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Original Article

Bio-efficacy of *Dioscorea pentaphylla* from Midmid-Western Ghats, India

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ABSTRACT

Antibacterial and antifungal activity of crude extracts of medicinally important and traditionally used yam plant, *Dioscorea pentaphylla*, from mid-Western Ghats was evaluated against 27 bacterial and 5 fungal clinical strains collected of the patients from infectious sources. The clinical strains belonging to their respective species showed concentration-dependent susceptibility toward crude petroleum ether extract, chloroform extract and methanol extract at 100 μg/100 μl. The extracts exhibited predominant antibacterial activity against *Staphylococcus aureus* (ATCC-20852), *Pseudomonas aeruginosa* (ATCC-29737) and *Klebsiella pneumoniae* (MTCC-618), respectively, and five clinically isolated pathogenic fungi, *Trichophyton rubrum, Microsporum gypseum, Tricophyton tonsurans, Microsporum audouini,* and *Candida albicans*, with antibacterial drug ciprofloxacin and antifungal drug fluconozole (50 μg/100 μl) as standards. Out of the three extracts, ethanol extracts possessed better minimum inhibitory concentration (MIC) against all the bacterial strains. All the three extracts showed significant activity against all the five fungal pathogen strains. The results are promising and support the traditional use of *D. pentaphylla* for the treatment of bacterial and fungal infections.

Key words: Antifungal, antibacterial, *Dioscorea pentaphylla*, minimum inhibitory concentration

INTRODUCTION

The practice of antimicrobial chemotherapy is one of the constant challenges, particularly in view of the rapid evolutionary changes and wide variety of pathogens encountered. Many investigators have evaluated the bioactivity of plant extracts and their constituents against serious infectious organisms.[1]

Prevalence of antibiotic-resistant strains of bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control the bacterial diseases. Numerous studies have been carried out in different parts of the globe to extract plant products for screening antimicrobial activity.[2]

Dioscorea yam is a member of the Yam family. The yams are vining plants with 600 known species, 71 of which are native to North America (67 species in Mexico).[3] In many species of yam, the rhizome (tuber) serves as both food and medicine. Many native Americans and South Asians used a syrup of the root to relieve labor pain and later physicians gave wild yam to patients with colic pain, morning sickness, asthma, hiccough, rheumatism and gastritis related to alcoholism.[4]

In the present investigation, *Dioscorea pentaphylla* was selected as one of the medicinally important plants, extensively consumed by local people as food. However, there are apparently no scientific reports on the antimicrobial properties of this plant. The lack of scientific knowledge has

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often exerted a major constraint on the use of traditional herbal remedies as an affordable alternative to orthodox medical treatment. Thus, the different solvent extracts of the tuber were screened for its activity against three bacterial pathogens and five fungal strains.

MATERIALS AND METHODS

Plant material

Tubers of *D. pentaphylla* were collected from the Lakkavalli reserve forest in and around the area of Bhadra Wildlife Sanctuary of the mid-Western Ghats region of Karnataka, India, and the species was identified by comparing with the authenticated specimen deposited at the Kuvempu University Herbaria (Voucher specimen KUDB/Ang/324). The leaves were washed in running tap water, shade dried, powdered mechanically and sieved (Sieve No. 10/44) and subjected to Soxhlet extraction using different solvents, viz., petroleum ether, chloroform and ethanol. The extracts were concentrated under reduced pressure at 40±5°C using a rotary flash evaporator (Buchi, Flawil, Switzerland).

Phytochemical analysis

Qualitative phytochemical analysis of *D. pentaphylla* tuber extracts was done as given below.

Tannins

Twenty milligrams of the extract was dissolved in 2 ml distilled water and filtered. Two millilitres of $FeCl_3$ was added to the filtrate. Blue-black precipitate indicated the presence of tannins.[5]

Alkaloids

Twenty milligrams of the extract was dissolved in 2 ml distilled water and filtered. To the filtrate, two to four drops of 1% HCl was added and steam was passed through it. To 1 ml of this solution, six drops of Wagner's reagent were added. Brownish-red precipitate indicated the presence of alkaloids.[5]

Saponins

To 0.5 ml of the filtrate obtained in alkaloids test, 5 ml distilled water was added. Frothing persistence indicated the presence of saponins.[5]

Flavonoids

Twenty milligrams of the extract was dissolved in 10 ml ethanol and filtered. Then, 0.5 ml of concentrated HCl and magnesium ribbon were added to 2 ml filtrate. Development of pink-tomato red color indicated the presence of flavonoids.[5]

Terpenoids

Salkovski test was performed using a small amount of extract solution. To this solution, five drops of concentrated H_2SO_4 and 1 ml chloroform were added. Change of yellow color into red indicated the presence of terpenoids.[6]

Phenols/polyphenols

A small amount of material was extracted in ethanol and evaporated to dryness. Residue was dissolved in distilled water and 0.5 ml Folin-ciocalteau reagent was added followed by 2 ml 20% Na_2CO_3 solution. Development of bluish color indicated the presence of phenols.[7]

Preparation of plant extracts for antimicrobial assay

Exactly 100 μg of all the crude extracts was dissolved in 100 μl of 10% dimethyl sulfoxide (DMSO). The standard antibacterial drug ciprofloxacin and antifungal drug fluconozole were also tested at a concentration 50 μg/100 μl of each.

Evaluation of minimal inhibitory concentrations

The minimal inhibitory concentration (MIC) of the different solvent crude extracts was determined by microdilution techniques in Luria-bertani medium (LB) broth, according to Clinical and Laboratory Standards Institute (CLSI), USA guidelines. The bacterial inoculates were prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard colony forming units and diluted 1:10 for the broth microdilution procedure. The microtiter plates were incubated at 37°C and MIC was determined after 24 hours of incubation. The highest activity of the isolated compounds compared to those of the crude extracts indicates that those compounds alone were solely responsible for antimicrobial activity.

Screening of antimicrobial activity

Twenty-seven clinical strains of three of the most common bacterial pathogens, *Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae* (*S. aureus* – ATCC-29737; *P. aeruginosa* – ATCC-20852 and *K. pneumoniae* – MTCC-618), strains of the corresponding bacteria, and five clinically isolated pathogenic fungi, *Trichophyton rubrum, Microsporum gypseum, Tricophyton tonsurans, Microsporum audouini,* and *Candida albicans*, were used as test organisms. Different pathogens and their serotypes were isolated from infected patients in the district health center of Annamali Nagar, and were identified in the Department of Zoology, Annamali University, India, with support from National Chemical Laboratory, Pune, India. The profile of bacterial species and their strains of different clinical origin are shown in Table 1. All the bacterial pathogens were maintained at −30°C in Brain Heart Infusion (BHI) containing 17% (v/v) glycerol. Before testing, the suspensions were transferred to

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LB broth and incubated overnight at 37°C. Inocula were prepared by adjusting the turbidity of the medium to match the 0.5 McFarland standards. Dilutions of this suspension in 0.1% peptone (w/v) solution in sterile water were inoculated on LB agar to check the viability of the preparations. In case of fungal stocks, cultures were stored on BHI (Merck Pvt. Ltd India) culture media (pH 6.5).

Antimicrobial assay

Agar radial well diffusion method[8] was used for the assessment of antimicrobial activity of the extracts of *D. pentaphylla*. Nutrient agar medium (tryptone 10 g/l, yeast extract 5 g/l, sodium chloride 10 g/l, agar-agar 15 g/l, pH 7.2) was poured into sterilized petri dishes (90 mm diameter). LB broth containing 100 μl of 24-hour incubated cultures of the respective clinical isolates and the ATCC and MTCC strains were spread separately on the agar medium. Wells were created using a sterilized cork borer under aseptic conditions.

Table 1: List of the clinical strains used for antimicrobial activity

Clinical strains	Clinical condition	Source
P. aeruginosa		
Ps-1	Bronchitis	Wounds
Ps-2	Otitis media	Pus
Ps-3	Burns	Sputum
Ps-4 and Ps-5	Upper UTI	Stool
Ps-6	Food poisoning	Hospital effluent
Ps-7	Cross infections in UTI	Hospital effluent
Ps-8	Septicemia	Old wounds
Ps-9	Unknown	Ear swab
K. pneumoniae		
Kp-1	Pneumonia	Mucus
Kp-2	Gram negative	Follicullitis stipules
Kp-3	Burns	Pus
Kp-4	UTI	Urine
Kp-5	Septicemia	Sputum
Kp-6	Cross infections in UTI	Urine
Kp-7	Abscess in immunodeficiency	Wounds
Kp-8	Upper UTI	Urine
Kp-9	Unknown	Hospital effluent
S. aureus		
Sa-1	Abscess in immunodeficiency	Wounds
Sa-2	Burns	Pus
Sa-3	Septicemia	Old wounds
Sa-4	Food poisoning	Stool
Sa-5	Burns	Pus
Sa-6 and Sa-7	Unknown	Hospital effluent
Sa-8	Abscess in immunodeficiency	Sputum
Sa-9	Otitis media	Ear swab
Fungal strains		
T. rubrum	Cutaneous mycoses	Skin
T. tonsurans	Scaring of the scalp	Scalp ringworm
M. gypseum	Ringworm	Infections skin
M. audouini	Cutaneous mycoses	Skin and hairs
C. albicans	Opportunistic mycoses candidosis	Lungs

Ps = clinical strains of *Pseudomonas aeruginosa*, Kp = clinical strains of *Klebsiella pneumoniae*, Sa = clinical strains of *Staphylococcus aureus, UTI = urinary tract infection*

In order to identify antifungal activity of total extracts and fractions against fungal pathogens, the agar diffusion assay was performed in BHI culture media (pH 6.5). Fungal spores were obtained by centrifugation at 1500× *g*/4°C for 15 min and diluted in phosphate buffer saline (PBS), pH 7.2. Spore count was performed using hemocytometer. After loading 10 μl of the cell suspension in PBS and number of spores/ml was calculated, the final concentration of each strain was identified to be 106 spores/ml. Cultures were incubated for 72 hours at 24°C. Then, 100 μl of fungal inocula was spread on the BHI agar plates and wells were made using cork borer and 50 μl of test compounds was loaded to each well. The plates were refrigerated for 2 hours in order to stop fungal growth and facilitate diffusion of the substances. The reference antibacterial agent, ciprofloxacin, and antifungal agent, fluconozole, were loaded in the corresponding wells in the bacterial and fungal culture plates. Bacterial culture plates were then incubated at 37°C for 24 hours, and fungal culture plates were incubated at 24°C for 48 hours. At the end of the incubation period, inhibition zones were observed and measured.

Statistical analysis

The results of these experiments are expressed as mean±SE of three replicates in each test. The data were evaluated by one-way analysis of variance (ANOVA) and mean separations were carried out using Duncan's Multiple Range Test (DMRT, Gomez and Gomez 1984) followed by Tukey's multiple comparison tests to assess the statistical significance. $P \leq 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Results of phytochemical analysis of *D. pentaphylla* tuber extracts are given in Table 2. All the three extracts were tested for the presence of phenols/polyphenols, flavonoids, terpenoids, tannins, alkaloids and saponins. Phenols and saponins were invariably present in all the solvent extracts. The analysis of different tuber extracts (viz., petroleum

Table 2: Phytochemical analysis of *Dioscorea pentaphylla* tuber extracts

Phytochemicals	Petroleum ether	Chloroform	Ethanol
Phenol/polyphenols	+	+	+
Terpenoids/steroids	–	+	+
Flavonoids	+	–	+
Saponins	+	+	+
Alkaloids	+	–	–
Tannins	–	+	+

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Table 3: Antibacterial activity of the crude extracts and their pure compounds of *Dioscorea pentaphylla* against clinical strains of *Pseudomonas aeruginosa*

Clinical strains	Diameter of zone of inhibition (mm)			
	Petroleum ether extract	Chloroform extract	Ethanol extract	Ciprofloxacin
Ps-1	07.40±0.10	12.83±0.20	17.23±0.14	23.30±0.15
Ps-2	09.00±0.12	10.16±0.16	16.33±0.24	20.50±0.28
Ps-3	11.23±0.15	11.73±0.33	19.23±0.14	22.23±0.14
Ps-4	10.23±0.15	13.50±0.17	18.43±0.23	20.20±0.26
Ps-5	12.30±0.10	13.23±0.14	18.06±0.18	23.30±0.15
Ps-6	7.40±0.10	17.33±0.20	20.50±0.28	24.33±0.20
Ps-7	10.60±0.12	16.00±0.28	17.00±0.28	20.00±0.28
Ps-8	08.53±0.29	12.13±0.13	19.23±0.17	23.16±0.16
Ps-9	11.37±0.19	13.33±0.16	19.12±0.00	24.50±0.28

Clinical strains of *Pseudomonas aeruginosa* from different clinical sources. The values are the mean of three experiments±SE; Means followed by the same letter were not significantly different on analysis by the DMRT test at 0.05% probability level

Table 4: Antibacterial activity of the crude extracts and their pure compounds of *Dioscorea pentaphylla* against clinical strains of *Klebsiella pneumoniae*

Clinical strains	Diameter of zone of inhibition (mm)			
	Petroleum ether extract	Chloroform extract	Ethanol extract	Ciprofloxacin
Kp-1	09.23±0.14	13.23±0.15	16.23±0.14	25.00±0.12
Kp-2	08.56±0.12	14.33±0.17	15.40±0.10	20.23±0.15
Kp-3	09.23±0.14	12.30±0.15	14.23±0.14	21.37±0.09
Kp-4	10.23±0.14	12.77±0.09	14.50±0.28	20.20±0.26
Kp-5	09.40±0.10	14.27±0.18	17.73±0.12	23.37±0.09
Kp-6	12.23±0.14	10.43±0.23	18.30±0.15	22.53±0.18
Kp-7	12.30±0.10	12.33±0.17	19.26±0.14	24.37±0.19
Kp-8	11.16±0.16	14.30±0.17	16.17±0.17	23.43±0.12
Kp-9	14.06±0.06	13.30±0.15	18.43±0.03	24.43±0.12

Clinical strains of *Klebsiella pneumoniae* from different clinical sources. The values are the mean of three experiments±SE. Means followed by the same letter were not significantly different on analysis by the DMRT test at 0.05% probability level

Table 5: Antibacterial activity of the crude extracts and their pure compounds of *Dioscorea pentaphylla* against clinical strains of *Staphylococcus aureus*

Clinical strains	Diameter of zone of inhibition (mm)			
	Petroleum ether extract	Chloroform extract	Ethanol extract	Ciprofloxacin
Sa-1	19.40±0.23	13.33±0.17	18.37±0.19	28.33±0.17
Sa-2	18.43±0.23	14.23±0.15	20.60±0.10	26.90±0.21
Sa-3	14.27±0.18	14.87±0.37	16.70±0.10	21.50±0.29
Sa-4	12.40±0.21	12.17±0.17	20.63±0.09	24.50±0.29
Sa-5	13.47±0.24	14.33±0.17	17.27±0.12	20.43±0.23
Sa-6	16.30±0.15	12.17±0.17	19.33±0.22	27.10±0.21
Sa-7	17.60±0.15	13.17±0.17	20.30±0.25	25.50±0.29
Sa-8	12.57±0.07	16.13±0.12	19.57±0.12	23.50±0.29
Sa-9	11.43±0.23	13.33±0.17	18.60±0.10	23.83±0.44

Clinical strains of *Staphylococcus aureus* from different clinical sources. The values are the mean of three experiments±SE. Means followed by the same letter were not significantly different on analysis by DMRT at the 0.05% probability level

ether, chloroform and ethanol) showed the presence of combinations of other phytochemical constituents as well.

The results of antimicrobial investigation revealed that the MIC of the petroleum ether, chloroform and ethanol extracts was 100 μl/100 μl for each. The zones of inhibition of the microbial colony are depicted in Tables 3-5. The petroleum ether extract demonstrated antibacterial activity against all the clinical strains of bacteria. It showed maximum activity against *S. aureus* (16.13 mm) followed by *P. aeruginosa* (12.30 mm), and *K. pneumoniae* (12.23 mm), and among fungal strains, it showed maximum activity against *M. audouini* (14.42 mm) when compared to standard. Chloroform extract showed least inhibition activity against all the strains of bacteria and fungi. It was 19.40 mm against *S. aureus*, 16.00 mm against *P. aeruginosa*, 14.33 mm against *K. pneumoniae* and 16.23 mm against the fungal strain *T. tonsurans*. Whereas the ethanol extract showed significant inhibition zone similar to standard. Ethanol extract illustrated inhibition zone against *S. aureus* (20.63 mm), *P. aeruginosa* (20.50 mm), *K. pneumoniae* (19.26 mm), and among the fungal strains against *M. gypseum* (20.37 mm) and *C. albicans* (18.13 mm).

Antifungal activity of the crude extracts and their pure compounds of *Dioscorea pentaphylla* against clinically isolated fungal pathogens

Among all the tested extracts, ethanol proved to be most potent bactericidal agent against all the strains as compared to other extracts, but it is not up to the standard drug, ciprofloxacin. Among the five dermatitis fungi cultured for antifungal assay, all the crude extracts showed zone of inhibition against all the strains of fungal colony [Table 6]. The ethanol extracts showed significant inhibition against in *C. albicans, T. rubrum, M. gypseum* and *T. tonsurans* on a par with the standard drug, fluconozole.

These results showed wide spectrum of antimicrobial properties for the petroleum ether, chloroform and ethanolic extracts of *D. pentaphylla*. The organic solvent extracts of *D. pentaphylla* tubers studied in the current work showed remarkable antibacterial activities against *P. aeruginosa, K. pneumoniae* and *S. aureus* responsible for causing diseases in animals and humans. These microorganisms pose important public health and economic concerns for the human society. However, the solvent extracts proved to be significant in their activity against the above bacterial strains.

There are reports showing that alkaloids and flavonoids are responsible for the antifungal activities in higher plants.[9]

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Table 6: Antifungal activity of the crude extracts and their pure compounds of *Dioscorea pentaphylla* against clinically isolated fungal pathogens

Clinical strains	Diameter of zone of inhibition (mm)			
	Petroleum ether extract	Chloroform extract	Ethanol extract	Fluconozole
Trichophyton rubrum	10.63±0.09	13.30±0.15	14.37±0.19	15.43±0.23
Microsporum gypseum	12.37±0.09	15.37±0.09	20.37±0.09	21.37±0.19
Tricophyton tonsurans	11.60±0.10	16.23±0.15	16.15±0.09	16.57±0.12
Microsporum audouini	14.42±0.09	12.77±0.15	10.37±0.19	16.40±0.10
Candida albicans	10.06±0.13	14.37±0.19	18.13±0.09	18.23±0.15

Clinically isolates fungal pathogens from different clinical sources. The values are the mean of three experiments±SE. Means followed by the same letter were not significantly different on analysis by DMRT at the 0.05% probability level

Moreover, secondary metabolites such as tannins and other compounds of phenolic nature are also classified as active antimicrobial compounds. Phenols, the aromatic compounds with hydroxyl groups, are widespread in plant kingdom. They occur in all parts of plants. Phenols are said to offer resistance to diseases and pests in plants. Grains containing high amount of polyphenols are resistant to bird attack.[7] Interestingly, phytochemical screening of the current investigation revealed that extracts from both plant parts and the tuber possess at least three to four of the following classes of secondary metabolites: phenols, flavonoids, terpenoids, tannins, alkaloids and saponins. Therefore, the presence of these phytochemicals could justify the observed antifungal activities in the current study. These results are in agreement with earlier studies conducted on other plant species belonging to the euphorbiaceae[10] and asteraceae.[11] According to earlier reports Dioscorea attributing secondary metabolites which are proved to be very good antimicrobial Activities. Diosgenyl saponins, one of the most abundant steroid saponins, with diosgenin as the steroidal sapogenin, are reported to exert a large variety of biological functions such as antifungal, antibacterial, and anticancer.[12]

Earlier chemical investigation of yam tubers afforded two norclerodane diterpenoids.[13] Clerodane class of diterpenes is a group of compounds that has attracted considerable interest because of problems associated with their stereochemistry and because of their diverse biological activities. They are known to possess antitumor, antibacterial, antifeedant, and antifungal activities.[14] The studies of Quan *et al*,[15] reported efficient antibacterial activity against *Bacillus subtilis* and *S. aureus* of diosgenin deratives like 2,6-iodopseudogiosgenin and 2,6-iodopsuedogiosgenone. Sautour *et al*,[16] showed steroidal saponins from *Dioscorea cayenensis* to possess activity against *C. albicans* (IP 1180-79), *Candida glabrata* and *Candida tropicalis* (clinical isolates). The CH_2Cl_2-soluble portion of the crude extract and the two clerodanes showed significant activities against *P. aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi A and Salmonella paratyphi B*, which was reported by Teponno *et al*.[17]

To conclude, the traditional use of tubers of *D. pentaphylla* for the treatment of bacterial and fungal infections has been realized. For follow-up research, it is needed to determine the active components in each extract and confirm their mechanism of action.

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