ANTIMICROBIAL ACTIVITY OF SOME PLANT EXTRACTS USED BY BHIL AND BHILAL TRIBE FROM MADHYA PRADESH, INDIA

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Abstract: The antimicrobial activity of plant methanol extracts utilized for the treatment of a variety of diseases by various tribes of Bhil and Bhilal of Alirajpur. However, their effectiveness has not been tested so far. Therefore, the present study aimed to assess selected plant methanol extracts against six bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli and Pseudomonas fluorescense.* Minimal inhibitory concentrations (MIC) of the crude plant extracts were determined. Plants and their parts of medicinal use were collected from local forests on the basis of information obtained from the local traditional healers. The ethanolic extracts were obtained by cold percolation method and the antimicrobial activity was found using paper disc diffusion method. The selected microorganisms were purchased from commercial suppliers.
The results indicated that all the selected plants exhibited antimicrobial activity against one or more of the tested microorganisms at three different concentrations of 1.25, 2.5 and 5 mg/disc. Among the plants tested, **Acacia planifrons, Chlorophytum tuberosum and Polygala irregulgaris** were most active. In conclusion, the present study revealed that the selected plants could be used as potential sources of antimicrobial agents. However, further studies are required to isolate and identify the active components of these plant extracts for pharmacological evaluation.

Keywords: Antimicrobial Plants, Methanol Extracts, Tribal Plants.

Introduction: Even today 80 percent of the world's population still depends on traditional herbal medicine for their major health care needs [1].

Use of herbal medicines in Asia represents a long history of human interactions with the environment. Even in the modern era, the use of traditional herbal drugs and plant nutrients rapidly increasing [2]. Hence, the knowledge of plant parts and utilization against various diseases is still of great importance. The whole plant or their parts contain a variety of chemical substances like alkaloids, flavanoids, steroids, saponins, tannins and phenolic compounds [3], [4], [5]. As a consequence, past decade has noticeably witnessed a fabulous flow in acceptance and public interest in natural therapies which resulted the emergence of these herbal drugs not only in drug stores but also in food stores and supermarkets.

Therefore, it is crucial to set up the scientific basis for the beneficial actions of conventional plant drugs which may serve as the source for the expansion of more effective drugs. Many traditional plants have been utilized as traditional medicines by the tribe of Alirajpur [6], [7]. The research reports on anti-bacterial effects of local traditional herbs of Alirajpur (Madhya Pradesh, India) were limited. Therefore, it was aimed to find out antibacterial activity of ethanol extract 10 plant species which are prevalent in this area.

Material and Methods: Plant parts like, leaves, seeds, stem bark and/or root of medicinal plants were collected from in and around Alirajpur town (M.P.India). We selected 6 tribal plants known as **Acacia planifrons, Abutilon indicum, Chlorophytum tuberosum, Polygala irregularis, Psilotum nudum and Ceropegia odorata.**

Preparation of the Plant Extracts: The plant parts of medical importance were collected and were washed thoroughly under running tap water to remove the surface contaminants. All the plant parts were sun dried under shade for five days and powdered with help of a blender. The powdered samples were subjected to

ethanol extraction using soxhlet apparatus. The powdered material was made aqueous using distilled water and filtered. The obtained ethanol extracts were labelled was used for antibacterial tests.

Extraction: Plant samples (7– 15g) were extracted successively with methanol at room temperature with constant shaking on a shaker for two days (48 hours). The process was again repeated. On subsequent filtration of the suspension through Whatman filter paper No. 2 the crude alcohol extracts were evaporated at 40 °C and put in a vacuum oven to near dryness to yield the plant extract. The solvent extracts, were concentrated by applying reduced pressure and stored at 5°C in sealed bottle until further use.

Growth and Maintenance of Test Microorganism: Bacterial cultures of *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), *Pseudomonas fluorescens* (*P. fluorescens*) and *Staphylococcus aureus* (*S. aureus*) were obtained from the culture centre of Government Veternaray College, Mhow, India, and used as test organisms.

Preparation of Inoculums: The gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli* and *Pseudomonas fluorescens* were pre cultured in nutrient broth overnight in a revolving shaker at 37°C, centrifuged at 10,000 rpm for 5 min. The pellet obtained by the above process was suspended in double distilled water. The cell density was normalized spectrophotometrically at 610 nm.

Anti-Bacterial Activity: The methanol extracts of various plant parts of **Acacia planifrons, Abutilon indicum, Chlorophytum malabaricum, Chlorophytum tuberosum, Polygala irregularis, Psilotum nudum and Ceropegia odorata were** tested by the disc diffusion method [8]. Different concentration of the extracts (100 µg ml) was prepared by adding methanol. The test microorganisms were sown into relevant medium by spread plate method 10 µl (10 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification, the filter paper discs (5 mm in diameter) were soaked with the extracts and were placed on test organism plates. The assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in nm.

Results: Results of the present study clearly relieved that the selected six tribal plants extracts have shown potential antibacterial activity against *B. subtilis, E. coli, S. aureus, and P. fluorescens* (Table 1). The methanol leaf extracts of **Abutilon indicum** showed highest antibacterial activity of 20 mm in *B. subtilis* and least activity recorded in *Pseudomonas. fluorescense* measured 14 mm. But significant activity was also found against *E. coli and S. aureus* around 18 mm. The Bark extract of *Acacia* **planifrons** exhibited highest activity against *B. subtilis* and *S. aureus* (15 mm) and lowest in *P. fluorescens*. *The tuber* extracts of **Chlorophytum tuberosum, Polygala irregularis and Ceropegia odorata** posses maximum 18 mm activity against *B. subtilis* and *S. aureus* and the 16 mm activity against E. *coli* and *P. fluorescens*. But the root extract of **Chlorophytum tuberosum** have shown highest inhibitory activity against *B. subtilis* and *S. aureus* and least activity observed in *E. coli* compared to other plants. The aerial parts extraction of **Psilotum nudum** shown almost similar zone of inhibition against all the tested bacteria except, *S. aureus* which showed highest activity (17 mm).

Table.1. Antimicrobial Activity of some Medicinal Plant Methanol Extracts (100 µg Ml) and Antibiotic (10 µg Ml) Against Bacterial Species Tested by Disc Diffusion Method

		Bacteria Species (Zone of inhibition (mm)			
Plant Species	**Extraction**	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas. fluorescense***	***Staphylococcus aureus***
Abutilon indicum	Leaf	20±0.66	16±1.20	14±0.33	18±0.57
Acacia planifrons	Bark	17±0.66	20±1.20	15±0.33	18±0.57
Chlorophytum tuberosum	Tuber	18±0.66	13±1.20	14±0.33	18±0.57
Polygala irregulgaris	Root	19±0.66	14±1.20	13±0.33	17±0.57
Psilotum nudum	Aerial parts	14±0.66	12±1.20	13±0.33	17±0.57
Ceropegia odorata	**Tuber**	15±0.66	12±1.20	11±0.33	15±0.57

Values are mean inhibition zone (mm) ± S.D of three replicates

Among the above six plants namely, **Acacia planifrons, Abutilon indicum, Chlorophytum malabaricum, Chlorophytum tuberosum** and *Psilotum* **nudum**, tuber and root extracts showed significant antibacterial activity against all the experimental bacteria. Tuber extracts have shown significant activity when compared with the extracts other plant parts.

Discussion: Plants form an important resource for the production of new chemotherapeutic drugs. The number of diseases and disorders are increasing day by day and prices spent on medicines are also increasing. Hence the restoration of tribal medicine is very important to combat the cost and effective therapy. The knowledge of plants has the potential for isolation of safe and effective drugs and for sustainable utilization of medicinal plant genetic resources and their conservation [7]. The first step towards this goal is to find out their potential in antibacterial activity [9]. Several researchers reported antimicrobial, anthelmintic and anti-inflammatory properties of various plants [10], [11], [12], [13], [14], [15], and [16]. Many these results have facilitated in identifying the accurate principle in the development of new drugs for the therapeutic use for human beings. On the contrary, only few reports are available on the development of antifungal or antibacterial property of plants for developing accurate formulations in crop protection.

In the present study, the methanol extracts of all the selected plant products **shown the highest activity against B.subtilis, E.coli, P.fluorescens, and S.aureus which thus** effectively proven **the use of methanol extracts of these plants** for their utilization as source for antimicrobial compounds.

Tepe, B et al [17] evaluated methanolic extracts of *Salvia cryptantha* and *Salvia multicaulis* for their potential antimicrobial and anti oxidative activities. The results confirmed antimicrobial and anti oxidative activities abilities of tested plants. They also recommended the use of methanolic extracts of tested plants for antimicrobial and antioxidative agents in the food industry.

For instance, methanol extracts of *Salvadora persica* L. (Salvadoraceae), *Colophospermum mopane* (J.Kirk ex Benth.) J. Léonard (Leguminosae) and *Dichrostachys cinerea* (L.) Wight & Arn. (Leguminosae) crude extracts were exhibited potential antibacterial activity against clinical isolates of *Staphylococcus aureus,* and *Escherichia coli* [18] (Mudzengi, C.P., *et al, 2017).*

Parekh, J.,et al (2006) [19] tested both methanol and aqueous extracts of 12 plants against five bacterial strains for potential antibacterial activity against 5 medically important bacterial strains, namely Bacillus subtilis, Staphylococcus epidermidis, Pseudomonas pseudoalcaligenes, Proteus vulgaris and Salmonella typhimurium. They found that methanol extracts are more active than the aqueous extracts.

Biswas, B., et al [20] investigated the antimicrobial efficacy of guava (Psidium guajava) leaf extracts against two gram-negative bacteria (Escherichia coli and Salmonella enteritidis) and two gram-positive bacteria (Staphylococcus aureus and Bacillus cereus). Their findings revealed that the both methanol and ethanol leaf extracts showed inhibitory activity against gram-positive bacteria, while the gram-negative bacteria resistance to all the solvent extracts.

The present study exhibits the antimicrobial potential of six selected plant extracts by using methanol solvents. The results clearly point out that the antibacterial activity varies with the species of the plants and plant material used. Thus, the study establishes the value of plants used in medicine, which could be of considerable interest to the development of new drugs. Further, this work provides a scientific perception and recommends for further studies to isolate and identify the active components of these plant extracts for pharmacological evaluation.

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