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Antimicrobial potential of *Alhagi maurorum* against clinically important microbes

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Abstract

Alhagi maurorum Medic (Fabaceae) is the single species of genus Alhagi found in India. Various studies have shown A. maurorum used in folk medicines. However, its potential health benefits have not been studied in details. Methanol extracts of A. maurorum from the aerial part were screened for antimicrobial. Antimicrobial activities were tested against eight microorganisms using well diffusion method. In the present study, antimicrobial activity of leaves and stem extracts of Alhagi maurorum was evaluated against four fungus Aspergillus niger, Penicillium funiculosum, Fusarium oxysporum and Trichoderma ressei and four bacterial strains Streptomyces grisveus, Staphylococcus aureus, Bacillus subtilis and Escherichia coli. It was observed that maximum zone of inhibition was in leaves (18mm) against Streptomyces grisveus while rest of the strains were found to be resistant. In case of fungus, maximum zone was observed in stem against Trichoderma ressei and Fusarium oxysporium (14mm) while other fungal colonies were found to be resistant.

Keywords: Alhagi maurorum, antibacterial, anti fungal, phytochemical analysis, agar well diffusion, zone of inhibition

Introduction

Natural plant products important role play in various functions, and how much of them have useful biological activities ^[1]. According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. *Alhagi maurorum* Boiss is customarily used in folk medicine as a remedy for rheumatic pains. Oil from the leaves of the plant is used for the treatment of rheumatism while the flowers of the plant are used for the treatment of piles migraine and warts ^[2] including liver disorders and for various types of gastrointestinal discomfort ^[3] but there is no scientific background that supports this use. The aim of the present work was to study the effect of antimicrobial activity of methanolic extracts of *Alhagi maurorum*.

Herbal medicines have made large contributions to human healthiness ^[4], and provided a good source of anti infective agents in the fight against microbial infections. Phytoremedies have also shown great promise in the treatment of intractable infectious diseases ^[5]. Antimicrobial chemotherapy made remarkable advances, resulting in the overly optimistic view that infectious diseases would be conquered in the near future. However, in reality, emerging and re-emerging. Since the advent of new mighty drugs is highly difficult, the proper use of currently available antimicrobial agents as well as efforts to minimize the spread of resistant bacteria through appropriate infection control would be quite important and may represent a first step in solving the issue of resistant microorganisms ^[6].

Material and methods

Experimental section

Collection of Plant material was authenticated by Department of Botany, University of Rajasthan. A. maurorum was

collected from Ajmer in Rajasthan (India). Preparation of the crude methanol extract was obtained by macerating 10 gm of dried plant powder in 95% methanol and kept on a rotary shaker for 24 hr. The extract was filtered, and will be dried. The extract will be stored at 4° C in airtight bottles. The dried extracts were dissolved in dimethyl sulfoxide to make the final concentration which kept in refrigerator till used.

The extracts were filtered, dried and then stored at 4^{0} C in airtight bottles.

Collection of Plant

The plant *Alhagi marorum* has been collected from Ajmer in Rajasthan in India. The stem and leaves of the plant washed in tap water and finally were made to shade dried.

Microorganisms Used

Clinical laboratory bacterial isolates of *Streptomyces grisveus*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* and fungal isolates viz. *Aspergillus Niger*, *Fusarium oxysporium*, *Penicillium funiculosum* and *Trichoderma reesei* were collected from the stock cultures of Microbiology Laboratory, SMS Medical College Jaipur, and India.

Preparation of Extract

The crude methanolic extract obtained by macerating 30 g of dried plant powder in 95% methanol and kept on a rotary shaker for 24 h. The extract was filtered, centrifuged at 5000 g for 15 min. and dried under reduced pressure then stored at 4°C in airtight bottles.

Culture and Maintenance of Bacteria

The bacteria were grown in Nutrient agar medium (prepared by autoclaving 8% Nutrient Agar, in distilled water at 15 lbs

psi for 25-30 min) and incubating at 37°C for 48 h. Each bacterial culture was further maintained on the same medium after every 48 h of transferring. Fresh suspension of test organism in saline solution was prepared from a freshly grown agar slant before every antimicrobial assay.

Determination of Antibacterial Assay

In vitro antibacterial activity of the crude methanol extract was studied against gram positive and gram negative bacterial strains by the agar well diffusion method ^[7]. The extracts were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations of 5 mg/mL. Wells (about 6mm in depth) were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (6 mm). The plates were incubated overnight at 37°C. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, streptomycin. For each bacterial strain controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed three times to minimize the error and the mean values were presented.

Determination of Antifungal Assay

Anti fungal activity of the experimental plant was investigated by agar well diffusion method ^[8]. The fungi were subculture onto Potato dextrose agar, PDA (Merck, Germany) and respectively incubated at 37°C for 24 h and 25°C for 2 - 5 days. Suspensions of fungal spores were prepared in sterile water and adjusted to a concentration of 106 cells/ml. The plates were dried at room temperature for 15 min. Wells of 6 mm in diameter were punctured in the culture media using sterile glass tube. 0.1 ml of several dilutions of fresh extracts was administered to fullness for each well. Plates were incubated at 37°C. After incubation of 24 h bioactivities were determined by measuring the diameter of inhibition zone (in mm). All experiments were made in triplicate and means were calculated.

Result and discussion

Plants synthesize variety of phytochemicals as part of their normal metabolic activities. Chemical profile of a single plant may vary over a time, as it reacts to changing conditions. In 2010 a survey of 1000 plants was done out of which, 156 clinical trials for evaluation of their pharmacological activities and therapeutic applications gave encouraging results ^[9]. This led to the new search for drugs and dietary supplements derived from plants. During the last 10 years pace of development of new antimicrobial drugs has slowed down, while prevalence of resistance has increased multifold ^[10]. The problem of microbial resistance of growing and outlook for the use of antimicrobial drugs in future is still uncertain therefore, action must be taken to reduce this problem, such as controlling the use of antibiotics and carrying out research for better understanding of genetic mechanism of resistance. This prompted to evaluate plants as source of potential chemotherapeutic and antimicrobial agent along with their ethnomedicinal use [11].

In the present investigation the methanolic extracts of *Alhagi* marorum were found to have maximum antibacterial against *Streptomyces grisveus* (18mm). Stem showed potent activity against *Bacillus subtilis* (14mm) while rests of the bacterial strains were found to be resistant (Table and Fig. 1).

Similarly against fungal strains maximum activity was observed in leaves against *Penicillium funiculosum* (17mm). It was observed that stem showed similar activity against two strains viz. *Trichoderma reesei* and *Fusarium oxysporium*. The other two strains of the fungal colonies were found to be resistant against both the plant parts of *Alhagi marorum*. (Table and Fig. 2)

Serial no.	Microbial colonies	Leaves (zone in mm)	Stem (zone in mm)	Standard as Ciprofloxacin
1	Escherichia coli	NA	NA	22
2	Staphylococcus aureus	NA	NA	22
3	Streptomyces grisveus	18±0.92	NA	22
4	Bacillus subtilis	NA	14±0.38	22

Table 1: Antibacterial activity of Alhagi marorum against various clinical isolates.





Fig 1: Antibacterial activity of experimental plants

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Serial no.	Name of fungus strain	Leaves (zone in mm)	Stem (zone in mm)	Standard as ketokenazole
1	Trichoderma ressei	13±0.67	14±0.82	31
2	Aspergillus niger	NA	NA	31
3	Penicillium-funiculosum	17±0.91	NA	31
4	Fusarium oxysporium	12±0.54	14±0.82	31



NA= No Activity,



Fig 2: Antifungal activity of experimental plants

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