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Phytochemical analysis and antibacterial activity of *Biophytum sensitivum*

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ABSTRACT

World is rich in knowledge with a wide diversity of medicinal plants which provide people with traditional healing methods for diseases in general healthcare area. The agar diffusion method was used to examine the antibacterial activity of different solvent extracts of *Biophytum sensitivum* tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Staphylococcus aureus*. For qualitative phytochemical investigation, several solvent extracts of the complete *Biophytum sensitivum* plant were investigated. The minimum inhibitory concentration (MIC) of *Biophytum sensitivum* ethanol extract had significant antimicrobial effectiveness against *Escherichia coli* (456 µg/ml), and *Pseudomonas aeruginosa* (419 µg/ml). The ethanolic extract showed (419 µg/ml and 398 µg/ml) antimicrobial activity against *Klebsiella pneumonia* and *Staphylococcus aureus* respectively. The lowest antibacterial activity was found in acetone extract with MIC (198µg/ml) of *Biophytum sensitivum*. Various parts of the plant has the presence of variety of phytoconstituents such as flavonoids, saponins, tannins, terpenes, steroids, amino acids, essential oil, polysaccharides and pectin.

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INTRODUCTION

In recent years, herbal products have gained popularity in industrialized countries as well as a number of other nations. According to the World Health Organization, 80 percent of people worldwide currently utilize herbal medicine for various kinds of primary healthcare (Mazid *et al.*, 2012). In ethnomedicine, a lot of the plants are utilized to treat a variety of illnesses. Antimicrobial drugs either eradicate or prevent the growth of microorganisms. Disinfectants are antimicrobial chemicals used on no-living items or outside the body parts.

The genus Biophytum is a member of the family Oxalidaceae and distributed in tropical Asia, Africa, America and the Philippines. In India, nine species of Biophytum are prominently found and out of these, three species viz. *Biophytum sensitivum* DC. Syn. *Oxalis sensitivum* Linn., are reported to have ethnomedicinal potential. *Biophytum sensitivum* (L.) DC (*B. sensitivum*), commonly known as 'Life plant', is a mesophytic under-shrub growing in slightly moist places. The plant is distributed up to an altitude of 1,800 m and is available during the rainy season in moist shady places (Warrier *et al.*, 1994). The plant is traditionally used for centuries in the treatment of various health aliments. It is an important medicinal plant in the Indian traditional system of medicine like Ayurveda (The Wealth of India, 1988).

Microorganisms play an important role in the manufacture of bioactive small molecules from natural resources for the prevention of several diseases and the creation of effective medications. The acetone extract of leaves of *B. sensitivum* had significant antifungal activity. The leaf extract of *B. sensitivum* inhibited the growth of fungal pathogens *A. fumigatus, A. niger, C. neoformans* and *Norcardia* sp. in disc method (Vijayan *et al.*, 2010).

Numerous medications have caused microbes to become resistant, which presents a substantial therapeutic issue in the management of infectious diseases. The overuse of commercially available antimicrobials, which are routinely used to treat illnesses, led to the development of the bacteria' tolerance (Lewis and Ausubel, 2006). In order to find new antimicrobial chemicals, researchers were motivated to investigate in other sources, particularly herbal resources

MATERIALS AND METHODS

Collection and identification of plant material

For the study, the whole plant of *Biophytum sensitivum* belongs to Oxalidaceae family was collected from Kerala, South India.

The whole plant were identified taxonomically and authenticated according to various literatures, Flora of Madras Presidency and Wealth of India including other pertinent taxonomic literature.

Preparation of plant materials and extract

The leaves were carefully cleaned, shade dried and powdered. The powdered material was stored in a closed air-tight plastic container at low temperature. The powdered plant material (50 g) was extracted with 300 mL of each solvent ethanol by maceration (3×24 h) at room temperature. The collected solvents were concentrated by rotary vacuum evaporator at 45°C and then dried using a freeze dryer. All extracts and acyclovir (extracted from commercial tablet) were dissolved in dimethyl sulphoxide (DMSO). The final concentration of DMSO was 0.1% v/v in cell culture environment.

Phytochemical analysis

The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc. Chemical tests were identifying the phytochemicals as described (Trease and Evans, 1983; Harborne, 1973). Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Test microorganisms

Escherichia coli, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Staphylococcus aureus* were used as test organisms in the current study. For the current experiment, the obtained cultures were repeatedly subcultured.

Antimicrobial activity by agar diffusion method

The antibacterial efficacy of several *Biophytum sensitivum* solvent extracts was assessed using the agar diffusion method. For spreading agar media, a subcultured microbial suspension (100 µl) was prepared. Various concentrated varied extracts were used to measure antimicrobial activity (Magaldi *et al.*, 2004). The plates were filled with the sample and then left to allow for an hour to enable the extract to disperse. The plates were maintained in an incubator for 24 hours at 37°C, and the inhibitory zone was measured in millimeters (mm). Results are compared with those of conventional antibacterial drugs.

RESULTS AND DISCUSSION

Phytochemical studies of *B. sensitivum* showed that it contains a number of phenolic and polyphenolic compounds, saponin, essential oil, polysaccharides and pectin. The main bioactive constituents found are bioflavonoid, amentoflavone with minute amount of cupressoflavone (Abinash *et al.*, 2012). All of the extracts from the *Biophytum sensitivum* contained saponin, phenols, tannins, glycosides, terpenoids, flavonoids, alkaloids, and coumarins, according to a preliminary phytochemical examination. With the exception of the chloroform extract's lack of saponins, glycosides, and coumarins and the extract from ethyl acetate's absence of saponin. The results of the phytochemical analysis are displayed in Table 1. A higher degree of biological activity derives from the presence of a high concentration of phytochemicals in the plant.

This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized (Sofowora, 1993). In the present study, it was clearly understood that the ethanolic extracted maximum amount of the different type of metabolites present in the Biophytum sensitivum. Boominathan and Ramamurthy (Boominathan and Ramamurthy, 2009) reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

Table 1. Qualitative phytochemical screening on extracts of *Biophytum sensitivum*

Name of test	Test applied / Reagent used	Ethanol	Water	Chloroform	Hexane	Acetone	Ethyl acetate
Alkaloids	A] Mayer's	+++	++	++	++	+++	++
	B] Wagner's	+++	++	++	++	+++	++
	C] Hagner's	+++	++	++	+++	+++	++
	D] Dragndorff's test	++	++	++	++	++	+
Flavonoids	HCl and magnesium turnings	+++	++	+	++	+	++
Carbohydrate	Molisch's test	+	+	+	+	+	+
Tannins & phenols	A] 10% Lead acetate	+++	+	++	++	++	++
	B] $Fecl_3$	+++	+	++	++	++	++
Test for steroids	A] Salkowski's test	++	++	++	++	++	++
	B] Libermann-Burchard's test	++	++	++	++	++	++
Gums & mucilages	Alcoholic precipitation	-	-	-	-	-	-
Fixed oil & fats	Spot test	+	-	+	+	-	-
Saponins	Foam test	+	+	+	+	+	+
Phytosterols	LB test	+	+	+	+	+	+
Volatile oils	Hydro distillation method	+	+	+	+	+	+
Protein & free amino acids	A] Biuret test	++	++	++	++	++	++
	B] Ninhydrin test	+++	++	++	++	++	++
	C] Xanthoprotein test	+++	++	++	++	++	++

Table 2. Antimicrobial activity of the extracts *Biophytum sensitivum*

Microorganism	Minimum inhibitory concentration (MIC)							
	Extract of *Biophytum sensitivum* (µg/ml)							
	Ethanol	Water	Chloroform	Hexane	Acetone	Ethyl acetate	Gentamicin (µg/ml)	Cephalosporins (µg/ml)
P. aeruginosa	456	368	279	245	206	298	ND	48
K. pneumonia	419	348	267	228	198	256	ND	46
E. coli	423	352	311	236	217	312	ND	56
S. aureus	398	325	246	217	201	248	34	ND

For instance, the presence of flavonoids suggest that the plant have been reported to exert multiple biological effects including, anti-allergic, anti-inflammatory, anti- microbial antioxidant, anti-cancer activity (Kunle and Egharevba, 2009). It also suggests that the plant might have diuretic properties (Jayvir *et al.*, 2002). The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and anti-bacterial activities and can relief in wound healing and burns (Haslem, 1989). Saponins and glycoside are also very important classes of secondary metabolites as some are cardio-active and used in treatment of heart conditions (Oloyode, 2005). Some researchers have also investigated that some saponins have anti-cancer and immune modulatory properties (Evans, 2002). Volatile oils are used in the industries for various purposes, both as a pharmaceutical/ cosmetic raw material for production of emollients and active ingredient for the respiratory tract infections.

The antibacterial properties of various solvent-based extracts of *Biophytum sensitivum* are displayed in Table 2. The ethanol extract of *Biophytum sensitivum* had the highest antimicrobial activity with MIC (423 µg/ml) against the *Escherichia coli*, (456 µg/ml) against the *Pseudomonas aeruginosa*, (419 µg/ml) against the *Klebsiella pneumonia* and (398 µg/ml) against the *Staphylococcus aureus*. The various extracts of *Biophytum sensitivum* tested against *Escherichia coli* and showed considerable MIC results in water extract (352 µg/ml), chloroform extract (311 µg/ml), ethyl acetate extract (312 µg/ml), acetone extract (217 µg/ml), hexane extract (236 µg/ml). The results were compared with standard Cephalosporins as reference compounds with MIC (56 µg/ml). The different extracts of *Biophytum sensitivum* were checked against the *Pseudomonas aeruginosa* and exhibited significant MIC values in water extract (368 µg/ml), chloroform extract (279 µg/ml), ethyl acetate extract (298 µg/ml), acetone extract (206 µg/ml), hexane extract (245 µg/ml). The obtained results were compared with Cephalosporins with MIC (48 µg/ml).

The individual extract of *Biophytum sensitivum* was checked against *Klebsiella pneumonia* and found impressive MIC values in water extract (348 µg/ml), chloroform extract (267 µg/ml), ethyl acetate extract (256 µg/ml), acetone extract (198 µg/ml), hexane extract (228 µg/ml). The different solvent extract of *Biophytum sensitivum* was evaluated against *Staphylococcus aureus* and found impressive MIC values in water extract (325 µg/ml), chloroform extract (246 µg/ml), ethyl acetate extract (248 µg/ml), acetone extract (201 µg/ml), hexane extract (217 µg/ml). The gentamicin (34 µg/ml) was used as a standard compound.

A considerable inhibitory zone may also be caused by the variety of phytochemicals present in the extract. The presence of different flavonoids, alkaloids, terpenoids, phenols, saponins, and coumarins has bactericidal properties (Kalidindi *et al.*, 2015). According to various scientific studies, high concentrations of phytochemicals and bioactive compounds are thought to have a stronger potential for treating a variety of pathogenic bacteria. Numerous plants and their various portions of them have historically been used to treat a variety of chronic illnesses, such as gastrointestinal problems, urinary tract infections, skin conditions, and various respiratory issues, etc. (Alzoreky and Nakahara, 2003). Several chronic illnesses caused by various bacteria may be prevented and managed with the use of plant-based remedies. Many societies still employ

ethnomedicines to treat illnesses and overcome obstacles without creating negative side effects. The inclusion of several phytoconstituents, including alkaloids, flavonoids, coumarins, saponins, polyphenols, tannins, and terpenoids, is what gives herbal preparations their therapeutic effects (Bhalodia and Shukla, 2011). The presence of secondary metabolites prevents the growth of harmful microorganisms causing serious diseases (Mathew *et al.*, 2016). The microorganisms are resistant to many antibiotics that is very harmful to humans. The researchers are finding an alternative to commercial antibiotics to prevent harmful infections against a variety of microorganisms using plant-based medicines (Khan *et al.*, 2013). The higher concentration of crude extracts sometimes may cause cytotoxicity in humans hence the dose-dependent values are determined using in vitro cell cytotoxicity assay (Mathew *et al.*, 2016). As compared to commercial antibiotics, plant-based medicines have very small side effects if they are consumed in excess quantity (Talib and Mahasneh, 2010). In the world, 80 % of different pharmaceuticals are prepared from plant-based medicines and which are effective to cure any chronic disease.

CONCLUSION

Biophytum sensitivum used for its wide therapeutic potential of antimicrobial agents. According to the studies, ethanol extract has the most potential, which may be because it includes the majority of the phytochemical compounds and bioactive compounds that have antibacterial activity. The complete plant extract of *Biophytum sensitivum* has to be further studied in order to identify and purify chemicals that might be used as natural medicinal alternatives to synthetic commercial ones. The future aspects of the plant can be anti microbial as it contains many of the phytochemicals and work has not been performed yet.

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