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RESEARCH ARTICLE

STUDIES ON THE ANTI-BACTERIAL AND ANTI-FUNGAL ACTIVITIES OF PLANT SAFED MUSLI
(*CHLOROPHYTUM BORIVILIANUM*)

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ABSTRACT

Chlorophytum borivilianum family Liliaceae is a traditional rare Indian medicinal herb widely used in the treatment of many clinical conditions in India. It is an important drug commonly known as 'Safed Musli'. It has many therapeutic applications in Ayurvedic, Unani, Homeopathic and Allopathic system of medicine. In the Ayurvedic literature, Safed Musli is celebrated as a Divya Aushad with unparalleled medicinal properties. *Chlorophytum borivilianum* is widely cultivated throughout India. Major antibacterial and antifungal activities reported from the roots of *C. borivilianum* include mainly antibacterial activities of *E.fecalis* cold, *E. fecalis* ethanolic and *E.coli* methanolic and antifungal activities of *A. fumigatus* methanolic, *A. fumigatus* ethanolic and *C. albicans* cold, *C. albicans* methanolic, *C.albicans* ethanolic. In this paper, an attempt has been made to explore various dimensions of the drug including antibacterial and antifungal activities studies carried out on this drug.

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INTRODUCTION

To trace the history of phytotherapy is to trace the history of humanity itself. Herbal medicine has evolved over centuries, depending on local flora, culture, and religion (Cassileth, 1998; Lans *et al.*, 2001; Cragg and Newman, 2001). This knowledge of plant-based drugs developed gradually and was passed on, thus laying the foundation for many systems of traditional medicine all over the world. During the past decade, there has been increasing acceptance and public interest in natural therapies in both developing and developed countries. Due to poverty and limited access to modern medicine, about four billion people, 80% of the world's population, living in developing countries use herbal medicine as their source of primary health care (Bisset, 1994; Farnsworth *et al.*, 1985). These medicinal plants species belong to a wide range of plant types, including trees, herbs, shrubs, lianas, woody climbers, Herbaceous climbers and twiners (Singh *et al.*, 2003).

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Safed musli (*Chlorophytum borivilianum*) is a herb, belongs to family Liliaceae. It was originally grown in thick forests of India. About 300 species are distributed throughout the tropical and subtropical parts of the world. Tropical and subtropical zones of Africa are the probable centres of origin of the genus. Seventeen species of *Chlorophytum* had been reported in India (Oudhia, 2007). All differ in medicinal properties but due to lack of correct information, all of them are called safed musli. In India, it is considered as a valuable medicinal herb, whereas in other parts of the world it is being used as ornamental plant. The roots (tubers) are rich in alkaloids, vitamins, minerals, proteins, carbohydrates, saponins, polysaccharides and steroids. It has various therapeutic values as total rejuvenator, antioxidant and Immunomodulator. It is being used as an anti arthritic and anticancer drug. Because of its aphrodisiac properties, it is mainly identified as 'Herbal viagra'. Safed musli is found in tropical moist and dry deciduous forests (Thakur *et al.*, 2009). It is widely distributed in India mainly in Southern Rajasthan, Western Madhya Pradesh, North Gujarat and few parts of Karnataka. But, continuous exploration has decreased its frequency, distribution and the quality.

The 'Safed Musli' complex is generally supposed to consist of *Chlorophytum borivilianum*, *C. arundinaceum*, *C. Tuberosum* and *Asparagus adscendens*. Among all these varieties *C. borivilianum* is cultivated on large scale in many parts of the county because it produces the highest yield and highest saponin content and used as Safed Musli. Although Indian forests are rich in 'Safed Musli', its demand is increasing rapidly in the Indian and international drug markets. According to a report in 2005-06, the demand for dry Safed musli is in the order of 35,000 tonnes per annum, the supply stands at 5,000 tonnes per year. Hence there is a strong need to understand the current scenario of its cultivation, description and its new medicinal properties.

Vernacular Name

Sanskrit : Swetha musli.
 Hindi : Safed musli, Hazarmuli, Satmuli
 Gujrati : Ujlimusli, Dholi musali
 Malayalam: Shedeveli, Shedheveli.
 Marathi : Safed musli, Sufed Musli, Kuli.
 Tamil : Tannirvittang, Tannirvittan-Kizhangu,
 Vipurutti, Taniravi thang
 Telgu : Tsallogadda, Swetha musli.
 Arabic : Shaqaqule-hindi
 Sinhalese : Hirtha-wariya, Mushali
 Garhwal : Jhirna
 U.P : Khairuwa
 Arabic : Shaqaqule.
 English : India spider plant, Spider plant
 (India), White musale.
 French : Chlorophytum medicinal

Ayurvedic description

Botanical name: *Chlorophytum borivilianum*

Sanskrit name : Swetha musli

Synonyms : Safed musli

Properties :

Rasa : Sweet, Bitter

Guna : Moist, Unctuous,

Heavy Virya : Cold

Vipaka : (post-digestive effect):

Sweet (Toth, 1994; Paques and Boxus, 1987).

Traditional uses

Traditionally, tubers are used in the treatment of rheumatism and the leaves as vegetable in various culinary preparations. It is traditionally used for its aphrodisiac properties in lack of libido male impotency, oligospermia. It is also widely used as a general health promotive tonic and for delaying the ageing process. Dried root powder increases the lactation amongst the feeding mothers and lactating cows. It also removes the knee pains within a week if taken daily with milk (Elizabeth, 2001, Singh and Chauhan, 2003). Leaves are eaten by the tribal people of Western Ghats as an expectorant. In the traditional diet of nursing mothers (after confinement) its powder is added in the preparation of laddoos (sweet prepared in ball form) to be taken as a energizing food. Efforts are on in countries like the USA and England to make chips/flakes with the tubers to use it as a nutritious item in breakfast. *C. borivilianum* has been described in ancient Indian literature such as Bhavaprakash nighantu, Rasendra Sarsangrah, Raja Ballabh Nighantu as 'Vajikaran' or aphrodisiac.

The roots of *C. borivilianum* are a constituent of 'Chyawanprash' an outstanding rejuvenator (Haque et al., 2011). It is known as the Indian Ginseng because of great therapeutic importance and its tubers are the major constituents of more than 100 ayurvedic preparations (Oudhia, 2000).



Whole plant of *C. borivilianum*



Roots of *C. borivilianum*

MATERIALS AND METHODS

Collection and identification of the Research Plant

The fresh roots of 'Safed musli' (*Chlorophytum borivilianum*) were collected from the local area surrounding Institute of Biotechnology, Patwadangar, Nainital. The plant material was washed first with running tap water and subsequently with RO water. The material was then spreaded over a blotting paper sheet and left for shade drying at room temperature. After complete drying, the dried leaves were powdered, weighed 65.6 gm and was kept safe in an air tight plastic container till used for further study.

Extraction of the *Chlorophytum borivilianum* roots

The roots were dried completely under shade for 20 days till the moisture content lost from the roots. Then, the roots were powdered by using a mixer till fine powder was obtained.

The powder weighed for and extractions were done by using organic and polar solvents. The organic solvents used were methanol and ethanol, whereas, cold aqueous and warm aqueous extraction were carried out on other hand. Five grams of powder was accurately weighed and filled in the Soxhlet tumbler, and Soxhlet apparatus based extractions were done for each organic solvent used here. The extraction was carried out till the colorless solution was noticed in the middle-chamber, understanding that the soluble component(s) were eluted with the action of the used organic solvent. Five grams of the fine powder was used for single extraction, with a solvent volume of 150ml to carry out the concerned extraction. For the cold aqueous type extraction, four grams of root powder was weighed and placed in a flask with 100 ml of the distilled water, kept in the shaker for 48 hours at 28° C. In contrary, for the warm water extraction with the same conditions maintained except the temperature was maintained at 50° C and kept for 1 hour.

Weight of extracts in different solvents

Solvent	Weight of extract
Cold	0.39g
Hot	0.76g
Ethanol	0.38g
Methanol	0.036

Screening of Antibacterial activity Microorganisms used

Strains of microorganisms used to susceptibility test were performed using five strains of microorganisms including Gram positive and Gram negative bacteria. These are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogens*, *Escherichia fecalis*.

Biochemical based screening of the desired Bacteria

The biochemical tests were performed next to the morphology observation of the microorganisms.

The test organisms were biochemically identified clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumonia*. They were obtained from the urine and throat samples of the people from Patwadangar and some biochemical tests were carried out to confirm the authenticity of their identities. Proper steps were taken to perform and obtain the biochemical tests results. For the TSI and Simmon Citrate tests, gram negative bacteria were inoculated in slants (table 4), whereas for the gram positive bacteria catalase, coagulase and oxidase tests were done for the right identification. The bacterial isolate was sub-cultured in nutrient broth for 24 hours. The plate 1, plate 2, plate 3 and plate 4 describes the gram staining of all the four test bacteria taken by an inverted microscope (model: Nikon 80i eclipse) and their biochemical test results are shown separately in plate 5 for TSI test, plate 6 and plate 7 for simmon's citrate test, plate 8 and plate 9 for MRVP tests, plate 10 and plate 11 for Indole tests, plate 12 and plate 13 for Urease tests done combined for *E.coli* and *P. aeruginosa*, and catalase tests were conducted for *S. pneumonia* (plate 14) and *S. aureus* (plate 15).

Appearance of the test Bacteria on MHA

In order to identify the colony morphology of the five test microorganisms, each isolate was streaked on the MHA plate to identify the growth features.

Identification tests for desired Microorganisms

Gram Staining

The Gram stain is used to classify bacteria on the basis of their forms, sizes, cellular morphology, and Gram reactions. It is a critical test for the rapid presumptive diagnosis of infectious agents and serves to assess the quality of clinical specimens.

Materials:

- Glass slides with frosted ends.
- Sterile normal saline
- Microbiological loops
- Immersion oil
- Stain solutions
 - a) Crystal Violet
 - b) Gram Iodine solution
 - c) Decoloriser
 - d) Safranin

Procedure

Smear Preparation: Take a loopful of specimen and spread it on the glass slide.

Smear Fixation:

- Airdry the smear on a flat surface.
- When smears are air dried, pass them two or three times through a flame. To avoid distortions do not overheat.
- Allow slide to cool before staining.

Staining

- Place the slide on staining glass rod
- Cover the smear with crystal violet stain and leave for 1 minute
- Wash with tap water
- Flood the smear with grams iodine solution and leave for 1 minute
- Decolorize the stain with alcohol for 20-30 seconds
- Wash with tap water
- Counter stain the smear with safranin for 10 seconds
- Wash with tap water
- Allow the test slide to dry
- Observe it under high power and finally under oil immersion.

Screening of Antifungal Activity Microorganisms used

Strains of microorganisms used to susceptibility test were performed using two strains of *Aspergillus fumigens*, *Candida albicans*.

RESULTS

Antimicrobial activity of Medicinal Plant

The results of antimicrobial activity of ethanol, methanol and aqueous extracts of *C. borivilianum*, agar well diffusion method.

Our study showed that aqueous extracts gave maximum zone of inhibition against *E. coli*, *C. borivilianum* in different concentration methanol 100mg/ml(18mm), methanol 75mg/ml(16), methanol 50 mg / ml (15) , methanol 25 mg / ml (14).

Antifungal Activity of Medicinal Plant

The results of antifungal activity of ethanol, methanol and aqueous extracts of *C. borivilianum*, agar well diffusion method.

Our study showed that aqueous extracts gave maximum zone of inhibition against *Candida albugens*, *C. borivilianum* in different concentration cold 100 mg / ml (24mm), cold 75mg / ml (23), cold 50 mg / ml (22), cold 25 mg / ml (21).

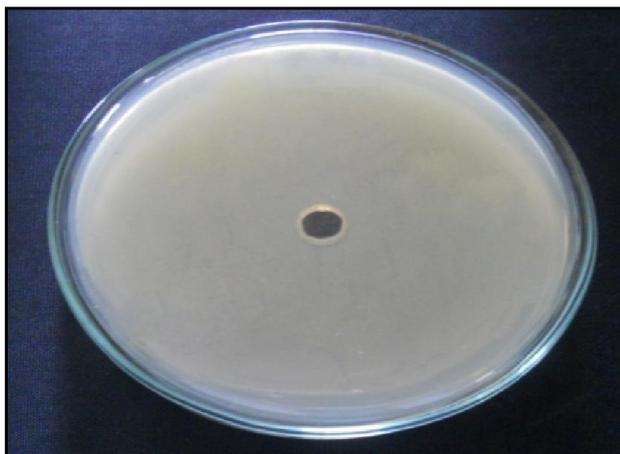
Anti Bacterial Sensitivity Test

The antibacterial activity of 1 commercial drug was assayed by Kirby-Bauer disc diffusion method. Approximately 80% of the recovered isolates were showed the sensitive activity against ciprofloxacin.

Antibacterial activity of various Plant Extracts

S.No.	Bacteria	Aqueous extracts				Organic extracts											
		Hot (mm)		Cold (mm)		E (mm)				M (mm)							
Zone inhibition of different concentration of plant extracts																	
		100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25
1.	<i>E.coli</i>	-	-	-	-	-	-	-	-	-	-	-	-	18	16	15	14
2.	<i>P.aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	<i>staphylococcus aureus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	<i>E. fecalis</i>	-	-	-	-	16	15	14	13	11	10	10	9	-	-	-	-
5.	<i>streptococcus pyogenes</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

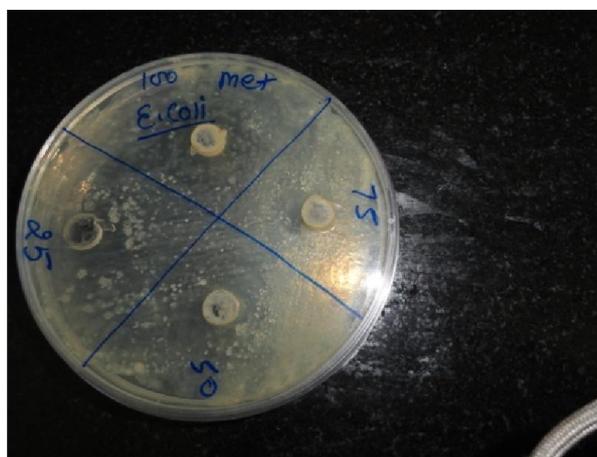
(-) indicates absence of antibacterial activity, (+) indicates presence of antibacterial activity
 Hot indicates cold water extract (10-15°C)
 Cold indicates hot water extract (65-75°C)
 (E) indicates ethanolic extract
 (M) indicates methanolic extract



Control



E. fecalis Cold



E. coli methanol



E. fecalis ethanol

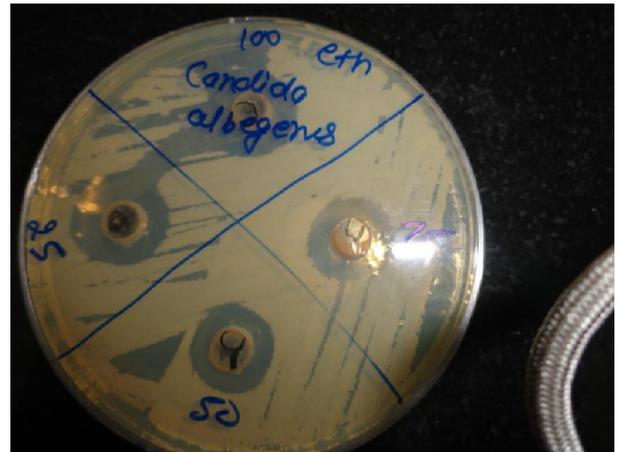
Antifungal Activity of plant extract

Fungus	Aqueous extracts						Organic extracts										
	HOT (mm)			COLD (mm)			E (mm)			M (mm)							
Zone inhibition of different concentration of plant extract																	
1. Aspergillus fumigatus	-	-	-	100	75	25	-	-	-	100	75	50	25	100	75	50	25
2. Candida albicans	-	-	-	24	23	22	21	18	17	16	15	22	21	20	19		

(-) indicates absence of antifungal activity, (+) indicates presence of antifungal activity
 Hot indicates cold water extract (10-15°C)
 Cold indicates hot water extract (65-75°C)
 (E) indicates ethanolic extract
 (M) indicates methanolic extract



Aspergillus ethanol



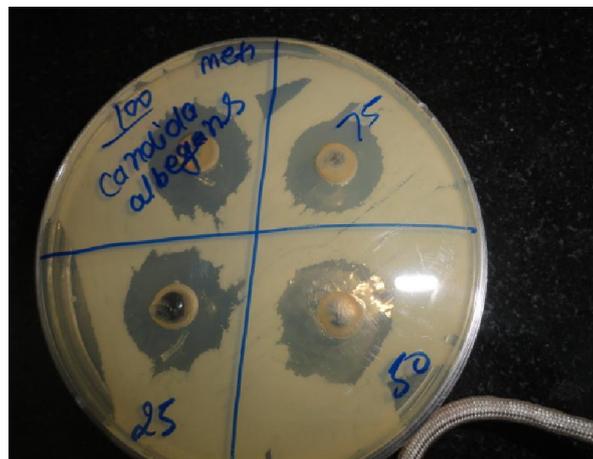
Candida albicans ethanol



Aspergillus Methanol



Candida albicans Cold

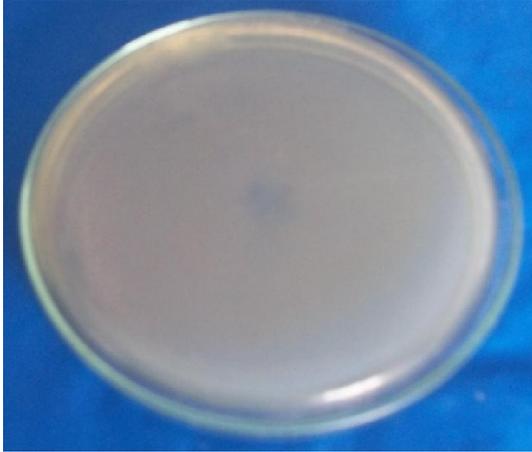


Aspergillus Methanol

The diameter of inhibition zones observed in 2 drugs namely ciprofloxacin. Among the antibacterial drugs tested ciprofloxacin showed maximum zone of inhibition against *Streptococcus pyogen* (22mm), *Staphylococcus aureus* (22mm), *Pseudomonas auregenosa* (26mm), *E. coli* (22 mm), *E. faecalis* (16 mm). *Streptococcus pyogen* have been found to be most susceptible against ciprofloxacin as revealed by the data, the maximum zone of inhibition was found in (22 mm).

Antifungal Sensitivity Test

The antifungal activity of 1 commercial drugs was assayed by Kirby-Bauer disc diffusion method. Approximately 80% of the recovered isolates were showed the sensitive activity against fluconazole. The diameter of inhibition zones observed in 1 drugs namely fluconazole (18mm) showed no inhibition zones against the growth of *Candida albicans*, *Aspergillus fumigens*.



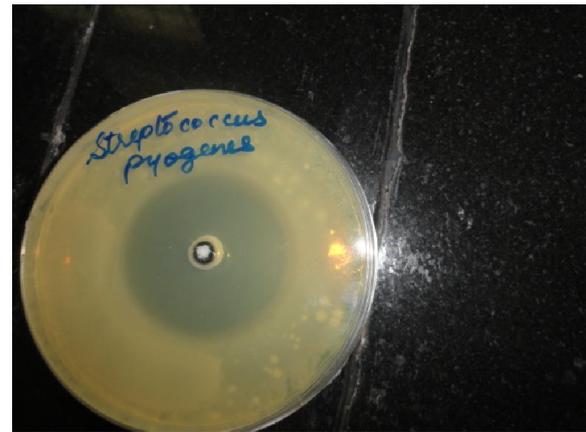
Control



E.coli



S.aureus



S.pyogen



E.faecalis

S. No	Isolated Bacteria	Zone of inhibition diameter(mm)		
		Ciprofloxin	Amoxicillin	Ofloxacin
1.	<i>E.coli</i>	22		19
2.	<i>P.aurogenosa</i>	26	19	15
3.	<i>S.pyogen</i>	22	18	18
4.	<i>S.aureus</i>	22	15	10
5.	<i>E.fecalis</i>	16	22	13



Candida albicans

S.No	Isolated Fungi	Zone of inhibition
		Fluconazole
1.	Aspergillus fumigens	18
2.	Candida albicans	24

DISCUSSION

Chlorophytum borivilianum family Liliaceae is a traditional rare Indian medicinal herb widely used in the treatment of many clinical conditions in India. It is an important drug commonly known as 'Safed Musli'. It has many therapeutic applications in Ayurvedic, Unani, Homeopathic and Allopathic system of medicine. In the Ayurvedic literature, Safed Musli is celebrated as a Divya Aushad with unparalleled medicinal properties. It is a chief ingredient in the preparation of over a hundred Ayurvedic formulations. *Chlorophytum borivilianum* is widely cultivated throughout India. The present study carried to determine the antibacterial and antifungal activity of *Chlorophytum borivilianum* (safed musli). In our study fresh plant part (rizomes) was collected from the natural habitat (IBT, Patwadangar, G.B.Pant University). *Chlorophytum borivilianum* antibacterial and antifungal activity checked out against *E. coli*, *Streptococcus pyogenes*, *E. fecalis*, *Pseudomonas*, *S. aureus*. This plant shows the antibacterial activity against *E. coli*, *E. fecalis* and have no activity against *Pseudomonas*, *S. Aureus*, *Streptococcus pyogenes*. In antifungal activity only *Aspergillus fumigans* and *candida albicans* shows the activity. Antibiotics ciprofloxacin and fluconazole used against bacteria and fungi respectively. Both show the positive results. The aqueous and ethanolic extracts of safed musli are antibacterial against *Bacillus subtilis* and *Staphylococcus aureus* (Sundaram *et al.*, 2011). According to Guno Sindhu Chakraborty 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. According to Dabur R

Water extracts of *Chlorophytum borivilianum* showed antimicrobial activity in a range of 75-1200 µg/m. Gugulothu Valya studied that invitro antimicrobial study indicated maximum range for the methanol extract of the five pathogenic bacteria tested while the minimum range of inhibitory activity 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 showed any antibacterial activity against *Bacillus subtilis*. In our study only methanol (100 mg/ml), ethanol (100 mg/ml) and cold (aqueous) extracts(100 mg/ml) of safed musli are antibacterial against *E. coli* and *E. fecalis*.

According to Gugulothu Valya in vitro antifungal study indicated maximum activity in methanol extract except the methanol extract for *A. fumigatus* against in which ethyl acetate showed maximum efficacy. According to Guno Sindhu Chakraborty the leaf extract showed inhibitory activity against *C. albicans* and *A. niger*. But in our study safed musli hot (100 mg/ml), methanol (100 mg/ml), ethanol (100 mg/ml) and cold (aqueous) extracts (100 mg/ml) were active against *Aspergillus fumigans* and *candida albicans*. This therefore becomes more relevant as the current antibiotics in use are of fast loosing effectiveness due to its emergence of resistant microorganisms. The isolation of the components of the rhizomes of *C. borivilianum* organic (ethanol and methanol) extract is in progress as very potent antimicrobial and antifungal agents.

Conclusion

Under the Indian system of medicine, safed musli has emerged out to be an extremely valuable gift of nature to mankind. As it has tremendous properties which can be utilised for health improvement of human beings, a special care should be taken in cultivation of *Chlorophytum borivilianum*, isolation of different phytoconstituents specially saponin, so true medicinal value of our indigenous medicinal plant can be explored. Thus from the above investigation it can be concluded that the plant *Chlorophytum borivilianum* can be used as a potent antimicrobial and antifungal agent for the treatment of diseases.

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