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Antimicrobial activity of *Gymnema sylvestre* (Asclepiadaceae)

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ABSTRACT

Objective: To evaluate antimicrobial activities of aqueous, methanol, chloroform and hexane extract of leaves plant of *Gymnema sylvestre* (*G. sylvestre*). **Methods:** The antimicrobial screening of the extracts of *G. sylvestre* against most prevalent microbes like *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Escherichia coli* (*E. coli*), *Candida albicans* (*C. albicans*), *Candida tropicalis* (*C. tropicalis*), *Candida krusei* (*C. krusei*) and *Candida kefyr* (*C. kefyr*) by agar well diffusion method, minimum inhibitory concentration, minimum bactericidal concentration, minimum fungicidal concentration were carried out. **Results:** The aqueous and methanol leaf extract showed significant antibacterial and antifungal activities against the selected microorganisms when compared to the standard drugs respectively. **Conclusions:** The dried scale leaves of *G. sylvestre* might represent a new antimicrobial source with stable, biologically active components that can establish a scientific base for the use in modern medicine.

1. Introduction

Plants have been used for the treatment of various diseases all over the world before the advent of modern clinical drugs and are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs[1]. Thus over 50% of these modern drugs are of natural products origin and as such play an important role in drug development in the pharmaceutical industry[2].

Infectious diseases are the number one cause of death world-wide, and in tropical countries it accounts for approximately 50% of deaths. This may be due to poverty and increasing incidence of multiple drug resistance. Bacterial resistance to almost all antibacterial agents has been reported[3]. This resistance is largely due to indiscriminate use of antimicrobial drugs commonly used in treatment of infectious diseases. Apart from resistance, some antibiotics have serious undesirable side effects which limit their applications, so there is urgent need to develop new antimicrobial agents that are very effective with minimal side effects, and represent a potential source of novel antibiotic prototypes[4].

Gymnema sylvestre (*G. sylvestre*) (Asclepiadaceae) is a large tropical liane native to central and western India and can be also found in tropical Africa and in Australia[5]. *G. sylvestre* is one of the important anti-diabetic medicinal plants. There is a growing demand for *G. sylvestre* leaves in pharmaceutical trade. Gymnemic acid, the active ingredient of this plant, is extracted from leaves and used widely as an anti-diabetic[6], anti-sweetner[7] and antihypercholesterolemia[8]. It also has stomachic, diuretic and cough suppressant properties[9]. The plant has been reported to possess antimicrobial[10] and ethnoveterinary medicinal properties[11].

In addition, it possesses hepatoprotective, and anti-saccharine activities[12]. Hence, because of these properties, *G. sylvestre* is most important for plant prospecting. In the present study, the selection of this plant for evaluation was based on its traditional usages. Although very limited work has been done on the antimicrobial activity of this endangered medicinal plant, it needs further study for verification of its activity against disease-causing microorganisms.

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2. Materials and methods

2.1. Plant material

The *G. sylvestre* were leaves collected during June-July of 2012 in and around Vellore, Tamilnadu and authenticated by Department of Botany. The voucher specimens were kept in the Department of Botany in Voorhees College Vellore, Tamilnadu, India.

2.2. Extraction procedure

Shade dried leaves (200 g) were coarsely powdered and subjected to successive solvent extraction by continuous hot extraction (soxhlet). The extraction was done with different solvents in their increasing order of polarity such as Aqueous, methanol, chloroform and Hexane. Each time the marc was air dried and later extracted with other solvents. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The extracts were preserved in airtight containers and kept at 4-5 ℃ until further use.

2.3. Test organisms

The microorganisms used for the test were *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), *Candida albicans* (*C. albicans*), *Candida tropicalis* (*C. tropicalis*), *Candida krusei* (*C. krusei*) and *Candida kefyr* (*C. kefyr*). All the stock cultures used in the study were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India.

2.4. Culture media and inoculums preparation

Nutrient agar/Potato Dextrose Agar (Himedia, India.) was used as the media for the culturing of strains. Loops full of all the microbial cultures were inoculated in the nutrient broth (NA) at 37 ℃ for 72 h.

2.5. Antimicrobial activity study

Antimicrobial activity of the *G. sylvestre* leaves extracts viz. aqueous, methanol, chloroform and Hexane were determined, using the agar well diffusion assay method[13]. Approximately 20 mL of molten and cooled media was poured in sterilized petri dishes. The plates were left overnight at room temperature to check for any contamination to appear.

The test organisms were grown in broth for 24 h. A 100 mL broth culture of each test organism (1×10^5 cfu/mL) was used to prepare lawns. Agar wells of 5 mm diameter were prepared with the help of a sterilized stainless steel cork borer. Five wells were prepared in the agar plates. The wells were labeled as A, B, C, D and E. 'A' well was loaded with 10 μL of aqueous leaves extracts, 'B' well was loaded with 10 μL of methanol leaves extracts, 'C' well was loaded with 10 μL of chloroform leaves extracts, 'D' well was loaded with hexane leaves extracts and 'E' well was loaded with positive control drugs.

Various bactericides/fungicides (Table 1) were used as positive controls. The plates containing the organisms and leaves extracts were incubated at 37 ℃. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells[14]. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

Table 1
Antimicrobial activity of different extracts of leaves of *G. sylvestre* against test organisms.

Microorganisms	Zone of inhibition in mm (mg/mL)				
	AE	ME	CE	HE	Reference drug
S. aureus	7	–	–	7	18
E. coli	–	15	–	–	21
B. cereus	–	8	–	9	20
K. pneumoniae	–	–	–	–	17
C. albicans	–	12	–	–	19
C. tropicalis	–	–	–	–	20
C. kefir	8	–	–	–	16
C. krusei	10	11	–	–	23

AE-Aqueous extract, ME-Methanol extract, CE-Chloroform extract, HE-Hexane extract.

2.6. Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

Antimicrobial activity was measured using a dilution technique[15]. The plant extract (100 mg) was solubilized in 1 mL of dimethyl sulfoxide (DMSO) and serially two fold diluted Nutrient broth (Himedia, India) to obtain a concentration range of 12.5-100.0 mg/mL.

Nutrient broths containing only DMSO diluted in the same way, which did not influence microbial growth, were included as controls. The test strains were suspended in sterile physiological Tris buffer (pH 7.4, 0.05 M), homogenized and adjusted to an optical density of 0.05 at 530 nm (equivalent to 1×10^6 CFU/mL).

This suspension was used asthe inoculums for the test in the agar plates. Culture suspensions (100 μL) were inoculated using a micropipette.

The minimal inhibitory concentration (MIC) was defined

as the minimal concentration of the plant extract which completely inhibited the visible growth (turbidity) of the bacteria in tubes.

The minimal bactericidal concentration (MBC), minimal fungicidal concentration (MFC) was defined as the minimal concentration of the extract which completely inhibited the visible growth of the test strains on solid media in petri dishes that were incubated at 37 ℃ for 72 h.

3. Results

In the present study the antimicrobial activity of leaf extracts viz. aqueous, methanol, chloroform and hexane were evaluated against eight test spp. (Table 1). In the first stage the leaf extracts of *G. sylvestre*, *i.e.* aqueous, methanol, chloroform and hexane applied on isolates of each test strains. Methanol leaf extract of *G. sylvestre* showed significant antimicrobial activity against *E. coli*, *B. cereus*, *C. albicans* and *C. kefyr*. The aqueous leaves extract recorded an intermediate antimicrobial activity against *S. aureus*, *C. krusei* and *C. kefyr* while on the other hand the hexane leaf extract revealed antimicrobial effect on *S. aureus*, *B. cereus* but chloroform leaf extract did not show significant antimicrobial effect on test strains. The inhibitory activities of all the four extracts of the leaf reported in the present study were compared with standard antimicrobics, Chlorompenicol, Ketoconazole and Itraconazole.

The results on MIC studies of leaf extract (Methanol), against 8 isolates of test organisms recorded lowest (50 mg/mL) for *E. coli* and *C. krusei* when compared with that of other species viz. *S. aureus* (75 mg/mL), *B. cereus* (75 mg/mL) and *K. pneumoniae* (75 mg/mL) whereas the MIC for *C. albicans*, *C. tropicalis* and *C. kefyr* were recorded as 100 mg/mL. Further the MIC studies of aqueous leaf extract against test organisms recorded lowest for *C. krusei* (50 mg/mL) when compared with that of other species viz *S. aureus* (75 mg/mL) and *C. kefyir* (50 mg/mL). The leaf extract (hexane) MIC was recorded for *B. cereus* and *S. aureus* as 50 mg/mL; 75 mg/mL respectively (Tables 2 & 3).

Table 2
Determination of minimum inhibitory concentration (MIC) for *G. sylvestre*.

Microorganisms	MIC (mg/mL)				
	AE	ME	CE	HE	Reference drug
S. aureus	75	75	>100	75	12.5
E. coli	>100	50	>100	>100	12.5
B. cereus	>100	75	>100	50	12.5
K. pneumoniae	>100	75	>100	>100	12.5
C. albicans	>100	>100	>100	>100	12.5
C. tropicalis	>100	>100	>100	>100	12.5
C. kefir	75	>100	>100	>100	12.5
C. krusei	50	50	>100	>100	12.5

AE-Aqueous extract, ME-Methanol extract, CE-Chloroform extract, HE-Hexane extract.

Table 3
Determination of minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) for *G. sylvestre*.

Microorganisms	MBC/MFC (mg/mL)				
	AE	ME	CE	HE	Reference drug
S. aureus	75	75	>100	75	12.5
E. coli	>100	50	>100	>100	12.5
B. cereus	>100	75	>100	50	12.5
K. pneumoniae	>100	75	>100	>100	12.5
C. albicans	>100	>100	50	>100	12.5
C. tropicalis	>100	>100	>100	>100	12.5
C. kefir	75	>100	>100	>100	12.5
C. krusei	50	50	>100	>100	12.5

4. Discussion

The therapeutic value of medicinal plants lies in the various chemical constituents' presents in them. The bioactivity of plant extracts was attributed to phytochemical constituents. For instance, plant rich in tannins have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane[16]. Flavonoids are a major group of phenolic compounds reported for their antiviral[17], antimicrobial[18] and spasmolytic properties[19]. Alkaloids isolated from plant are commonly found to have antimicrobial properties[20]. Extract of the seeds of *Vitex agnus-castus* (Family:Lamiaceae) was reported to possess antimicrobial activity which was associated with its alkaloids, saponins, tannins, flavonoids, and glycosides contents[21].

The antimicrobial activity of the leaf extracts of *G. sylvestre* as recorded in present study might therefore be attributed to the presence of above phytochemicals *i.e.* flavonoids, terpenoids, amino acids, glycosides, tannins, amino acids and carbohydrates.

From the above study, it is concluded that the dried scale leaves of *G. sylvestre* might represent a new antimicrobial source with stable, biologically active components that can establish a scientific base for the use in modern medicine. Further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs from *G. sylvestre*.

Conflict of interest

The authors declare they have no conflict of interests.

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