29.00	28.00	26.00	30.00
<i>Arnebia nobilis</i>	IZ (mm)	0.00	10.00	7.00	0.00	9.00
	AI	0.00	0.34	0.25	0.00	0.30
<i>Garcinia indica</i>	IZ (mm)	23.00	0.00	12.00	23.00	9.00
	AI	0.85	0.00	0.42	0.88	0.30
<i>Boerhavia diffusa</i>	IZ (mm)	11.00	10.00	10.00	12.00	12.00
	AI	0.40	0.34	0.35	0.46	0.40
<i>Solanum albicaule</i>	IZ (mm)	11.00	15.00	0.00	9.00	14.00
	AI	0.40	0.51	0.00	0.34	0.46
<i>Vitex nigundu</i>	IZ (mm)	10.00	18.00	10.00	0.00	0.00
	AI	0.37	0.62	0.35	0.00	0.00
<i>Bunium persicum</i>	IZ (mm)	10.00	11.00	14.00	0.00	15.00
	AI	0.37	0.37	0.50	0.00	0.50
<i>Acacia concinna</i>	IZ (mm)	18.00	14.00	18.00	6.00	8.00
	AI	0.66	0.48	0.64	0.23	0.26
<i>Albizia lebbek</i>	IZ (mm)	13.00	10.00	10.00	6.00	10.00
	AI	0.48	0.34	0.35	0.23	0.33

IZ = Inhibition zone, AI = Activity index Standard = tetracycline

While screening of ethanolic extract of Ratanjot the antibacterial activity against selected test bacteria showing very good inhibitions zones. Ethanolic extract have the potentials to make inhibition zone against *Staphylococcus* positive strain (IZ = 10 mm); *E. coli* (IZ = 7 mm) and activity against fungi the inhibition zone against *Candida albicans* is (IZ = 9 mm). These results showed that the given test extracts have maximum activity against *S. aureus* and minimum against *E. coli* and nil activity against *Pseudomonas aeruginosa* and *staphylococcus negative strain*, whereas ethanolic extract of *G. indica* inhibition zone against *Pseudomonas aeruginosa* (IZ = 23mm); *E. coli* (IZ = 12 mm) and *Staphylococcus aureus* negative strain (IZ = 23mm) and no activity as

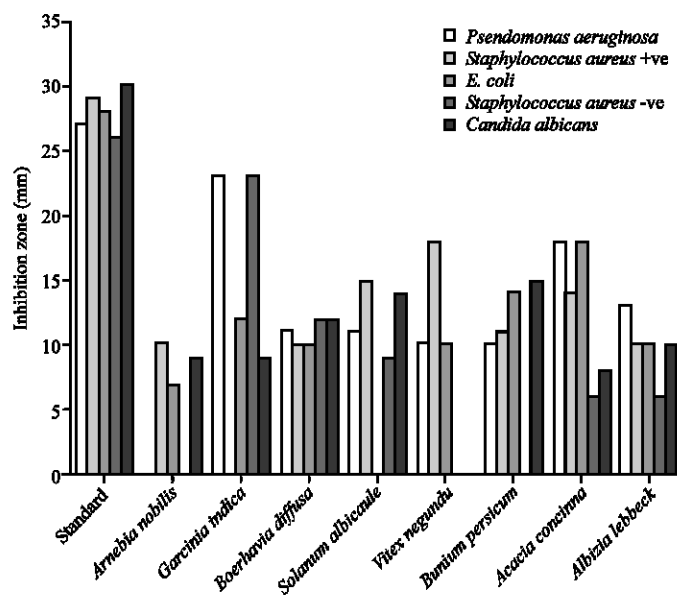


Fig. 1: Antimicrobial potentials of certain Indian Medicinal Plants against selected microorganisms in terms of inhibition zone (mm)

antifungal agent. These results showed that the given test extracts have maximum activity against *P. aeruginosa* and minimum against *E. coli* and nil activity against *Candida albicans* and staphylococcus positive strain.

While screening of ethanolic extract of *Solanum albicaule* showed efficacy against *Pseudomonas aeruginosa* (IZ = 11 mm), *Staphylococcus* positive strain (IZ = 15 mm) and *Staphylococcus aureus* negative strain (IZ = 9 mm) and in anti-fungal activity the inhibition zone against *Candida albicans* is (IZ = 14). These results showed that the given test extracts have maximum activity against *Staphylococcus aureus* positive strain and minimum against *S. aureus* negative and nil activity against *E. coli*.

Ethanolic extract of *Boerhavia diffusa* have inhibition zones against *Pseudomonas aeruginosa* (IZ = 11 mm), *Staphylococcus* positive strain (IZ = 10 mm); *E. coli* (IZ = 10 mm), *Staphylococcus aureus* negative strain (IZ = 12 mm) and in antifungal activity the inhibition zone against *Candida albicans* is (IZ = 12). These results showed that the given test extracts have maximum activity against *S. aureus* negative and minimum against *Staphylococcus* Positive Strain and *E. coli*. It is worth mentioning here that *B. diffusa* has the potentials more or less against all the selected test bacteria and fungi.

*Vitex negundu* ethanolic extract showed inhibition zone against *Pseudomonas aeruginosa* (IZ = 10 mm), *Staphylococcus* positive strain (IZ = 18 mm) and *E. coli* (IZ = 10 mm) whereas in antifungal activity the inhibition zone against the *Candida albicans* was nil. These result showed that the given test extract have maximum activity against *Staphylococcus aureus* positive and minimum against *Pseudomonas aeruginosa* and *E. coli* and nil against *Candida albicans*.

*Bunium persicum* ethanolic extract showed inhibition zone against *Pseudomonas aeruginosa* (IZ = 10 mm), *Staphylococcus aureus* positive strain (IZ = 11 mm), *E. coli* (IZ = 14 mm) and in antifungal activity the inhibition zone against *Candida albicans* is (IZ = 15 mm). These result showed that the given test extract have maximum activity against *E. coli* and *Candida albicans* and minimum against *Pseudomonas aeruginosa*.

*Acacia concinna* ethanolic extract possessed inhibition zone ethanolic extract have the potentials to make inhibition zone against *Pseudomonas aeruginosa* (IZ = 18 mm), *Staphylococcus positive* strain (IZ = 14 mm), *E. coli* (IZ = 18 mm) and in antifungal activity the inhibition zone against the *Candida albicans* is (IZ = 8 mm). These result showed that the given test extract have maximum activity against *Pseudomonas aeruginosa* and *E. coli* and minimum against *Candida albicans*. Albizzia lebbeck ethanolic extract have the potentials to make inhibition zone against *Pseudomonas aeruginosa* (IZ = 13 mm), *Staphylococcus positive* strain (IZ = 10 mm), *E. coli* (IZ = 10 mm) and in antifungal activity the inhibition zone against the *Candida albicans* is (IZ = 10 mm). These result showed that the given test extract have maximum activity against *Pseudomonas aeruginosa*.

## DISCUSSION

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance (Okeke *et al.*, 2005). Use of ethnopharmacological knowledge is one attractive way to reduce empiricism and enhance the probability of success in new drug-finding efforts (Patwardhan, 2005). Validation and selection of primary screening assays are pivotal to guarantee sound selection of extracts or molecules with relevant pharmacological action and worthy following up (Cos *et al.*, 2006).. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection (Graybill, 1988; Ng, 1994; Dean and Burchard, 1996; Gonzalez *et al.*, 1996). In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections (Sieradzki *et al.*, 1999a).

The present results therefore offer a scientific basis for traditional use of ethanolic extracts *Arnebia nobilis*, *Garcinia indica*, *Boehavia diffusa*, *Solanum albicaule*, *Vitex negundu*, *Bunium persicum*, *Acacia concinna* and *Albizia lebbeck*. These results explain that *G. indica* is showing potential antimicrobial activity against *S. aureus* negative can be used as a very good treatment for acne if added to daily diet and *V. negundu* showed a potentials against *S. aureus* positive. Further, *B. diffusa*, *A. concinna* and *A. lebbeck* have also possessed antimicrobial potentials against all test bacteria and fungi which explains that their use in daily life will generate a resistant or immunity to fight against microorganisms.

Ethanolic extracts of certain Indian Medicinal Plants showed promising antimicrobial potentials against selected test bacteria and fungi. The main aim of these studies is to validate and authenticate the antimicrobail potentials of certain plants and simultaneously, justify their use in the daily diet to cure mankind from certain ailments.

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