

Phytochemical Analysis and Antimicrobial Activity of *Chlorophytum borivilianum* against Bacterial Pathogen causing disease in Humans

Syed Rehan Ahmad¹, Abul Kalam² and Kishan Pal³

^{1,2}Dept. of Microbiology, Bidhannagar Govt College, Sector-1, Salt Lake City, Kolkata, WB-64.

³Dept of Microbiology, Shri Venkateshwara University, Gajraula, U.P., 244236, India.

Corresponding author : Syed Rehan Ahmad; rehanbiotech1@gmail.com

Abstract

The present communication deals with in vitro analysis of Antibacterial activity of extract of leaves and stem of *Chlorophytum borivilianum* Santapau and Fernandez (Liliaceae). The result of the preliminary investigation revealed the presence of alkaloids, glycosides nucleus, Saponins and tannins in leaves as well as in stem. The antimicrobial activity of leaves and stem extract of *Chlorophytum borivilianum* was studied against four bacteria among them two are Gram -ve bacteria (*Escherichia coli* and *Klebsiella pneumonia*) and two are Gram +ve positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*.) by agar disc cup diffusion method. Zone of inhibition produced by different extracts was tabulated. Only the aerial parts of plant inhibited the growth of bacteria at the concentration of 1000mg/ml and 500 mg/ml respectively. Extract Showed maximum antibacterial activity against all organisms tested in order of sensitivity as *Staphylococcus* > *Bacillus subtilis* > *Klebsiella* > *Escherichia coli* .

Keywords: *Chlorophytum borivilianum*, safed musli, leaves and stem extract, antibacterial activity, zone of inhibition.

The WHO estimated that 80% of the world's population depends on traditional medicines for meeting their primary health care needs[1]. *Chlorophytum borivilianum* is a wonderful medicinal herb with sub-erect lanceolate leaves and tuberous root system belonging to the family Liliaceae. It can grow upto a maximum height of 40 to 47 cm. Tubers can grow upto a depth of 26 cm. *Chlorophytum borivilianum* is a little annual herb that grows proficiently in tropical and sub-tropical climates with altitudes upto 1500 meters (Kaushik 2005). In India 17 species of *Chlorophytum borivilianum* are found and 256 species are found over the home planet, and out of 17 species, 3 species namely as *Chlorophytum borivillianum*, *Chlorophytum arundinaceam* and *Chlorophytum tuberosum* are commercially cultivated by the Indian farmers but *Chlorophytum borivillianum* is the only species, which is under commercial cultivation.

Chlorophytum borivilium has good market both indigenously and globally. It is an annual crop capable of giving good returns to farmers under irrigated conditions. Safed musli is found growing in thick forests in its natural form. The roots of safed musli are reported to contain 2-15% saponin, which has the medicinal property of enhancing vitality and immunity to human beings. Because of its medicinal property, safed musli is known as *divya aushadhi* and ayurvedic plant. Mainly its tuberous roots are used in ayurvedic medicines. Safed musli is cultivated in most states of the country, the prominent amongst them being Madhya Pradesh, Maharashtra, Punjab, Andhra Pradesh etc. Based on agro climatic suitability, it can be cultivated in Eastern, Western, Central and Southern Plateau and Hill regions, East and West Coast Plains and Hill regions and Gujarat Plains and Hill regions comprising the states of Bihar, Orissa, Uttar Pradesh, Rajasthan, Karnataka, Kerala, Tamilnadu and Gujarat. The Medicinal Plants Board has recognized Safed musli as 6th important herb to be protected and promoted. The Board encourages mainstream cultivation of Safed musli by farmers by extending a subsidy of 20% through National Horticultural Board on project cost.

C. borivilium is a plant well known for its aphrodisiac as well as immunodilatory activity [2]. *C. borivilium* is traditionally used for treating oligospermia, pre- and postnatal infections, arthritis, diabetes and dysuria [3–5]. Its antiviral, anticancer, immunomodulatory, antidiabetic, antistress, and anti-inflammatory properties have been evaluated [6–11].

Safed Musli contains carbohydrates (35-45%), fiber (25-35%), alkaloids (15-25%), saponins (2-20%), and proteins (5-10%). It is a rich source of over 25 alkaloids, vitamins, proteins, carbohydrates, steroids, saponins, potassium, calcium, magnesium, phenol, resins, mucilage, and polysaccharides and also contains high quantity of simple sugars, mainly sucrose, glucose, fructose, galactose, mannose and xylose. Among them Saponin and alkaloids are chief medicinal compounds presents in the roots. As a lot research work have been done on roots of *C. borivilium* that is why our interest restricted in extract of leaves and stem of the said plant and its efficacy on bacteria .

Materials and Methods

Plant collection and authentication

The leaves (120 g) and stem (550 g) of *C. borivilium* was collected from the herbal garden of Shri Venkateshwra University, Gajraula, U.P and authenticated by Prof, Krishan Pal, Dept. Microbiology, Shri Venkateshwra University, Gajraula, U.P, India - 244236.

Plant preparation and extraction

The leaves and stem of *C. borivilium* was washed thoroughly under running tap water dried on paper towel then aerial parts of it blender, it was 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 (13.5% w/v), stems (21.4% 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 by^[3,4].

Microorganisms

Four human pathogenic bacteria made up of two Gram- positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram- negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) were used for the antibacterial assay, All the microorganisms were obtained from the laboratory stock, Dept. of Microbiology, Shri Venkateshwra University, Gajraula, U.P

Media

For culturing the bacteria we have used different media such as Nutrient broth, nutrient agar, sabouraud dextrose agar (SDA), tryptone soya broth, tryptone soya agar (Oxoid Laboratories, U.K) 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

We have used Ampicillin, 1mg/ml, as the standard reference drug for antibacterial assays

Preparation of bacterial cultures^[7, 8]

We have used the agar cup diffusion method 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.

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. When seeded with bacteria, each plate had wells filled with DMSO. Ampicillin was used as a reference drug for antibacterial studies.

Result

From the Table 1 it has been calculated that the leaves and stem of *C. borivilianum* contain the presence of alkaloids, glycosides, saponin glycosides, steroids and tannins. For the antimicrobial activity the diameters of the inhibition zones were measured and recorded in the Table 2, 3, 4, 5, and 6. The comparative study for diameter of inhibition zone for all four bacteria in different extract have been measured and recorded in Figure 1.

Table 1. Phytochemical analysis of extract

Phytochemical	<i>C. borivilianum</i> Leaf	<i>C. borivilianum</i> Stem
Alkaloids	+++	++
Glycosides	++	++
Saponins Glycosides	+++	+
Steroids	+++	+++
Phenols	++	-
Tannins	++	+++

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

Table 2. Antimicrobial Activity of Leaf extracts *C borivilianum*

<i>C.borivilianum</i> Leaf	Extract Conc. Mg/ml	Staphylococcus aureus	Escherichia coli	Klebsiella	S .sabtilis
	250	+	-	-	+
	500	++	-	+	-
	1000	+++	++	++	+++

(-) : No Inhibition (< 10 mm), (+) : Low activity (10- 13 mm), (++) : relative high activity (14-20 mm), (+++) : High Activity (> 20 mm), Not Done (ND)

Table 3 : Antimicrobial Activity of Stem extracts *C borivilianum*

<i>C.borivilianum</i> Stem	Extract Conc. Mg/ml	Staphylococcus aureus	Escherichia coli	Klebsiella	S. sabtilis
	250	+	+	+	+
	500	++	++	++	-
	1000	+++	+++	+++	+++

(-) : No Inhibition (< 10 mm), (+) : Low activity (10- 13 mm), (++) : relative high activity (14-20 mm), (+++) : High Activity (> 20 mm), Not Done (ND)

Table 4 : Antimicrobial Activity of extracts (Petroleum Ether)

<i>C. borivilianum</i> Petroleum Ether	Extract Conc. Mg/ml	Staphylococcus aureus	Escherichia coli	Klebsiella pneumoniae	S .sabtilis
	250	-	-	-	ND
	500	-	-	-	ND
	1000	-	-	+	ND

(-) : No Inhibition (< 10 mm), (+) : Low activity (10- 13 mm), (++) : relative high activity (14-20 mm), (+++) : High Activity (> 20 mm), Not Done (ND)

Table 5. Antimicrobial Activity of extracts (Methanol)

<i>C.borivilianum</i> Methanol	Extract Conc. Mg/ ml	Staphylococcus aureus	Escherichia coli	Klebsiella pneumoniae	S .sabtilis
	250	-	-	-	ND
	500	+	-	-	ND
	1000	++	-	+	ND

(-) : No Inhibition (< 10 mm), (+) : Low activity (10- 13 mm), (++) : relative high activity (14-20 mm), (+++) : High Activity (> 20 mm), Not Done (ND)

Table 6. Antimicrobial Activity in Ampicillin

Control Ampicillin 1mg/ml	Staphylococcus Aureus	Escherichia coli	Klebsiella Pneumonia	S. sabtilis
	+++	++	+++	+++

(-) : No Inhibition (< 10 mm), (+) : Low activity (10- 13 mm), (++) : relative high activity (14-20 mm), (+++) : High Activity (> 20 mm), Not Done (ND)

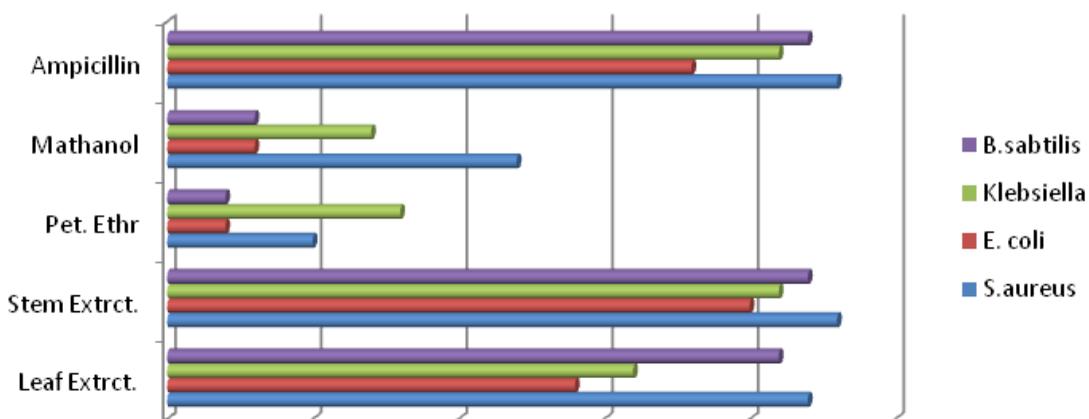


Figure 1. Comparative antimicrobial activity against different extracts of *C. borivilianum* in 1000 mg/ml

Discussion

In the present investigation strongly demonstrated that the *C. borivilianum* has potent antibacterial activity. The above result show that 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 6. 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 4). 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 not very impressive antibacterial properties (Table 5). This therefore becomes more relevant as the current antibiotics in use are of fast losing effectiveness due to its emergence of resistant microorganisms. The isolation of the components of the aerial parts of *C. borivilianum* methanol extract is in progress as very potent antimicrobial agents.

Conclusion

The above result in the table 1 to 6 and in the chart -1 showed that *C. borivilianum* have very potent antibacterial agent can be used as a potent antimicrobial agent for the treatment of diseases. Thus further work can be carried out on the isolation procedure for finding out the exact active moiety responsible for the biological activity

The extract of leaves and stem was tested against the two Gram positive and two Gram negative bacteria .

Finally it can be concluded that the leaves and stem extract of *Chlorophytum borivilianum* can be used effectively against certain bacteria causing disease in human beings as it is a potent antimicrobial agent .

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