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Research Article

Phytochemical Screening and Antibacterial Activity of Leave and Stem Extracts of Chlorophytum Borivilianum

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ABSTRACT

Safed musli (chlorophytum borivilianum) holds great importance in traditional ayurvedic practices. The utilization of organic products is gaining in popularity. In many underdeveloped countries, traditional remedies and medicinal plants are commonly relied upon as a normative basis for promoting good health. Nevertheless, the identification of numerous medications and chemotherapy drugs derived from plant species, in addition to traditional herbal remedies, has been associated with an increasing dependence on the utilization of medicinal. The results revealed the presence of alkaloids, phenols, flavonoids, saponins, and glycosides. Phytochemical screening of Chlorophytum borivilianum (Safed Musli) reveals the presence of various secondary metabolites like alkaloids, glycosides, saponins, steroids, and tannins. The roots of this medicinal plant are a rich source of these compounds, with saponins being a key pharmacologically active constituent. The plant also contains free sugars, mucilage, hemicelluloses, and other polysaccharides. The extract of leaves and stem was tested against the two Gram positive and two Gram negative bacteria. It can be inferred that the leaves and stem extract of chlorophytum possess certain properties. Borivilianum can be used effectively against certain bacteria causing disease in human beings as it is a potent antibiotic.

INTRODUCTION

In India, extracts from many types of local plants are used in traditional manner for treatments of various ailments. The question is whether they are produced by the plant itself or are produced as a consequence of a mutualistic relationship with beneficial organisms in their tissue. There are many reports which show that in a microbe-plant relationship, endophytes contribute substances that possess various types of bioactivity, such as antimicrobial and antifungal. Exploitation of such endophyte - plant interactions can result in the promotion of plant health and can play a significant role in low input sustainable agriculture applications (Ryan et al., 2007).

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Though the utilization of this vast resource of poorly understood microorganisms had just begun, it has already become obvious that an enormous potential for organism, product and utilitarian discovery in this field holds exciting promise. In the present times, the medicinal and aromatic plants have attracted attention throughout the world for their therapeutic, flavour and perfume values. Since, not much of work has been done on the medicinal plants regarding endophytic microbe–plant interactions, the present study was proposed on one of the medicinal plant i.e. Chlorophytum borivilianum Sant et Fernand (Family: Liliaceae) commonly known as Safed musli in Indian Systems of Medicine is extensively used as a nutritive tonic for various purposes by the tribal and local people. While working on the documentation of Traditional uses and controversial identity of Indian Medicinal Plants, it came across that Safed musli is one of a very popular herbal drug used in variety of diseases and ailments. It also has an exhaled position as a Rasayana drug of Ayurvedic System of Medicine. The Rasayana groups of drugs are considered as adaptogen for promoting the longevity, prevent aging, and provide positive health and mental faculties (Anonymous, 1978). Under the name Safed musli, several plant species such as Asparagus adscendens and species of Chlorophytum namely C. tuberosum, C. borivilianum, C. arundinaceum etc. are used and sold in the market, fetching an attractive price Rs. 600-1200 per kg in the Indian Market (Maiti and Geetha, 2005). However, in the recent times, the roots / tubers / fingers of C. borivilianum are more acceptable source of Safed musli and cultivated on large scale in different parts of country for internal consumption and export purposes. Chlorophytum borivilianum is a small perennial herb with a condensed stem disc from which a whorl of leaves originates. It grows in moist places in plains and lower hills slopes and the natural distribution of C. borivilianum is Gujarat (Ahwa in Dangs) and Maharasthra (Borivilli, Kanheri Caves) and this species is considered endemic to these areas. Until few years back, the forests were very rich for these species. However, the continued and indiscriminate collection of plants resulted into fast depletion of natural populations. The drug is considered a valuable nervine and general tonic for strength and vigor. It is also used as a remedy for diabetes and arthritis and for natal and post natal problems. Recently the drug has been investigated for various pharmacological activities and chemical constituents. The ethanolic extracts of roots and sapogenins were evaluated for immunomodulatory activity and the effect of ethanolic extract was more pronounced as compared to sapogenins. The extracts not only potentiate non specific immune response but are also effective in improving humoral as well as cell-mediated immunity (Thakur et al, 2006). Safed musli (Chlorophytum borivilianum) is a commercially important medicinal plant in India. Cultivation of this crop in Lucknow, Pantnagar and adjoining areas in northern India was severely affected by a leaf blight disease in 2003 and 2004. Initial symptoms were the appearance of minute, pin-head, circular, reddish-brown lesions on the leaves, which form longitudinal streaks along the midrib, veins and margin of the infected leaves. Severely infected leaves dry prematurely and plants fail to produce healthy fingers (tubers) used for medicinal purposes. The disease occurred in August and September, after the onset of the rainy season, and caused losses of up to 30% in severely affected fields (Sattar et al., 2005). The crop is affected by many pests and pathogens (Banginwar et al, 2012) and the role of microbes especially the endophytic ones is poorly understood. Chlorophytum borivilianum is a small perennial herb. It has a condensed stem disc from which a whorl of leaves originates. It is generally found in moist places of plains and lower hills slopes and is presently cultivated in Gujarat, Madhya Pradesh and Maharasthra. Chlorophytum borivilianum is considered endemic to Maharasthra and Gujarat. Until few years back, the forests were the rich source for these species, however, continued and indiscriminate collection of plants resulted into

fast depletion of natural populations of this drug considered as a valuable nervine and general tonic.

Fig 1: Chlorophytum borivilianum (Safed musli)

Traditionally, Safed musli is well known for its therapeutic nature in the treatment of male impotency in India. Its remedial and nutritional importance is attributed by phytochemicals constituents particularly saponins (Thakur, et al., 2009). In recent times, C. borivilianum has achieved a well-established national and international market for being the herbal alternative of "Viagra" without any side

Cultivation

Safed musli is cultivated in most states of the country, the prominent amongst them being Madhya Pradesh, Maharashtra, Punjab, Andhra Pradesh, Tamil Nadu, Kerala, Karnataka, Rajasthan and Gujarat. Based on agro climatic suitability, it can be cultivated in eastern, western, central and southern plateau and also on hill regions. It can also be cultivated on east and west coast plains and hill regions comprising the states of Bihar, Orissa, Uttar Pradesh, Rajasthan, Karnataka, Kerala, Tamil Nadu 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 and even supports the project cost.

Phytochemical Composition of Safed Musli:

- Alkaloids:

  C. borivilianum is known to contain a variety of alkaloids, including over 25 different types.

- Glycosides:

  These are also present, contributing to the plant's medicinal properties.

- Saponins:

  Saponins are a crucial class of compounds in C. borivilianum, known for their unique "soap-like" properties and potential therapeutic applications.

- Steroids:

  Steroids, including beta-sitosterol and stigmasterol, have been identified in the plant.

- Tannins:

  Tannins are also present, contributing to the plant's potential antioxidant and antimicrobial activities.

- Other Compounds:

  The plant also contains free sugars, mucilage, hemicelluloses, and other polysaccharides.

MATERIALS AND METHODS:

Plant collection and authentication

The leaves and stem of C. borivilianum was collected from the Raisen district of Madhya Pradesh in the moth of Oct- Nov 2024 and authenticated by Dept. of Botany, SAM Global University, Raisen, M.P.

Plant preparation and extraction

The leaves and stem of C. borivilianum was cleaned under running tap water, dried on a paper towel, and then blended. The aerial parts were extracted using petroleum ether and methanol, and the mixture was macerated at room temperature for 72 hours. The macerated product was strained through a vacuum filter, and the resulting liquid was then dried at a lower pressure. The yield percentages of extracts from the leaves (13.5 % w/v) and stems (21.4 % w/v) were determined.

Phytochemical screening

The plant materials were dried and ground into a fine powder, then examined for the presence of any substances. Phytochemicals, glycosides, saponin glycosides, steroids and tannins using the method adopted by Trease, Evans and Harbourne.

Antimicrobial Analysis

The plant material (leaf and stem) of C. borivilianum allowed to dry and grinded by using mortar and pestle. Five to ten (5–10) grams of grinded material was dissolved in 50–100 mL of solvent (Petroleum ether, Benzene, Chloroform and Methanol) and kept overnight in an orbital shaker at 100 rpm and 28ºC. The extracts were then filtered with Whattman No. 42 filter paper (125 mm) and the resulting extract filtrate was used for further investigation,

Methods for Phytochemical Analysis

Test for alkaloids A small portion of the alcoholic extract was stirred separately with 1 ml of dilute Hydrochloric acid and filtered. The filtrate was treated with Dragandroff's reagent. Appearance of organic precipitate shows the presence of alkaloids.

Test for Cardiac Glycosides

Legal's Test Plant extracts were treated with sodium Nitro Pruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Test for Carbohydrates

Fehling's Test About 2mg of plant extracts was shaken with 10 ml of distilled water, filtered and filtrate was concentrated in separate test tube. An equal part of Fehling's Solution A and B (1 ml) were added to each and allowed for boiling for few minutes. Formation of red or brick colour precipitate indicated the presence of the reducing sugar.

Test for tannins and phenolic compounds

10 mL of extract was allowed to dry and later treated with10 mL of heated0.7% NaCl solution. It was subdivided into three portions. Toone of the portion of the test extract 1% NaCl solution was added, gelatine salt was added to another portion; precipitation in the second one indicates the presence of tannins. Addition of FeCl3 solution to the extract and gives green or blue green coloured precipitate, which is a positive result.

Test for phytosterols

1 g of the extract was treated with few drops of glacial acetic acid; followed by 3 mL of acetic anhydride, and at last few mL of concentrated sulfuric acid was added. Green colour formation is the positive test.

Determination of Antimicrobial Activity Testing of antimicrobial activity

4 different pathogenic bacteria consist 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. The cultures were obtained from NCIM, Pune, India.

Culture 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

For this study Ampicillin, 1mg/ml, as the standard reference drug for antibacterial assays were used.

Antibacterial Assay

Entophytic fungal extracts were screened for their antibacterial activity by disc diffusion plate method. All the test strains of bacteria were sub-cultured and maintained in nutrient agar media. Streptomycin (0.1µg/ml) was used to compare the antibacterial activity in fungal extract through disc diffusion method as described by Devi et al.

RESULTS AND DISCUSSION:

It has been observed that the leaves and stem of C. borivilianum contain the presence of alkaloids, glycosides, saponin glycosides, steroids and tannins (table 1) The extract of leaves and stem was tested against the two Gram positive and two Gram negative bacteria. The comparative study for diameter of inhibition zone for all four bacteria in different extract have been summarized in table 2 and table 3. This study 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 4. Only the ethanol extract of the aerial parts of the plant inhibited the growth of bacteria at concentration of 250 mg/ml and 500 mg/ml respectively. The petroleum extract of C.borivilianum was less sensitive to the bacteria at the test concentrations, therefore becomes more relevant as the current antibiotics in use are of fast loosing effectiveness due to its emergence of resistant microorganisms. The results of this study confirm the use of this plant as remedies for analgesic, anti-inflammatory and arthiritic conditions. There is an absolute need for bioactivity guided fractionation and isolation of the active components in the plant extracts.

Table.1 Phytochemical analysis of extract

Phytochemical	C. borivilianum Leaf	C. borivilianum Stem
Alkaloids	+++	++
Glycosides	++	++
Saponins Glycosides	+++	+
Steroids	+++	+++
Phenols	+	-
Tannins	+++	++
(-) : Absent , (+) : Slightly present , (++) Fairly present , (+++) Abundant		

Table.2 Table.1 Phytochemical analysis of extract

	Extract Conc. Mg/ml	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella*	*S .sabtilis*
C.borivilianum Leaf	250	++	-	+	++
	500	+++	-	+	+
(-) : No Inhibition (< 10 mm) , (+) : Low activity (10- 13 mm) , (++) : relative high activity (14-20 mm), (+++) : High Activity (> 20 mm)					

Table.3 Antimicrobial Activity of Stem extracts C borivilianum

	Extract Conc. Mg/ml	***Staphylococcus aureus***	***Escherichia coli***	***Klebsiella***	***S .sabtilis***
C.borivilianum Stem	250	+	-	+	+
	500	++	+	-	+
(-) : No Inhibition (< 10 mm) , (+) : Low activity (10- 13 mm) , (++) : relative high activity (14-20 mm), (+++) : High Activity (> 20 mm)					

Table.4: Antimicrobial Activity Of Ampicillin

	Staphylococcus Aureus	***Escherichia Coli***	***Klebsiella***	***S .Sabtilis***
Control Ampicillin 1mg/Ml	+++	++	++	+++
(-) : No Inhibition (< 10 Mm) , (+) : Low Activity (10- 13 Mm) , (++) : Relative High Activity (14-20 Mm), (+++) : High Activity (> 20 Mm)				

CONCLUSION:

Phytochemical screening of Chlorophytum borivilianum (Safed Musli) reveals the presence of various secondary metabolites like alkaloids, glycosides, saponins, steroids, and tannins. The roots of this medicinal plant are a rich source of these compounds, with saponins being a key pharmacologically active constituent. The plant also contains free sugars, mucilage, hemicelluloses, and other polysaccharides. The result of the antibacterial study as shown in table1 to table 4 exhibits that C. borivilianum is very potent antibacterial for the treatment of diseases. Further studies 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. It can be inferred that the leaves and stem extract of chlorophytum possess certain properties. Borivilianum can be used effectively against certain bacteria causing disease in human beings as it is a potent antibiotic.

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