Antimicrobial activity of *Crotalaria burhia* Buch.-Ham. root

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*Crotalaria burhia* Buch.-Ham. (Family- Fabaceae) is used traditionally in the treatment of eczema. Petroleum ether, chloroform, methanol and aqueous extracts of root were screened for antimicrobial activity against different strains of bacteria and fungi. The tests were carried out using agar disc diffusion method at four different concentrations of the crude extracts. All the extracts inhibited the growth of both Gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus*) and Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium*). The Gram positive bacteria appeared to be more susceptible to the extracts than the Gram negative bacteria. Methanol extract at the concentration ranging between 250 µg/ml and 1000 µg/ml showed inhibitory activity against all bacteria while petroleum ether and chloroform extract showed moderate antimicrobial activity. Aqueous extract was found neutral against all bacteria. The extracts also showed significant antifungal activity against *A. niger* and *C. albicans*. All the microorganisms showed dose dependent susceptibility towards the extracts used in the study. The antibacterial and antifungal activity of the extracts and standard drugs were statistically significant. The results indicate the potential of *C. burhia* root in treating microbial infections, thus, justifying their traditional use in the treatment of eczema, which are of infectious origin.

**Keywords:** *Crotalaria burhia*, Fabaceae, Eczema, Antimicrobial, Antibacterial, Antifungal.

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**Introduction**

Medicinal plants have long been the subject of human curiosity and need. Plant-derived products are present in 14 of the 15 therapeutic categories of pharmaceutical preparations that are currently recommended by medical practitioners and they form an important part of the health-care system in the western world. It is estimated that, there are about 2,50,000 species of higher plants and the majority of these have not been evaluated scientifically in detail for their pharmacological activities. The antimicrobial properties of certain Indian medicinal plants were reported based on folklore information and a few attempts have been made on inhibitory activity against certain pathogenic bacteria and fungi. Infectious diseases, particularly skin and mucosal infections are common in most of the tribal inhabitants due to lack of sanitation, potable water and awareness of hygienic food habits. An important group of these skin pathogens are the fungi, among which dermatophytes and *Candida* spp., besides certain pathogenic bacteria are the most frequent. Furthermore, in the last few years, the numbers of immunosuppressed and immunocompromised patients, who frequently develop opportunistic systemic and superficial mycoses such as candidiasis, dermato-mycosis, fungal infections, etc., have increased dramatically. This is mainly due to the non-availability of effective antifungal drugs for systemic fungal infections and toxicity of available drugs like amphotericin-B. Thus there is an increased need for the development of alternative antipathogenic substances. One possible approach is to screen local medicinal plants in search of suitable chemotherapeutic antibacterial and antifungal substances. The herbalists prescribed various preparations of medicinal plants in treating ailments such as itching, eczema, scabies and skin diseases.

*Crotalaria burhia* Buch.-Ham. (Family-Fabaceae), commonly known as *Khip* is an undershrub, fibrous plant, found all over the desert extensively growing on sand dunes, common in the arid parts of West Pakistan, India (Punjab, Rajasthan and Gujarat) and Afghanistan. Fresh plant juice is applied on swelling. It is very useful in gout, hydrophobia and swelling. Studies have shown that the plant possesses anti-cancer property and roots are good coolant.

The present study was conducted to investigate antimicrobial properties of different extracts of its root against wide range of bacterial and fungal species.
Materials and Methods

Plant material
Root was collected during February-March 2009 from Jaipur, Rajasthan, India. The plant material was authenticated by the Joint Director, Botanical Survey of India (BSI), Jodhpur (Rajasthan, India) and the voucher specimen (JNU/JPR/PC/SK-1) has been deposited in their herbarium.

The shade dried powdered root was successively extracted with petroleum ether, chloroform and methanol using Soxhlet apparatus. The extracts were filtered and evaporated to dryness in vacuo at 40°C. Finally, the marc left was extracted with water under reflux. The water phase was filtered and freeze-dried. Percentage yield of the extracts are present in results section. The dried extracts were dissolved in dimethyl sulfoxide and used for the present study.

Microorganisms and media
Gram-positive bacteria: Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus
Gram-negative bacteria: Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium
Fungi: Aspergillus niger, Candida albicans.

Bacteria and fungi were procured from the stock cultures of the Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial and fungal stock cultures were maintained on Muller Hinton agar and Sabouraud-dextrose agar slants, respectively, which were stored at 4°C. Eight microorganisms maintained on nutrient agar base were used to assess the antimicrobial activity of the plant extracts. The fungi were maintained on Sabouraud-dextrose agar, which is often used with antibiotics for the isolation of pathogenic fungi.

Antimicrobial screening
Agar cultures of the test microorganisms were prepared as described by Mackeen et al. Three to five similar colonies were selected and transferred to 5 ml broth with a loop and the broth cultures were incubated for 24 h at 37°C. The extracts were dissolved in dimethyl sulfoxide with a magnetic stirrer. For screening, sterile 6 mm diam. filter paper discs were impregnated with 100-1000 µg of different extracts and then placed in Muller Hinton agar medium. The inoculums for each organism were prepared from broth cultures. The concentration of culture was 1×10^5 colony forming units/ml. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicate antimicrobial activity. All data are the average of quadrato analysis. Antibiotic Amikacin (10 µg/ml) and Griseofulvin (20 µg/ml) were used as reference standards as recommended by the National Committee for clinical laboratory standards.

Statistical analysis
Data are presented as the mean ± SD of four measurements. Statistical analysis was performed by student’s t-test.

Results and Discussion
The percentage yield of the petroleum ether, chloroform, methanol and aqueous extracts after successive extraction of root powder were 0.30, 0.20, 1.92, 2.00%, respectively. It was observed that all the extracts showed (Table 1) antibacterial activity against all the microorganisms. However, no activity was observed against M. luteus, E. coli, P. aeruginosa and S. typhimurium at 100 and 250 µg/ml concentration. It was also observed that all the extracts exhibited (Table 2) antifungal activity against A. niger and C. albicans in a dose dependent manner. These extracts showed a broad spectrum of activity against the bacterial strains at the concentration of 100-1000 µg/ml. Amikacin (10 µg/ml) and Griseofulvin (20 µg/ml) were used as positive controls for bacteria and fungi, respectively.

The preliminary phytochemical screening of C. burhia root indicated the presence of the group compounds such as alkaloids, phenols, polyphenols, saponins, tannins, triterpenes, anthraquinones, flavonoids and steroids. Many compounds belonging to these secondary metabolite groups have been reported to their antimicrobial activities.

In general, among the tested microbial strains, bacteria were found to be more sensitive to many of the test agents than fungi. The antibacterial activity was more pronounced on the Gram-positive bacteria (Staphylococcus aureus) than the Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa). The reason for the difference in sensitivity between Gram-positive and Gram-negative bacteria might be ascribed to the differences in morphological constitutions between these microorganisms, Gram-negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances. The Gram-positive bacteria on the other hand are more susceptible having only an outer peptidoglycan layer which is not an.
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Effective permeability barrier. Therefore, the cell walls of Gram-negative microorganisms, which are more complex than the Gram-positive ones, act as a diffusion barrier and making them less susceptible to the antimicrobial agents than are Gram-positive bacteria.

Conclusion

C. burhia root investigated possesses activity against at least one strain of bacteria and/or fungi. The extensive use of this herbal drug by the local people in treating various types of skin disorders like eczema might therefore, be justified by their antimicrobial activities against different strains of bacteria and fungi, which are known to be responsible for causing various skin infections. The results also indicate that scientific studies carried out on root having traditional claims of effectiveness might warrant fruitful results. The antimicrobial activity may be due to the presence of active principles like alkaloids and polyphenols present in the root. Further studies are needed to isolate active component to develop new natural drugs for infectious diseases. It is believed that screening of root for other biological activities including anti-inflammatory, wound healing and antioxidant activities are essential.

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