



Research Journal of
**Medicinal
Plant**

ISSN 1819-3455



Academic
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Antibacterial Activities of Crude Extracts of *Chlorophytum borivilianum* to Bacterial Pathogens

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ABSTRACT

Antibacterial properties of different extracts of *Chlorophytum borivilianum* were studied. Ethanol, ethyl acetate, acetic acid and water were used to prepare the extract. The antibacterial activity of different extracts were carried out against four bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*, by agar cup diffusion method. Zone of inhibition produced by different extracts were measured. Acetic acid extract of *C. borivilianum* showed antibacterial activity against all the tested bacteria in the order of sensitivity as *Staphylococcus aureus*>*Pseudomonas aeruginosa*>*Escherichia coli*>*Bacillus subtilis*. The antibacterial activity of *Staphylococcus aureus* was sensitive with 6, 24, 12 and 8 mm zone of inhibition at 10 mg mL⁻¹ of water, acetic acid, ethanol and acetone extract respectively. For, *Pseudomonas aeruginosa* zone of inhibition is 8, 20, 12 and 10 mm for water, acetic acid, ethanol and acetone. *Escherichia coli* revealed no zone of inhibition for water extract whereas it possess 18, 10, 2 mm zones of inhibition at 10 mg mL⁻¹ for acetic acid, ethanol and acetone respectively. *Bacillus subtilis* showed 3, 20, 9 and 4 mm zone of inhibition at 10 mg mL⁻¹ for different extracts. These results showed that the extract has a wide range of antibacterial property than the other extracts.

Key words: *Chlorophytum borivilianum*, safed musli, antibacterial activity, medicinal plants, herbal extracts, minimum inhibitory concentration, agar well cup diffusion, zone of inhibition

INTRODUCTION

The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparation in ancient Indian literature and the preparations have been found to effective in the treatment of diseases (Sampathkumar *et al.*, 2008). Different antibiotics are available to cure different antibacterial diseases, but bacteria develop resistance against these antibiotics or they show side effects. Majority of medicinal plant species are rich in biomolecule contents which can cope with health hazard and recently, antibacterial activity of many plant species have been reported (Pandey and Mishra, 2010). Among these medicinal plants, Safed musli or *C. borivilianum* has been used therapeutically for centuries and is of particular interest due to lengthy historic reputation as a curative agent and its widespread use in complementary therapies. It is a well-known natural dietary supplement and chemopreventive agent. *Chlorophytum borivilianum*, (Liliaceae) commonly known as Safed musli is a genus of about

200-220 species of evergreen perennial flowering plants in the Liliaceae family, native to the tropical and subtropical regions of Africa and Asia (Kaushik, 2005). It is found in the oldest mountain ranges on the continent, the Aravalis from where it spread to the near-by areas of the sub-continent, presently known as the states of Gujarat, Rajasthan, Madhya Pradesh and the Central Deccan Plateau. They grow to 10-60 cm tall, with a rosette of long, slender leaves 15-75 cm long and 0.5-2 cm broad, growing from a thick, fleshy rhizome. The flowers are small, usually white, produced on sparse panicles up to 120 cm long; in some species the panicle also bears plantlets, which take root on touching the ground. Its tubers are used in Ayurvedic medicine. It contains about 30% alkaloids, Natural steroid saponin (10-20%), polysaccharides (40 to 45%), carbohydrates and proteins (5 to 7%) (Habeeb *et al.*, 2007; Tandon *et al.*, 1992; Deore and Khadabadi, 2008; Mayank and Dixit, 2008).

These compounds are the source of polysaccharides and possess antibacterial, antiviral, antifungal, antioxidant, antistress, angiogenic, immune system modulator, antidiabetic, hypolipidemic, antihypertensive, analgesic, antiinflammatory, antihepatitis, antigastric ulcer and aphrodisiac activities (Li *et al.*, 1990). Nevertheless, very few studies have been carried out on root extract of *C. borivillianum*. In this study, we determine the antimicrobial activities of *C. borivillianum* extract. Although a lot of work has been carried out on the medicinal applications of *C. borivillianum*, there is still little information on the uses of the root (O'Donnell *et al.*, 2006; Chakraborty and Aeri, 2009). This study therefore provides information on the antibacterial activity (against the microorganisms causing skin, upper respiratory tract, gastrointestinal and urinogenital tract infection) of *C. borivillianum* extract.

MATERIALS AND METHODS

Collection of plant material *C. borivillianum*: The plantlets were collected from local market in Allahabad and grown in Centre for Biotechnology Garden, University of Allahabad.

Preparation of extracts: The plantlets of *C. borivillianum* was washed thoroughly under running tap water dried on paper towel, then kept in oven at 60°C for proper drying and finally powdered. The dried powder of the plant (20 g) was dissolved in 100 mL of different solvents water, glacial acetic acid, ethanol, acetone (from higher polarity to lower polarity) and kept for 72 h. The extract was collected and filtered. This procedure is repeated three times for proper extraction. The extracts collected were pooled, evaporated to dryness by rotary evaporator. Then the residues were resuspended in DMSO stock concentration of 100 mg mL⁻¹ (Chakraborty and Aeri, 2009).

Microorganisms tested: The following microorganisms were used: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from National Collection of Industrial Microorganisms (NCIM) National Chemical Laboratory, Pune in December 2009 and were maintained on nutrient agar (Ghosh *et al.*, 2008). All the four bacterial pathogens are responsible for nosocomial diseases.

Determination of antimicrobial activity: The antibacterial susceptibility tests were carried out using agar diffusion method (Perez *et al.*, 1990; Deore and Khadabadi, 2009; Zhang *et al.*, 2004; Bonjar and Nik, 2004). The microorganisms were cultured overnight at 37°C in nutrient agar. The final cell concentrations of bacterial inoculants were 10⁶-10⁷ CFU mL⁻¹. Petri dishes with 60 mL of sterile nutrient agar were selected with the appropriate bacterial suspension. The extracts were

delivered into wells and the plates were incubated at 37°C for 24 h. The presence of zone of inhibition was regarded as the presence of antimicrobial action and antimicrobial activity was expressed in terms of average diameter of the zone of inhibition measured in millimeter. Each test was carried out in triplicate.

RESULTS

Whole plant extracts of *C. borivillianum* (both aqueous and ethanolic) tend to inhibit gram positive bacteria, *S. aureus* and *B. subtilis*. However the inhibitory activity was very low in aqueous extract (6.3 mm, Table 1) in comparison to glacial acetic acid extract (24-20 mm), ethanol extract (12-9 mm) and acetone extract (8-4 mm). Same pattern was also observed with gram negative bacteria, *E. coli*, *P. aeruginosa* (Table 1). Relatively higher MIC concentrations were obtained for gram negative bacteria *E. coli* (Table 1) with glacial acetic acid extract.

Surprisingly, no inhibitory effect has been noted for aqueous extract, this could be attributed to the extraction of active component of *C. borivillianum* in glacial acetic acid rather than water. Results show that glacial acetic acid extracts possess great inhibitory effect for gram positive bacteria, *S. aureus* followed by *B. subtilis* (Fig. 1). Among gram negative bacteria highest inhibitory effect was observed with *P. aeruginosa*, followed by *E. coli* (Fig. 1).

In this study, we have reported that glacial acetic acid extract of *C. borivillianum* plant has high antibacterial activity for gram negative as well as gram positive bacteria with a very low MIC.

Table 1: Antibacterial activity of *Chlorophytum borivillianum* extracts : S1 Water, S2 glacial acetic acid, S3 ethanol, S4 Acetone. Minimum inhibitory concentration (MIC) of S2 plant extracts against human pathogenic bacteria

Type	Bacteria zone of inhibition (mm)	Minimum inhibitory concentration of bacteria				MIC for S2 extract (mg mL ⁻¹)
		S1	S2	S3	S4	
Gram positive	<i>Staphylococcus aureus</i>	6.0	24	12	8.0	0.349
	<i>Bacillus subtilis</i>	3.0	20	9.0	4.0	0.273
Gram negative	<i>Escherichia coli</i>	- ^a	18	10	2.0	0.78
	<i>Pseudomonas aeruginosa</i>	8.0	20	12	10	0.349

-^a: No zone of inhibition

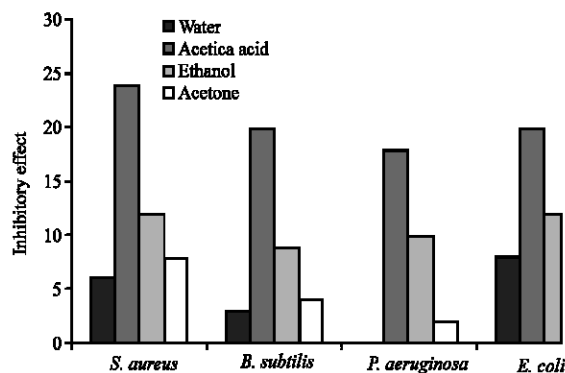


Fig. 1: Inhibitory effects of water, acetic acid, ethanol and acetone extracts of *Chlorophytum borivillianum* on different bacterial pathogens

DISCUSSION

The present study strongly demonstrated that the *C. borivillianum* has potent antibacterial activity. The above results show that glacial acetic acid extract has maximum antibacterial activity. The polar extract of roots and stems showed very good antibacterial than nonpolar extract (Chakraborty and Aeri, 2009). It has been reported that the methanolic extract of *C. arundinaceum* showed maximum inhibitory activity than chloroform extract (Valya *et al.*, 2009). According to, O'Donnell *et al.* (2006), the methanolic extract *Chlorophytum inornatum* shows maximum antibacterial activity. The different solvents hexane, chloroform, acetone and methanol were used to prepare extracts in which methanolic extract showed potent antibacterial activity (Dabur *et al.*, 2007). These findings show that phytoconstituents responsible for antibacterial activity are polar in nature and extracted in polar solvents only. The chemical compounds, mannans and saponins are the main phytochemicals which are responsible for the different medicinal property present in *C. borivillianum* (Mayank and Dixit, 2008).

CONCLUSION

The above results showed that *C. borivillianum* have very potent antibacterial agent. *Chlorophytum borivillianum*, has mannose and glucose which makes a mucilaginous layer around the urinogenital, gastrointestinal and respiratory tract when consumed orally. The layers trap the microbial flora and make them unable to invade the system. Therefore, the bacteria cannot grow in the media containing *C. borivillianum* extract. Thus from the above investigation it can be concluded that the plant *C. borivillianum* 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.

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