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SCREENING OF INDIAN MEDICINAL PLANTS AND THEIR POTENTIALS AS ANTIMICROBIAL AGENTS

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Screening of Indian Medicinal Plants and their potentials as Antimicrobial Agents

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Abstract-Ethanol extracts of certain Indian Medicinal Plants Curculigo orchioides. Symplocos racemosa. Pueraria tuberosa, Scindapsus officinarum, Luffa acutangula and Acacia nilotica 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 ethno-medicinal 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 Curculigo orchioides, Symplocos racemosa, Pueraria tuberosa, Scindapsus officinarum, Luffa acutangula and Acacia nilotica. These results explain that certain plants showed potential antimicrobial activity against S. aureus negative can be used as a very good treatment for acne if added to daily diet. Further, almost all the selected plants 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.

I. INTRODUCTION

A sture has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Ayurveda is ancient health care system and is practiced widely in India, Srilanka and other countries (Chopra and Doiphode, 2002). Ayurveda system of medicine use plants to cure the ailments and diseases. Despite the availability of different approaches for the discovery of therapeutically, natural products still remain as one of the best reservoir of new structural types. They are used directly as therapeutic agents, as well as starting material for the synthesis of drugs or as models for pharmacologically active compounds (Cowan, 1999). In modern time plants have been sources of analgesics, anti-inflammatory, anti-neoplastic drugs, medicine for asthma, anti arrhythmic agents and anti hypertensive.

Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Shariff, 2001).

In last three decades numbers of new antibiotics have produced, but clinical efficacy of these existing antibiotics is being threatened by the emergence of multi drug resistant pathogens (Bandow et al., 2003). In general, bacteria have the genetic ability to transmit and acquire resistance to drugs (Cohen, 1992). According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs (Santos et al., 1995).

Antibiotic resistance has become a global concern (Westh et al., 2004). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants. Search for new antibacterial agents should be continued by screening many plant families. Recent work revealed the potential of several herbs as sources of drugs (lwu, 2002). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003).

Numerous studies have identified compounds within herbal plants that are effective antibiotics. Traditional healing systems around the world that utilize herbal remedies are an important source for the

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discovery of new antibiotics. Some traditional remedies have already produced compounds that are effective against antibiotic resistant strains of bacteria (Kone et al., 2004). The results of this indicate the need for further research into traditional health system. It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity. The need of the hour is to screen a number of medicinal plants for promising biological activity.

Therefore, in present project attempts have been made to six medicinal plants Curculigo orchioides, Symplocos racemosa, Pueraria tuberosa, Scindapsus officinarum, Luffa acutangula and Acacia nilotica each belonging to different families were evaluated for antibacterial potentials. 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.









Luffa acutangula F tuberosa Aca Orchioides

a Picrorhizza kurroa Acacia nilotica Pueraria Curculigo

II. MATERIALS AND METHODS

Collection: Plant samples (Curculigo orchioides, Symplocos racemosa, Pueraria tuberosa, Scindapsus officinarum, Luffa acutangula and Acacia nilotica) 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 Curculigo orchioides, Symplocos racemosa, Pueraria tuberosa, Scindapsus

officinarum, Luffa acutangula and Acacia nilotica 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⁻¹ 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 4 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).

III. RESULTS

The profile of six medicinal plants used in present investigation. The results of antimicrobial activity of the crude extracts of Selected Indian Medicinal Plants

(Curculigo orchioides, Symplocos racemosa, Pueraria tuberosa, Scindapsus officinarum, Luffa acutangula and Acacia nilotica) showed good antimicrobial activity against selected test bacteria and intermediate against fungi (Table 1). 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: 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

Ethanolic extract	Measures	Pseudomonas aeruginosa	Escherichia coli	S. aureus positive	S. aureus negative	Candida albicans
	Standard IZ	27	28	29	26	30
Curculigo archioides	IZ (mm)	12	11	10	8	11
	AI	0.63	0.647	0.45	0.347	0.5
Symplocos racemosa	IZ (mm)	10	11	10	10	9
	AI	0.526	0.647	0.45	0.43	0.33
Puraria tuberosa	IZ (mm)	7	10	13	12	9
	AI	0.368	0.35	0.59	0.521	0.33
Scindapsus officinarum	IZ (mm)	10	12	8	9	10
	AI	0.526	0.705	0.363	0.391	0.45
Luffa acutangula	IZ (mm)	10	9	8	8	8
	AI	0.526	0.34	0.363	0.347	0.266
Acacia nilotica	IZ (mm)	7	19	11	11	8
	AI	0.368	0.67	0.37	0.42	0.266

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

While screening of ethanolic extract of Curculigo orchioides the antibacterial activity against selected test bacteria showing good inhibition zone. The ethanolic extract have the potential to make inhibition zone against Pseudomonas aeruginosa (IZ=12mm), GPB (IZ=10mm), Staphylococcus aureus positive strain (IZ=10mm), E.coli (IZ=11mm), Staphylococcus aureus negative strain (IZ=8mm) & in antifungal activity the inhibition zone against Candida albicans is11 mm. These results showed that the given test extracts have maximum activity against Pseudomonas aeruginosa & minimum against Staphylococcus aureus negative strain. While screening of ethanolic extract of Symplocos racemosa the antibacterial activity against selected test bacteria showing good inhibition zone. The ethanolic extract have the potential to make inhibition zone Pseudomonas aeruginosa (IZ=10mm), against GPB(IZ=10mm), Staphylococcus aureus positive strain

(IZ=10mm), *E. coli* (IZ=11mm), *Staphylococcus aureus negative strain* (IZ=10mm) & in antifungal activity the inhibition zone against *Candida albicans* is 9mm. These results showed that the given test extracts have maximum activity against *E.coli* & minimum against *Candida albicans*.

While screening of ethanolic extract of *Pueraria tuberosa* the antibacterial activity against selected test bacteria showing very good inhibition zone. The ethanolic extract have the potential to make inhibition zone against *Pseudomonas aeruginosa* (IZ=7mm), *GPB*(IZ=8mm), *Staphylococcus aureus positive strain* (IZ=13mm), *E. coli* (IZ=1mm), *Staphylococcus aureus negative strain* (IZ=12mm) & in antifungal activity the inhibition zone against *Candida albicans* is 9mm. These results showed that the given test extracts have maximum activity against *Staphylococcus aureus positive strain* & minimum against *E. coli*. While screening of ethanolic extract of *Scindapsus officinarum* the antibacterial activity against selected test bacteria showing good inhibition zone. The ethanolic extract have the potential to make inhibition zone against *Pseudomonas aeruginosa* (IZ=10mm),

GPB (IZ=9mm), *Staphylococcus aureus* positive strain (IZ=8mm), *E. coli* (IZ=12mm), *Staphylococcus aureus* negative strain (IZ=9mm) & in antifungal activity the inhibition zone against *Candida albicans* is10mm.



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

These results showed that the given test extracts have maximum activity against E. coli & minimum against Staphylococcus aureus positive strain. While screening of ethanolic extract of Luffa acutangula the antibacterial activity against selected test bacteria showing good inhibition zone. The methanolic extract have the potential to make inhibition zone against Pseudomonas aeruginosa (IZ=10mm), GPB(IZ=8mm), Staphylococcus aureus positive strain (IZ=8mm), E. coli (IZ=9mm), Staphylococcus aureus negative strain (IZ=8mm) & in antifungal activity the inhibition zone against Candida albicans is 8mm. These results showed that the given test extracts have maximum activity against Pseudomonas aeruginosa & minimum against), GPB. Staphylococcus positive aureus strain, Staphylococcus aureus negative strain Candida albicans While screening of ethanolic extract of Acacia nilotica the antibacterial activity against selected test bacteria showing very good inhibition zone. The ethanolic extract have the potential to make inhibition zone against *Pseudomonas aeruginosa* (IZ=7mm), GPB(IZ=1mm), Staphylococcus aureus positive strain (IZ=11mm), E. coli (IZ=19mm), Staphylococcus aureus negative strain (IZ=11mm) & in antifungal activity the inhibition zone against Candida albicans is 8mm. These results showed that the given test extracts have maximum activity against E. coli & minimum against **GPB**

IV. DISCUSSION

Use of ethno-pharmacological 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). 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 infectionfighting strategies to control microbial infections.

The present results therefore offer a scientific basis for traditional use of ethanolic extracts *Curculigo* orchioides, *Symplocos racemosa, Pueraria tuberosa, Scindapsus officinarum, Luffa acutangula and Acacia nilotica.* These results explain that Indian Medicinal Plants have potential as antimicrobials against *S. aureus* negative can be used as a very good treatment for acne if added to daily diet and some other showed potentials

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against *S. aureus* positive. Further, more or less all the selected Indian Medicinal Plants 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 antimicrobial potentials of certain plants and simultaneously, justify their use in the daily diet to cure mankind from certain ailments.

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