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Research Paper

In vitro antimicrobial study of root extract of *Chlorophytum arundinaceum* Baker

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Abstract

The tubers of ***Chlorophytum arundinaceum* Baker** of Liliaceae family is a traditionally used medicinal plant with ethnic and/or folk claims like immunomodulatory, antidiabetic, anti-arthritic, aphrodisiac, etc. The *in vitro* antimicrobial activity of its root extract was evaluated against six bacterial species (*Bacillus subtilis, Escherichia coli, Proteus vulgaris*, *Pseudomonas aeruginosa, Shigella sonnei* and *Staphylococcus aureus*) and four fungal species (*Aspergillus fumigatus, A. niger, Candida albicans* and *Trichophyton rubrum*). The root extracts in solvents like petroleum ether/benzene, ethyl acetate, hydro-alcohol, methanol and chloroform showed moderate microbial activity whereas the aqueous root extract evinced no antimicrobial activity. None of the solvent extracts tested showed any activity against *B. subtilis.* The methanolic extract has shown maximum antibacterial as well as antifungal activity of the five solvent systems tried except *A. fumigatus*.

Keywords: Antimicrobial activity, *Chlorophytum arundinaceum,* Root-tuber, *Safed musli.*

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Introduction

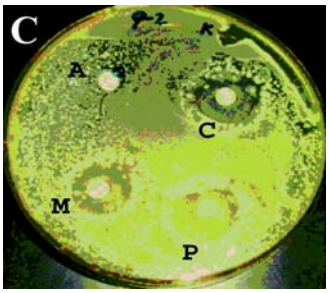
It is estimated that even in this modern age, 80% of the medical care is provided by homecare and traditional native systems of medicine and a major part of these therapies involve the use of plant extracts or their active principles[1]. In Traditional Health Systems of India a large number of medicinal plants have been used for many centuries for treating various diseases[2]. India, on account of its rich flora, is considered as the source of nearly three-fourth of plant-derived drugs of the world[3]. These medicinal plants have a wide variety of chemical constituents and some of them have the ability to inhibit the growth of microorganisms[4]. Experiments are in progress at several laboratories to isolate antimicrobial compounds from several plant species.

Chlorophytum* Ker-Gawl.** (Family — Liliaceae) is a pantropical genus, rich in both mono- and bi-desmosidic saponins[5]. *Safed musli* is a controversial Indian drug. Various species of ***Chlorophytum are supplied in the crude drug market as *Safed musli* and used by practitioners. One of the popular and marketed medicinal herbs/species under this name is ***Chlorophytum arundinaceum* Baker**[6,7].

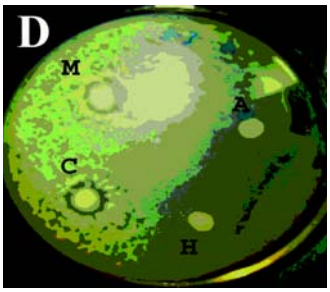
C. arundinaceum is a root-tuber geophyte found in India, Nepal and Myanmar. In India, it is found wild in natural forest of India right from Tamil Nadu in the south to foot hills of Himalayas in the north, Gujarat to Aravali Hills in the west to Assam in the east. It holds an important place in Ayurvedic and Unani Systems where it is mostly used to

Plant

Root tubers



Root extracts showing Antibacterial activity



Root extracts showing Antifungal activity

A= Aqueous extract; C= Control; H= Hydro-alcohol; M=Methanol; P= Petroleum ether

Fig. 1A-D: *Chlorophytum arundinaceum*

treat oligospermia (low sperm count), pre- and post-natal symptoms, arthritis, diabetes, dysuria, aphrodisiac, etc.[6-9]. Considering the medicinal importance and demand in the market, a large number of farmers took up its cultivation in different parts of the country, i.e. Andhra Pradesh, Assam, Maharashtra, Gujarat and Rajasthan[10]. A new bibenzyl compound, 2', 4, 4'-trihydroxy-2 xylopyranosyl-bibenzylenzyl has been isolated from its roots[11]. Besides, arundinoside A and B, docasonoic acid, n-nonacosane, pentacosonyl docosnonate, stigmasterol, tetracosanoic acid[6, 12, 13], etc., Rachchh *et al* reported its potential to cure gastric ulcer[14]. The present study is carried out to evaluate its antimicrobial activity so as to find scientific evidence for the folk claims.

Materials and Methods

Plant material

The roots (tubers) of ***C. arundinaceum*** were collected from Sukumamidi village in Chintur range of Khammam district of Andhra Pradesh during 2004-2005. The herbarium specimen of the species is deposited in Kakatiya University Herbarium (KUH). The live plants are also maintained in the pots (Fig. 1A). The roots (tubers) were washed thoroughly (Fig.1B) and made into small pieces. Then, they were shade dried at room temperature for a week and powdered to coarse size and stored in air tight container for extraction of active principles.

Extraction procedure

The powdered root material was first defatted with petroleum ether/ benzene. The extraction was done using ethyl acetate, methanol, chloroform and 80% alcohol in soxhlet apparatus. The different solvent extraction processes were completed by 72 cycles (8 h per day for 9 days). When the solvent was drained colourless, the extraction was stopped. The solvent was completely removed by using rotary flash evaporator or water bath to obtain semi-solid mass. On the other hand, the water extraction of the root was done by hot water maceration. Ultimately, the extract was obtained as dried powder.

Experimental procedure:
Well Diffusion Method

This method depends on the diffusion[15] of various extracts from a cavity through the solidified agar layer of petri-plates to an extent, so that the growth of the inoculated microorganisms is prevented entirely in circular area or zone around the cavity containing the extracts. Using micropipette, 0.4 ml of each the seeded broth containing 10^{-6} to 10^{-7} cfu/ml test organisms was inoculated on the four plates of solidified agar and spread uniformly with a glass spreader. Then, four wells were dug out in the agar layer of each plate with an aluminum bore of 5 mm diam. to incorporate 0.4 ml extract, standard drug, solvent DMSO and methanol. Then, the broth was kept in the freeze for one day. The extract is allowed to diffuse into the medium, and then incubated at 37-38ºC for 18 h for antibacterial and 48 h for antifungal activity. After the incubation period, the mean diameter of the zone of inhibition (in mm) formed around the well was measured (Table 1).

The testing of antifungal activity was carried out using the same procedure adopted for antibacterial study. The only difference was the medium used for antifungal study. Instead of nutrient agar medium, Sarboud's Dextrose Agar medium was used. The standards (controls) used were Gentamycin and Amphotericin B for antibacterial and antifungal (antibiotic) activity studies, respectively. The bacterial cultures (two positive and four negative) used in the present study were obtained from Microbial Type Culture Collection Centre (MTCC), Chandigarh, India whereas the four fungal strains tested were procured from culture collection of Department of Microbiology, Kakatiya University, Warangal. The culture accession numbers of bacteria and fungi tested are provided in Table 1.

The data were analyzed statistically and the mean ± SE, levels of significance (student's t-test), and percentage activity of different species of pathogenic bacteria and fungi in different root-tuber extracts in terms of controls used are calculated as per the standard procedures.

Results and Discussion

The phytochemical extraction of root-tubers of ***C. arundinaceum*** was carried out using five different solvent systems (chloroform, ethyl acetate, hydro-alcoholic, methanol and petroleum ether). The water extraction of the tubers of the taxon was also prepared. The six bacterial species used for the study are *Bacillus subtilis, Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa, Shigella sonnei* and *Staphylococcus aureus*; the four strains of the fungal species tested for the activity included *Aspergillus fumigatus*, *A. niger*, *Candida albicans*

Table 1: Antimicrobial activity of different extracts of *Chlorophytum arundinaceum* root-tuber

Test organism / Accession No.	Zone of inhibition (in mm) ± SE; inhibitory activity (%)						Standard drugs/controls	
	Petroleum ether	Ethyl acetate	Methanol	Hydro-alcohol	Chloroform	Aqueous	Gentamycin	Amphotericin B
Bacillus subtilis ATCC-6633	Nil	Nil	Nil	Nil	Nil	Nil	15.33	-
Escherichia coli ATCC-2343	5.7 ± 0.6 (32.9)	7.2 ± 0.8 (41.55)	7.5 ± 0.5 (43.28)	6.5 ± 0.1 (37.51)	6.6 ± 1.0 (38.08)	Nil	17.33 (100)	-
Proteus vulgaris ATCC-2027	8.7 ± 1.3 (54.37)	6.0 ± 0.5 (37.50)	9.7 ± 1.5 (60.62)	7.5 ± 0.5 (46.87)	7.6 ± 1.3 (47.50)	Nil	16.00 (100)	-
Pseudomonas aeruginosa MTCC-1034	7.6 ± 0.5 (48.50)	7.0 ± 1.3 (44.66)	7.8 ± 0.3 (48.75)	7.2 ± 0.3 (45.93)	6.5 ± 0.4 (41.47)	Nil	15.67 (100)	-
Shigella sonnei MTCC-2957	5.8 ± 0.4 (35.51)	6.3 ± 1.5 (38.55)	8.7 ± 0.3 (53.24)	5.6 ± 0.5 (34.27)	6.8 ± 0.6 (41.61)	Nil	16.33 (100)	-
Staphylococcus aureus ATCC-29737	9.8 ± 0.7 (59.97)	8.5 ± 0.3 (52.00)	10.3 ± 0.9 (63.00)	8.8 ± 0.6 (53.85)	7.2 ± 0.5 (44.06)	Nil	16.33 (100)	-
Aspergillus fumigatus KUCCC-A-9	7.5 ± 1.5 (48.92)	8.7 ± 0.0 (56.72)	6.0 ± 1.0 (39.12)	7.3 ± 0.6 (47.59)	6.8 ± 0.0 (44.33)	Nil	-	15.33 (100)
Aspergillus niger KUCCC-A-11	6.0 ± 0.0 (37.50)	5.7 ± 0.3 (35.62)	7.2 ± 1.3 (45.00)	6.0 ± 0.0 (37.50)	6.2 ± 1.0 (38.75)	Nil	-	16.00 (100)
Candida albicans KUCCC-C-8	7.3 ± 0.8 (44.70)	6.5 ± 0.6 (39.78)	7.7 ± 0.8 (47.12)	5.7 ± 1.0 (34.88)	5.8 ± 0.8 (35.49)	Nil	-	16.33 (100)
Trichophyton rubrum KUCCC-T-1	6.5 ± 1.3 (41.48)	5.3 ± 1.0 (33.81)	9.8 ± 1.5 (62.52)	8.5 ± 0.3 (54.23)	7.5 ± 1.6 (47.85)	Nil	-	15.67 (100)

All the values of inhibitory activity for the agents and extracts tested are significant at 0.05 levels except for the aqueous extract on one hand and for *Bacillus subtilis* on the other

and *Trichophyton rubrum* (Table 1). Earlier, the extracts of ***C. arundinaceum*** were shown to possess chemicals which exhibit adaptogenic activity, antiobesity and inhibition of lipid metabolism, antioxidant, antiulcer and immunomodulatory activities[13, 14].

In vitro antimicrobial study (Table 1; Fig. 1C) indicated maximum range (43.3-63.0%) for the methanol extract of the five pathogenic bacteria tested while the minimum range of inhibitory activity (32.9-44.06%) was exhibited in different solvent systems (petroleum ether for *E. coli*, ethyl acetate for *Proteus vulgaris*, hydro-alcoholic for *Shigella sonnei*, and chloroform for *Pseudomonas aeruginosa* and *Staphylococcus aureus*). However, no extract (of all the solvents used) showed any antibacterial activity against *Bacillus subtilis*.

In vitro antifungal study (Table 1; Fig. 1D) indicated maximum activity in the methanol extract (range: 45.0% against *A. niger* to 62.52% against *T. rubrum*) except the methanol extract for *A. fumigatus* against in which ethyl acetate extract showed maximum efficacy. The minimum efficacy was shown by ethyl acetate for *T. rubrum* (33.81%) and *A. niger* (35.62%), hydro-alcohol extract for *C. albicans* (34.88%) and methanol extract for *A. fumigatus* (39.12%). The curative property of ***C. arundinaceum*** for diarrhoea could be due to the presence of saponins[5].

Conclusions

The methanolic extract of root-tuber of ***C. arundinaceum*** has shown the maximum antibacterial activity regardless of the solvent system. It also showed maximum inhibitory activity against the fungal strains except *A. fumigatus* against which the ethyl acetate extract had shown highest activity. The antimicrobial activity exhibited by various extracts of tubers were, however, less than the standard drugs used.

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