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Bioactive Metabolites (Alkaloids, Flavonoids, Saponin, Steroids, Phenols and Tannins) Extracted from Stem and Roots of *Chlorophytum borivilianum* and their Antibacterial Activity

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Abstract: The present study deals with the role of certain bioactive metabolites (alkaloids, flavonoids, saponin, steroids, phenols and tannins) extracted from stem and root of *Chlorophytum borivilianum*, as well as *in vitro* analysis of the antibacterial activity of stem and root extracts of *Chlorophytum borivilianum*. Two gram-negative bacteria (*Escherichia coli* and *Klebsiella*) and gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) were taken to evaluate the effectiveness of alkaloids, flavonoids, saponin, steroids, phenols and tannins for antibacterial activity. Agar cup diffusion method and minimum zone of inhibition (MIZ) have been used to test the fractions for antimicrobial activity. It is concluded that both the extracts are very effective antibacterial agent, however, the stem extract are more potent as compared to root extract. It has also been noticed that both the extracts were effective against all four strain but it worked much better against gram positive bacteria as compared to gram negative bacteria. Further, it has been noticed that the order of susceptibility are as *Staphylococcus* > *Bacillus subtilis* > *Klebsiella* > *Escherichia coli*.

Keywords: *Chlorophytum borivilianum*, Safed musli, Stem extract, Root extract, Antibacterial activity, Zone of inhibition

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Introduction

According to WHO, eighty (80) per cent of world population depends on traditional drugs for their primary health care need (Purohit and Prajapati, 2003). Safed musli (*Chlorophytum borivilianum*; family Liliaceae) is a herb with sub-erect lanceolate leaves and tuberous root system. It can grow to a height of 45 cm. Tubers can grow to a

maximum depth of 25 cm. It is an annual herb, growing in tropical and sub-tropical climates having attitude up to 1500 meters. There are more than 200 species of *Chlorophytum*, but only 17 species are found in India. Among these 17 species, 3 species namely *Chlorophytum borivilianum*, *Chlorophytum arundinacean* and

Chlorophytum tuberosum are commercially cultivated by the Indian farmers. *Chlorophytum borivillianum* has market demand, both Indigenous and globally. It gives farmers a handsome returns annually. Safed musli grows in its natural form in thick forest. The roots of safed musli contains 2-15 % Saponin, which has medicinal property to enhance vitality and immunity in mankind. Safed musli is an ayurvedic plant, also known as divya aushadhi because of its medicinal properties. It is the root of safed musli which is mostly used in Ayurvedic medicines. Safed musli is cultivated in the entire country, but Madhya Pradesh, Maharastra, Punjab, Andhra Pradesh etc. produces major amount. It can also be cultivated in eastern, western, central and southern plateau and Hill regions, east and west coast plains and Gujrat Plains and Hills regions comprising the states of Bihar, Orissa, Uttar Pradesh, Rajasthan, Karnataka, Kerala, Tamilnadu and Gujrat depending on agro climatic suitability. Safed musli has to be protected and promoted as it is recognized as 6th important herbs by the Medicinal Plants Board. The Medicinal Plants Board encourages farmers for mainstream cultivation of safed musli, by extending a subsidiary of 20 per cent through natural Horticulture Board on project cost. *Chlorophytum borivillianum* is well known for its aphrodisiac as well as immunomodulatory activity (Shabi *et al.*, 1985). It has been traditionally used for the treatment of Oligospermia, pre- and post-natal infections, arthritis, diabetes and dysuria (Kokate, 1994; Harbone, 1998; Narayan and Shastry, 1988). The antiviral, anticancer, immunomodulator, antidiabetic, anti-stress and snit inflammatory properties of *Chlorophytum borivillianum* have been evaluated (Shabi *et al.*, 1985; Tandon *et al.*, 1990). It is rich in over 25 alkaloids, vitamins, proteins, carbohydrates, steroids, Saponins, Potassium, Calcium, Magnesium, Phenols, Resins, mucilage and polysaccharides and also contains high quantity of simple sugar, mainly sucrose, glucose, fructose, galactose, mannose and xylose (Indian Pharmacopoeia, 1996). Saponin and alkaloids are

prime medicinal components which are present in roots (Govindrajan *et al.*, 2005). There exists several researches on the roots of *Chlorophytum borivillianum* which provoked us to perform the present study which is restricted to the extract of leaves and stem of the plants and its effectiveness on bacteria (Jamal, 2005; Yunus, 2005)

Materials and Methods

Collection of Plant and Extract Preparation:

The plantlets were obtained from the medicinal garden of Shri Venkatesheara University, Uttar Pradesh, India. The verified plant samples were taken, washed with distilled water and cut into small pieces with help of sharp and sterilised scissor or blade. The small pieces of plant were dried, crushed and extracted with methanol in a 1: 10 ratio. Now the slury (a semi-liquid mixture) was filtered and the filtrate was subjected to evaporation under vacuum conditions. After evaporation a dried extract was obtained. The extract was weighed and resuspended in dimethyl sulphoxide (DMSO) and stored for future use. The percent yield was obtained after the extraction from the leaves (12 mg/g), stem (16 mg/g), root (14 mg/g) and purified saponin (11 mg/g).

Microorganisms Tested:

The bacterial strains used in the study included *Bacillus subtilis* (Bs), *Klebsiella pneumonia* (Kp), *Staphylococcus aureus* (Sa) and *Escherichia coli* (Ec) which were procured from IMTECH, Chandigarh, India.

Antimicrobial Activity Test:

Agar well diffusion method were used to test the antimicrobial activity test (Dabur and Gupta, 2001). During this test, methanol were used as negative and ampicillin were used as positive control. The bacterial inoculants concentration was 10⁶-10⁸ CFU/ml. For bacterial inoculants, Petri plates of nutrient agar were used. Plant extract of 0.5 mg were loaded in the wells and the plates were incubated at 37 °C for 24 h. Zone of inhibition (ZOI) was measured in millimeters

(mm) for antimicrobial activity .

MIC Test:

MIC determination was performed in 96-well microtiter plates using the broth dilution procedure (Dabur *et al.*, 2007). In this experiment, the appropriate microorganisms were inoculated into each well of the plate with 100 µl of culture medium, then 100 µl of antibiotic diluent was added and the plates were incubated at 37 C for 24 h for further experimental procedures to evaluate the minimum concentration of plant extracts for optimal suppression of bacterial pathogens. The study was carried out using a stock solution of 0.5 mg 5 mg/ml with a total volume of 100 µl of crude methanol extract and purified saponin together with control.

Results

Phytochemical analysis has been done for stem and root extract of *C. borivilianum* and it was found that the extracts contain the following bioactive metabolites: alkaloids, Flavonoids, Saponin, Steroids, Phenols and Tannins (Table 1). The diameters of the zone of inhibition (ZOI) were measured and recorded for the antimicrobial activity (Table 2). Antimicrobial activities of stem and root extract of *C. borivilianum* have been shown in Tables 3 and 4. Similar work has been done by Dabur *et al.* (2007). The antimicrobial activities Ampicillin for four microbes have been recorded in the Table 5. The performance of antimicrobial activity of extract in Petroleum Ether and Methanol were very poor and shown in Tables 6 and 7.

Discussion

The present study revealed that *Chlorophytum borivilianum* has potent antibacterial bioactive metabolites (Deora and Khadabadi, 2009). It has been noticed that the both stem and root extract showed antimicrobial property but stem extract was more potent than that of root extract. It has been noticed that methanol extract was better

Table 1: Phytochemical analysis of extract of stem and root of *C. borivilianum*

Phytochemicals	<i>C. borivilianum</i> Stem	<i>C. borivilianum</i> Root
Alkaloids	+++	++
Flavonoids	++	+++
Saponins	+++	++
Steroids	+++	++
Phenols	+++	+
Tannins	+++	+
(-) : Absent, (+) : Slightly present, (++) Fairly present, (+++) Abundant		

Table 2 : Zone of Inhibition (ZOI)

Bacterial Strain	Zone of Inhibition (ZOI)		
	Stem Extract (0.5 mg/ml)	Root Extract (0.5mg/ml)	Ampicillin 100 µg/ml
Gram-negative bacteria			
<i>Escherichia coli</i>	16.8±1.7*	18.5±0.5*	20.5±1
<i>Klebsiella pneumonia</i>	17 ± 1.1*	19.6±1.1*	22±1.2
Gram -positive bacteria			
<i>Bacillus subtilis</i>	24± 0.1 *	24.5± 0.6*	28 ± 1.4
<i>Staphylococcus aureus</i>	25.5±1.7*	28.5±1.3*	30 ± 1.2

* indicates significant responses antibacterial performer than that of petroleum ether. On comparison it has been evaluated that methanol showed relative high antimicrobial activity at higher concentration (500 and 1000) but petroleum ether extract did not give any response even at any concentration except *Staphylococcus aureus* which showed low activity at higher concentration (1000) (Tables 6, 7). The effects noticed in this study verify the use of this plant as treatments for analgesic, anti-inflammatory and arthritic conditions. There is an absolute need for bioactivity guided fractionation and isolation of the lively additives within the plant extracts. The results of present study showed that *C. borivilianum* has very potent antibacterial agent which can be used as a potent

Table 3: Antimicrobial activity of stem extract of *C. borivilianum*

<i>C. borivilianum</i> Stem	Extract Conc. mg/ml	Gram-negative bacteria		Gram-positive bacteria	
		<i>Klebsiella</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
	250	+	-	+	+
	500	++	+	++	++
	1000	+++	++	+++	+++

(-) : No Inhibition (< 10 mm) , (+) : Low activity (10- 13 mm) , (++) : relative high activity (14-20 mm),
(+++): High Activity (> 20 mm)

Table 4: Antimicrobial activity of root extract *C. borivilianum*

<i>C. borivilianum</i> Root	Extract Conc. mg/ml	Gram Negative Bacteria		Gram Positive Bacteria	
		<i>Klebsiella</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
	250	-	-	-	+
	500	++	+	+	++
	1000	+++	++	++	+++

(-) : No Inhibition (< 10 mm) , (+) : Low activity (10- 13 mm) , (++) : relative high activity (14-20 mm) ,
(+++): High Activity (> 20 mm)

Table 5: Antimicrobial Activity of Ampicillin

<i>Control</i> <i>Ampicillin</i> 1mg/ml	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumonia</i>	<i>Bacillus subtilis</i>
	+++	++	+++	+++

(++) : relative high activity (14-20 mm) , (+++): High Activity (> 20 mm)

Table 6: Antimicrobial Activity of extracts (Petroleum Ether)

<i>C. borivilianum</i> Petroleum ether	Extract mg/ml	Gram-negative bacteria		Gram-positive bacteria	
		<i>Klebsiella</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
	250	-	-	-	ND
	500	-	-	-	ND
	1000	+	-	+	ND

(-) : No Inhibition (< 10 mm) , (+) : Low activity (10- 13 mm) , (ND): Not Detected

Table 7: Antimicrobial activity of extracts (Methanol)

<i>C. borivilianum</i> Methanol	Extract mg/ml	Gram-negative bacteria		Gram-positive bacteria	
		<i>Klebsiella</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
	250	-	-	-	ND
	500	-	-	+	ND
	1000	+	-	++	ND

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

antimicrobial agent for the treatment of diseases.

Conclusion

The extracts of stem and root of *C borivilianum* were tested against the two gram-positive and two gram-negative bacteria. It is found that *C. borivilianum* is more active towards the gram – positive bacteria as compared to gram-negative bacteria. It has been also noticed that stem extract are more potent as compared to root extract. However, both the extracts can be used effectively against certain bacteria causing disease in human beings as both extract gave positive response and served as antimicrobial agent. It has been concluded that the antibacterial activity showed in order of susceptibility as *Staphylococcus* > *Bacillus subtilis* > *Klebsiella* > *Escherichia coli*.

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