Research Article

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ANTIBACTERIAL ACTIVITIES OF ETHANOLIC EXTRACTS OF PLANTS USED IN FOLK MEDICINE

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Received: 05-10-2010; Revised: 21-11-2010; Accepted: 28-11-2010

ABSTRACT

The antibacterial activity of the ethanol and aqueous extracts of the leaves of *Achyranthes aspera*, *Alternanthera pungens, Cynodon dactylon, Lantana camara* and *Tagetes patula* was investigated against *Bacillus subtilis* (MTCC 441) , *Staphylococcus aureus* (MTCC 3160) and *Pseudomonas aeroginosa* (MTCC 4673), using agar diffusion technique. Results showed that the only ethanolic extracts of 4 plants species except *Alternanthera pungens* were effective against all the test microorganisms. The minimum inhibitory concentrations (MIC) of the extracts of ethanol were found to be 25 to 125 mg/ml, while the water based extracts showed no inhibition. The results of the study provide scientific basis for the use of the plant extract in the treatment of wounds and skin diseases. Therefore it is concluded that the active principles possessing antibacterial activity may be extracted from the leaves of *Achyranthes aspera*, *Cynodon dactylon, Lantana camara* and *Tagetes patula* by ethanol.

KEYWORDS: *Achyranthes aspera*, *Alternanthera pungens, Cynodon dactylon, Lantana camara, Tagetes patula,* MIC.

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INTRODUCTION

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants are found in "Rigveda", which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge[1]. In India, the use of different parts of several medicinal plants to cure specific ailments has been in vague from ancient times. The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries[2]. From over 3, 00,000 species of higher plants to occur in nature, only about 2 percent have been screened so far. Extract of plants from 157 families has been reported to be active against microorganisms[3]. Nowadays multiple drug resistance microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease[4]. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants[5]. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced in the market are obtained from natural or semi synthetic resources. It has been reported that Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases[6]. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs[7].There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases[8]. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections[9]. Green plants represent a reservoir of effective chemo therapeutic agent and can provide valuable sources of natural pesticides[10]. Bio pesticides have been suggested as an effective substitute for chemicals[11]. Reports are available on the use of several plant by-products, which posses' antimicrobial properties, on several pathogenic bacteria and fungi[12].

In the present study, five plants species are used *Achyranthes aspera*, *Alternanthera pungens, Cynodon dactylon, Lantana camara* and *Tagetes patula* are used to control several diseases derived from microbial infections. The gram positive bacterium such as *Bacillus subtilis and Staphylococcus aureus* are mainly responsible for post operative wound infections, toxic shock syndrome, endocarditis, and food poisoning. The gram negative bacterium such as *Pseudomonas aeroginosa* causes generalized inflammation and sepsis. The main objective of the present study was to investigate the effects of ethanolic extracts and aqueous extracts of five plants on *Bacillus subtilis*, *S. aureus* and *Pseudomonas aeroginosa.*

MATERIALS AND METHODS

Plant materials

All plant materials are collected from the ITM Universe campus, Gwalior, Madhya Pradesh in the month of March and April. The selected plants were identified taxonomically and authenticated by Department of Botany, IASCA, ITM Universe, Gwalior and these are identified as *Achyranthes aspera*, *Alternanthera pungens, Cynodon dactylon, Lantana camara* and *Tagetes patula.* Fresh leaves and shoots are collected and shade dried under maintained temperature. The dried leaves and shoots are grained into a coarse powder and used for further studies.

Microorganisms used

Bacterial strains are obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India and used to study the antibacterial activity of various extracts of leaves of *Achyranthes aspera*, *Alternanthera pungens, Lantana camara, Tagetes patula* and leaves and shoots of *Cynodon dactylon* on bacterial strain of *Bacillus subtilis* (MTCC 441)*, Staphylococcus aureus* (MTCC 3160) and *Pseudomonas aeroginosa* (MTCC 4673).

Preparation of the Inoculums

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of 50 ml Nutrient Broth for

bacteria were incubated with agitation for 24 hours at 37°C on an shaking incubator at 110 rpm. Each test organism suspension was subsequently stroken out on Nutrient Agar media and incubated at 37°C for 24 hours. A single colony was transferred to Nutrient Agar media slants and incubated at 37 °C for 24 hours. These stock cultures were kept at 4°C. For use in experiments a loop of each test organism was transferred in 50 ml Nutrient Broth, and incubated separately at 37°C for 18-20 hours.

Extraction

The plants powder (30 g) was extracted using 70% ethanol (100 ml) in shaking incubator for 24 hours at 37°C and 110 rpm. The ethanolic extracts were centrifuged at 10000×g for 30 minutes and filtered using a Whatman filter paper to obtain the clear extracts. Remaining ethanol in the crude extract was removed by heating the extract in to the hot air oven at 50°C for 24 hours.

Determination of the Antimicrobial Activity

MHA plates were prepared by pouring 20 ml of molten media into sterile petriplates. After solidification of media 20µl inoculum suspension was swabbed uniformly. The 6 mm sterile disc which was earlier dipped into the ethanolic extract was placed on the surface of medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

RESULTS

The effects of different extracts of five test plants on *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeroginosa* shown in **Tables 1, 2 & 3**. The results clearly showed that plant extracts were specific in action against the growth of bacteria. Ethanol extract was most effective followed by aqueous extract. *S. aureus* and *Bacillus subtilis* were more sensitive for ethanol extract of leaves of all the tested plants rather than aqueous extracts when compared with ethanolic extract. In the present study, five plant species were screened to detect the presence or absence of several bioactive compounds which are reported to cure different diseases and ailments.

It was confirmed that the presence of antimicrobial substances in all the tested plants in one form (or) other. The antimicrobial compounds may be found as *anthroquinone, alkaloids, catachols, flavonoids, phenolic compounds, saponins, steroids, tannins* and *triterpenoids*[13], whose presence may be attributed to the medicinal properties of plants. In the present study, *Bacillus subtilis* and *Staphylococcus aureus* was sensitive to all the four and subjected to extensive scientific and pharmacological screening that can be used as sources for new drugs.

DISCUSSION

Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials[14]. The results of antibacterial activity of the crude Ethanolic extracts of the different species were summarized in Table 1, 2, & 3. The ethanolic extracts of *Achyranthes aspera*, *Tagetes patula, Cynodon dactylon* materials only showed antibacterial activity against gram negative (*Pseudomonas aeroginosa)* and gram positive (*Bacillus subtilis* and *Staphylococcus aureus)* bacteria i.e. (70% of Ethanolic extracts). It shows that the Ethanolic extracts of *Achyranthes aspera*, *Tagetes patula, Cynodon dactylon* & *Lantana camara* contains active compounds which are either in the forms of proteins or in form of any other organic compounds, because only the ethanolic extracts has showed the antimicrobial properties rather than aqueous extracts. The *Lantana camara* extracts exhibited no activity against *S. aureus*[15] while the ethanolic as well as aqueous extracts of *Alternanthera pungens* extract showed no sign of antibacterial activity.

Only the Ethanolic extracts of 4 plants showed significant antibacterial activity. The exact nature and mode of action of the active constituents is quite obscure at this stage. Further work may however reveal whether these components act as intracellular bacterial enzyme inhibitor or impair the cell wall synthesizing system of the cell, or any other biological reaction impairment which causes cessation and/or inhibition of growth of bacterial cells.

ACKNOWLEDGEMENTS
The authors are grateful to Management and Director of the institute for providing facility and support during study. Our thanks are also due to faculty members of Botany and Microbiology department for providing plant taxonomic identification and authentication for the present investigation.

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Table 1: Effect of ethanolic extract of stems and leaf samples of certain medicinal plants on *Bacillus subtilis*

Name of Plant(s)	*Bacillus subtilis*	
	Zone of inhibition of ethanol extract (in cm)	Zone of inhibition of aqueous extract (in cm)
Ciprofloxacin (Positive control)	1.8	1.0
Negative Control	-	-
Achyranthes aspera	1.2	-
Alternanthera pungens	-	-
Cynodon dactylon	1.0	-
Lantana camara	0.7	-
Tagetes patula	1.0	-

Table 2: effect of ethanolic extract of stems and leaf samples of certain medicinal plants on *staphylococcus aureus*

Name of Plant(s)	*Staphylococcus aureus*	
	Zone of inhibition of ethanol extract (in cm)	Zone of inhibition of aqueous extract (in cm)
Ciprofloxacin (Positive control)	1.8	2.1
Negative Control	-	-
Achyranthes aspera	1.7	-
Alternanthera pungens	-	-
Cynodon dactylon	1.6	-
Lantana camara	-	-
Tagetes patula	1.0	-

Table 3: Effect of ethanolic extract of stems and leaf samples of certain medicinal plants on *Pseudomonas aeroginosa*

Name of Plant(s)	*Pseudomonas aeroginosa*	
	Zone of inhibition of ethanol extract (in cm)	Zone of inhibition of aqueous extract (in cm)
Ciprofloxacin (Positive control)	1.8	1.9
Negative Control	-	-
Achyranthes aspera	1.5	-
Alternanthera pungens	-	-
Cynodon dactylon	-	-
Lantana camara	1.2	-
Tagetes patula	1.4	-

Values are the average of at least three determinations

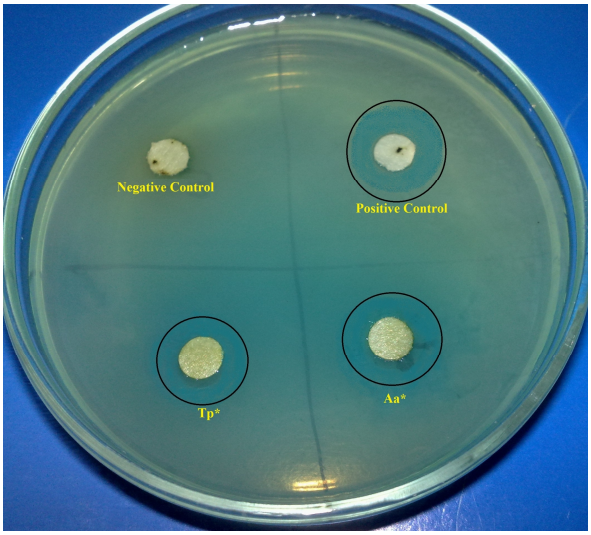

Figure 1: Showing ethanolic extract test of *Bacillus subtilis* on negative control (70% ethanol), positive control (ciprofloxacin), *Tegetes patula* and *Achyranthes aspera*.

Source of support: Nil, Conflict of interest: None Declared