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PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF *CHLOROPHYTUM BORIVILIANUM*

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ABSTRACT

Extracts of leaves and stems of *Chlorophytum borivilianum* San and Fern (aerial parts) were subjected to preliminary phytochemical screening for the presence of plant secondary metabolites and *in-vitro* antibacterial and antifungal studies. The results of the preliminary investigation revealed the presence of alkaloids, glycosides, steroidal nucleus, saponins and tannins in both parts. The methanolic extract of leaf and stems part were investigated *in vitro* antimicrobial activity using agar disc diffusion technique. Six clinical strains of human pathogenic microorganisms, comprising 3 Gram positive, 1 Gram negative and 2 fungi were utilized in the studies. The leaf extract of *Chlorophytum borivilianum* displayed overwhelming concentration dependent antimicrobial properties, inhibiting the growth of *Staphylococcus aureus* and *Bacillus cereus*, far above that of ampicillin used in the study at a concentration of 1.0 g/ml. The extract was less sensitive to the 2 Gram negative bacteria in the assay. In the antifungal assay, the growth of *Aspergillus niger* and *Candida albicans,* used were inhibited in the same manner comparable to voriconazole the reference drug included in the study. The methanol extract of stem also displayed a concentration related antibacterial activity, inhibiting the growth of *S. aureus* comparable to ampicillin at 1.0 g/ml. The extract was least active against *Escherichia coli* with a mild activity at 1.0 g/ml. The extract exhibited a weak activity against *C.albicans* as well as *A. niger.* Both plant parts seem to justify their ethnomedical uses.

Keywords: Antimicrobial activity, *Chlorophytum borivilianum*, Liliaceae, Antifungal agents.

INTRODUCTION

Chlorophytum borivilianum San. and Fern. (Liliaceae) is a traditional endangered perennial herbaceous medicinal plant commonly known as Safed Musli.[1] About 256 species are distributed in tropical and sub tropical Africa. 17 species of Chlorophytum are known to occur in India, *Chlorophytum borivilianum* is the most commercially exploited and widely growing species. Safed musli in traditionally used for lack of libido male impotency, oligospermia. It is also widely used as a general health promotive tonic and for delaying the ageing process. Varying its common use for health promotion, it is also used for increasing lactation, treating various gynecological disorders, arthritic conditions and to control diabetes mellitus.[2] *Chlorophtyum borivilianum* contains proteins (8-9%), carbohydrates (41%), root fibers (4%), saponins (2-17%). Saponin is the chief medicinal compound present in the roots. Saponins and alkaloids

present in the plant are the primary source of its significant medicinal properties.[3] Saponins of stigmasterol and sarsasapogrnin with sugars as xylose, arabinose and glucose were extracted from the methanolic fraction of the leaves.[4] In continuation of our interest in this family the preliminary phytochemical, antibacterial and antifungal properties of *Chlorophytum borivilianum* are presented.

MATERILAS AND METHODS

Plant Collection and Authentication

The leaves (100 g) and stem (500 gm) of *C. borivilianum* was collected from the herbal garden of Jamia Hamdard, New Delhi and authenticated by Prof, P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai. Voucher Specimen of *Chlorophytum borivilianum* was deposited under PARC / H 101 in the herbarium of Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard.

Plant Preparation and Extraction

Air- dried aerial parts of *C. borivilianum* was grounded into powder by means of mechanical grinder. It was successively extracted in petroleum ether and methanol by macerating at room temperature (30°C) for 72 hours respectively. The macerated product was filtered through vacuum and the filtrate was dried under reduced pressure. The percentage yields of extracts leaf (12.5 % w/v), stems (20.4 % w/v).

Preliminary Phytochemical Screening

Air-dried and powdered plant materials were screened for the presence of alkaloids, glycosides, saponin glycosides, steroids and tannins using the methods described.[5,6]

Microorganisms

Clinical strains of three human pathogenic bacteria made up of 3 Gram positive (*Staphylococcus aureus, Bacillus subtilis* and *B. cerues*) and 1 Gram negative bacteria (*Escherichia coli*) were used for the antibacterial assay, while for the antifungal assay, one yeast (*Candida albicans)* and one mold (*Aspergillus niger)* were used for the studies. All the microorganisms were obtained from the laboratory stock of Hamdard University.

Media

Nutrient broth, nutrient agar, sabouraud dextrose agar (SDA), tryptone soya broth, tryptone soya agar (Oxoid Laboratories, U.K) were used in the study. Dimethyl Sulfoxide DMSO) was used in solubilising the extracts and drugs and was used as the negative control in the studies.

Antimicrobial Agents

Ampicillin, 1mg/ml, was used as the standard reference drug for antibacterial assays while voriconazole were used as the standard reference drugs for antifungal assay.

Preparation of Bacterial Cultures

The agar cup diffusion method was used to test the fractions for antimicrobial activity. From stored slopes, 5 ml single strength nutrient broth was inoculated. The tubes were well shaken and incubated at 37°C for 18-24 hours.

Preparation of Fungal Cultures

From stored slopes 5 ml single strength tryptone soya broth was inoculated. The tubes were well shaken and incubated at room temperature for 2-3 days. Using sterile pipettes, 0.2 ml of 1 in 100 dilution of the bacterial culture were added to 20 ml of the melted and cooled (45-50°C) nutrient agar. The contents were mixed by gentle swirling movements before being poured into clean, sterile petri dishes. After agar in plates solidified, 6 wells (7 mm each) were bored in each plate using aseptic cork borer. 1000 mg/ml, 500 mg/ml and 250 mg/ml of each extracts reconstituted in DMSO were filled in to the wells with the aid of Pasteur pipettes. Diameters of zones of inhibition were determined as an indication of activity after incubating the plates at 37°C for 24 hours for bacteria and at 25°C for 72 h for fungi. When seeded with bacteria, each plate had wells filled with DMSO. The antibacterial and antifungal studies were done using the previous procedures.[7] Ampicillin was used as a reference drug for antibacterial studies and for antifungal studies, voricinazole were utilized.

RESULTS

The results of phytochemical screening indicated the presence of alkaloids, glycosides, saponin glycosides, steroids and tannins (table 1). For the

antimicrobial activity the diameters of the inhibition zones were measured and recorded (table 2).

DISCUSSION

The leaf and stem extract of *C. borivilianum* displayed concentration dependent antibacterial activities and this was comparable to that of the reference drug ampicillin at 1 mg/ml as shown in table 2. Only the ethanol extract of the aerial parts of the plant inhibited the growth of bacteria at concentration of 1000 mg/ml and 500 mg/ml respectively. The petroleum extract of *C. borivilianum* was less sensitive to the bacteria at the test concentrations (table 2). The leaf extract showed inhibitory activity against *C. albicans* and *A. niger*. The methanol extract showed the highest antifungal activity and its activity at 1000 mg/ml and 500 mg/ml was higher than that of the reference antifungal drug, voricinazole (table 2). The results of this study confirm the use of this plant as remedies for analgesic, anti-inflammatory and arthritic conditions. There is an absolute need for bioactivity guided fractionation and isolation of the active components in the plant extracts. The methanol extract of *C. borivilianum* had impressive antibacterial and antifungal properties and there by could lead to the discovery of new molecules of antibiotics.

CONCLUSION

Thus it becomes more relevant as the current antibiotics are in use as of fast loosing effectiveness due to its emergence of resistant microorganisms. Thus further the active constituents which showed positive results in phytochemical can be further investigated for proper isolation and the exact mechanism will be screened for the potent biological action.

Table 1: Preliminary phytochemical screening of extracts

Plant Extract	*C.borivilianum* Leaf	*C. borivilianum* Stem
Alkaloids	+++	++
Glycosides	++	++
Saponin Glycosides	+++	+
Phenols	++	-
Tannins	++	+++

(-): Absent, (+): Slightly present, (++): Fairly Present, (+++) Abundant

Table 2: Antimicrobial activity of extracts

Extract	Concentration mg/ml	*S. aureus*	*B. subtilis*	*B. cereus*	*E.coli*	*C.albicans*	*A. niger*
C.borivilianum							
Leaf	250	+	+	ND	-	+	+
	500	++	-	ND	++	+	+
	1000	+++	+++	ND	++	+	+
Stem	250	+	+	ND	-	+	+
	500	++	-	ND	++	+	+
	1000	+++	+++	ND	++	+	+
C.borivilianum							
Petroleum-Ether	250	-	ND	-	-	-	-
	500	-	ND	-	-	+	-
	1000	-	ND	-	-	++	+++
Methanol	250	-	ND	-	-	-	-
	500	+	ND	+	-	+++	-
	1000	++	ND	+++	-	+++	+++
Control							

Ampicillin	1mg/ml	+++	+++	++	++	ND	ND
Voriconazole	1% w/v	ND	ND	ND	ND	+++	+++
DMSO	-	-	-	-	-	-	-

(ND)= not done, (+++)= high activity (>20 mm), (++) = relative high activity (14-19 mm), (+)= low activity (10-13 mm), (-) = no inhibition (< 10 mm)

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