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Evaluation of phytochemical standardization and antioxidant activity of leaf part of *Biophytum sensitivum*

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Abstract

The present study undertaken to explore pharmacognostic profile of the medicinally important plant *Biophytum sensitivum* belongs to the family *Oxalidaceae*. The plant is extracted with petroleum ether, chloroform, ethyl acetate, ethanol and water using soxhlet extraction method. Various physicochemical characters are analysed as per WHO guidelines. Antibacterial activity of plant extract was tested against *Bacillus subtilis*, *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Proteus vulgaris*. Antioxidant activity was analysed by DPPH method. Antioxidant activity of ethanol extract of leaf part of *Biophytum sensitivum* shows maximum activity 82% at the concentration of 500µg/ml. The results revealed that the ethanolic extract of leaf part of *Biophytum sensitivum* posses potent antioxidant activity. From the result it also be concluded that the leaf extract of *Biophytum sensitivum* can be a potential candidate and suit to be an agent to reduce oxidative stress.

Keywords**:** *Biophytum sensitivum, phytochemical, antibacterial, antioxidant, physicochemical character*

1. Introduction

Medicinal plants in traditional systems of medicines produce a miscellaneous range of bioactive molecules, making them a rich source of drugs. Medicinal plants give slow recovery, the therapeutic use of medicinal plant is becoming popular because of their lesser side effects and low resistance in microorganisms (1-6). Medicinal activity of plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins and phenolic compounds. These phytochemicals present in the plants play a vital role in contributing them the antimicrobial action. Phytochemical function as antioxidants that react with the free oxygen molecules or free radicals in our bodies. Free radicals can damage the cell of our bodies and must be removed.

Biophytum sensitivum belongs to the family *Oxalidaceae* commonly known as "little tree plant" due to its miniature, tree like appearance is a reputed medicinal plant in traditional system of medicine especially Ayurveda. It is used in a variety of pathological complications namely stomach ache, asthma, insomnia, convulsions, cramps, chest complaints, inflammation, tumors and remedying chronic skin diseases. It posses various pharmacological activities including radio-protective activity, immune modulatory activity, cardioprotective, anti-tumor activity, wound healing activity (7-9).

2. Materials and Methods

2.1. Collection and Identification of plant material

Biophytum sensitivum was collected from Marthandam, Kanyakumari District, Tamilnadu, India in the month of December 2015 and identified using standard key and voucher specimen of this plant was deposited herbarium at Scott Christian College, Nagercoil (Voucher no. SCCN: 4402). The leaf part was washed and air dried over a period of one month. The dried samples were milled into a fine powder by pounding manually with a clean sterile mortar and stored in cellophane bags till further use.

2.2. Extraction

About 100 gm of leaf part of *Biophytum sensitivum* was extracted in a soxhlet apparatus successfully with petroleum ether, chloroform, ethyl acetate,

ethanol and water by increasing order of polarity for 24 hours. The sample is concentrated under reduced pressure using vaccum pump. The powdered forms of the extracts were obtained and it is weighed, kept in a labeled sterile specimen bottles (10-16).

2.3. Determination of physicochemical parameters

The physicochemical character of powdered leaves part of *Biophytum sensitivum* was determined as per World Health Organisation guidelines (17). Different extracts of plant material obtained were subjected to various phytochemical investigation to detect the chemical constituents present in them (11-16). Plant material is subjected to fluorescence powdered drug analysis which is performed under visible, short UV and long UV using Chase and Pratt method (18). The fluorescence character of different extract were observed using day light, short UV and long UV light (19).

2.4. Heavy metal analysis

About 0.2 gm of the sample is taken in a volumetric flask and 4 ml of concentrated nitric acid is added and the solution is allowed to stand for few hours. Then carefully heated over water bath till the red fumes are comes out from the flask carefully. The solution is allowed to cool and 4 ml of perchloric acid is added. Then the flask is heated over water bath to evaporate till small portion which is then filter through whatmann filter paper and made up to 100 ml using deionized water. The heavy metal analysis was carried out by using SL 243 Double beam Atomic Absorption Spectrophotometer at Scott Christian College, Nagercoil.

2.5 Antibacterial assay

Tested microorganisms:

The bacterial Microbial Type Culture Collection (MTCC) strains were used for antibacterial studies i.e. *Bacillus subtilis*, *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Proteus vulgaris* obtained from Chandigarh, India.

Disc Diffusion assay

About 0.2 ml of bacterial culture of respective strains poured in sterile 9 cm petridisc containing 10 ml of Nutrient agar medium and spread over agar plates using sterile glass L-rod. 0.2 ml of each extracts were applied per filter paper disc and allowed to dry. Then placed in the top layer of the agar plates and incubated at 37^{o}c for 24 hrs. Streptomycin is used as a positive control. The experiments were carried out in triplicate. The average diameter of zone of inhibition were recorded [20].

2.6 Antioxidant activity assay

DPPH assay: (2,2-diphenyl-1-picrylhydrazyl)

The Radical Scavenging Activity of different extracts were determined by using DPPH assay. The decrease of absorption at 517 nm of the DPPH solution after the addition of antioxidant was measured in a cuvette containing 200 µl of methanolic DPPH solution mixed with 100 to 500 µg/ml of plant extract and vortexes thoroughly. All the test solution is incubated at room temperature for 30 minutes. Ascorbic acid was used as a reference. The ability of the plant extract to scavenge DPPH radical was calculated by the following equation.

Radical Scavenging Activity (%) = (absorbance at blank – absorbance at test/absorbance at blank) × 100

3. Result and Discussion

3.1. Physico-chemical parameters

Moisture content of leaf part of *Biophytum sensitivum* is 6.1 %. *Biophytum sensitivum* contain total ash value is 11.4 %. Acid insoluble ash value and water insoluble ash value is 4.6% and 6.6%. Extractive value increases with increase in solvent polarity. Table 1 exhibit physicochemical parameter of powdered form of leaf part of *Biophytum sensitivum*.

Table 1: Physico-chemical parameters

WHO Parameters	Average values % w/w aerial
Moisture content	68.1
Total ash content	11.4
Acid insoluble ash	4.6
Water insoluble ash	6.6
Extractive values	
Petroleum ether	18.2
Chloroform	20.4
Ethyl acetate	24.7
Ethanol	31.5
Water	35.1

3.2. Elemental Analysis

Iron is an essential trace element for animals and human beings. Iron content is 0.007 ppm in *Biophytum sensitivum* leaf part. Recommended Dietary Allowance for iron is 10 mg/day for adult males and 15 mg/day for adult females with an additional 15 mg/day recommended during pregnancy. The important role of zinc is growth and development. Zinc metal concentration is 0.034 ppm. The concentration of copper was recorded in *Biophytum sensitivum* leaf part is 0.017 ppm. RDA of copper is 1.5 - 3.0 mg/day for adult. Estimated safe and adequate dietary intake of Cr III is 50-200 µg for adult. The concentration of chromium was recorded as 0.023 ppm. The presence of lead, nickel and cobalt concentration is 0.006, 0.001 and 0.300 ppm respectively.

3.3 Phytochemical screening

Biophytum sensitivum leaf part of ethanol extract contain alkaloid, carbohydrate, glycosides, flavonoid and tannins. Ethyl acetate extract contain more number of phytochemicals such as alkaloid, carbohydrates, glycosides, saponins, phytosterol, phenol and tannins. From the result ethyl acetate extract contain more number of phytochemical when compared with other four extract. But chloroform extract and water extract exhibit least number of phytochemicals. Plant material having phenol, tannin and flavonoid content are potential antioxidant agent and they neutralize the free radical through donation of hydrogen atom, quenching of oxygen and by chelation of metals that minimize oxidative stress (21-24).Qualitative phytochemical activity of five different extract are shown in Table 2.

Table 2: Qualitative phytochemical estimation

Chemical constituents	Tests	P	C	EA	E	W
Alkaloids	Mayer's test	+	+	+	+	-
	Wagner's test	+	+	+	+	-
	Dragendorff's tet	+	+	+	+	-
Carbohydrates	Molish's test	-	+	+	+	-
Glycosides	Modified Borntrager's test	-	-	+	+	-
Cardiac glycosides	Legel's test	+	+	+	-	-
Saponins	Foam test	+	+	+	-	-
Phytosterol	Libermann Burchard's test	+	+	+	-	-
Diterpene	Copper acetate test	+	-	-	-	-
Triterpene	Salkowski's test	+	-	-	-	-
Phenols	Ferric chloride test	-	+	+	+	-
Flavonoids	Alkaline reagent test	-	-	-	+	+
	Lead acetate test	-	-	-	+	+
	Zinc HCl test	-	-	-	+	-
Proteins	Xanthoproteic test	-	-	-	-	-
Amino acid	Ninhydrin test	-	-	-	-	-
Resin	Acetone-water test	-	-	-	-	-
Fixed oil & Fat	Stain test	-	-	-	-	-
Tannins	Gelatin test	+	-	+	+	+

P-Petroleum ether, C-Chloroform, EA-Ethyl acetate, E-Ethanol, W-Water

3.4 Fluorescence analysis

Fluorescence analysis is one of the important pharmacological parameter and exhibit quality and purity of the drug. Table 3 implies the fluorescence character of powder as well as extract form of *Biophytum sensitivum* using different reagents.

Table 3: Fluorescence analysis of leaf part of *Biophytum sensitivum*

Treatment	Visible	Short wave (254 nm)	Long wave (365 nm)
Powder as such	Green	Green	Green
Powder + HCl	Green	Green	Yellow
Powder + H_2SO_4	Brown	Light green	Yellow
Powder +NaOH	Green	Yellowish green	Pale green
Powder +H_2O	Green	Light green	Yellow
Powder +$FeCl_3$	Light green	Green	Dark green
Powder +HNO_3	Dark yellow	Green	Yellow
Powder + alc.NaOH	Green	Light green	Yellowish green
Powder + alc. KOH	Light green	Dark green	Dark green
Powder + acetic acid	Green	Green	Brown
Powder + picric acid	Green	Bluish green	Brown
Powder +iodine solution	Green	Dark green	Brown
Powder + ammonia	Green	Light green	Yellowish green
Powder + ethanol	Green	Dark brown	Reddish brown
Powder + methanol	Green	Dark green	Yellowish green
Pet.ether	Dark green	Green	Pale green
Chloroform	Dark green	Green	Pale green
Ethyl acetate	Brown	Green	Pale green
Ethanol	Brown	Green	Pale green
Water	Red	Green	Brown

3.5 Antibacterial activity

The five different solvents of *Biophytum sensitivum* crude extracts were selected for antibacterial activity on six different organisms are given in Table 4. All the tested extract shows maximum activity against *Staphylococcus aureus*, *Streptococcus mutans* and *Proteus vulgaris*. Ethanol extract of leaf part of *Biophytum sensitivum* exhibit more active against *Bacillus subtilis* (20 mm), *Streptococcus mutans* (18 mm), *Escherichia coli* (15 mm) and *Proteus vulgaris* (14 mm) when compared to control streptomycin.

Table 4: Antibacterial activity of leaf part of *Biophytum sensitivum*

S.No	Microorganism	P	C	EA	E	W	control
1	*Bacillus subtilis*	8	9	12	20	15	21
2	*Streptococcus mutans*	11	10	13	18	9	20
3	*Staphylococcus aureus*	12	13	15	14	8	18
4	*Escherichia coli*	9	11	13	15	10	22
5	*Klebsiella pneumonia*	12	14	10	11	8	17
6	*Proteus vulgaris*	13	12	11	14	9	19

P- petroleum ether, C- chloroform, EA – ethyl acetate, E- ethanol, W-water

3.6 Antioxidant activity

Radical scavenging activity of five different solvents of *Biophytum sensitivum* crude extracts were studied. Ethanol extract show activity 82% at lowest concentration (100µg/ml) and 93.3% at the highest concentration (500µg/ml). Ethanol extract shows more scavenging activity compared with other four extract and control at different concentration. Aqueous extract shows minimum scavenging activity 11.6% at lowest concentration (100µg/ml) and 39.7% at the concentration of 400µg/ml. Free radical scavenging activity of five different extract shown in Table 5. Antioxidants can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells prevent damage to lipids, proteins, enzymes, carbohydrates and DNA. A wide range of antioxidants from both natural and synthetic origin has been proposed for use in the treatment of various human diseases (25).

Table 5: Radical Scavenging Activity of leaf part of *Biophytum sensitivum*

Concentration (µg/ml)	Radical Scavenging Activity (%) *Biophytum sensitivum* Leaf					Standard (%)
	P	C	EA	E	W	
100	48.5	54.9	81.3	82.0	11.6	53.1
200	51.4	57.7	87.6	85.2	26.7	61.2
300	53.8	59.5	88.3	91.9	34.5	65.4
400	55.6	66.5	89.7	92.6	39.7	80.9
500	57.3	70.0	91.1	93.3	57.3	82.7

P- petroleum ether, C- chloroform, EA – ethyl acetate, E- ethanol, W-water

4. Conclusion

Based on result from this study, it can be concluded that the ethanolic extract of *Biophytum sensitivum* leaf protects and exhibit antioxidant activity. This effect may be attributed to its phytochemical constituents which might include phenolics, flavonoids and heavy metals. This approach might be useful in the management of oxidative stress.

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