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Pharmacognostic Standardization of *Biophytum Sensitivum* Dc

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Abstract *Biophytum sensitivum* DC. (Family – Oxalidaceae) is a traditional drug used in treating various ailments especially in mental disorders. The present study was envisaged to establish pharmacognostic standards of the plant so that authentic plant can be selected for research purpose. Heavy metal content, microbial load, aflatoxins contamination and pesticide residues were also examined to substantiate the standardization data. Transverse section of the root was cut and showed the presence of root hair, cork, cortex, pericyclic region, vascular bundles and pith whereas the stem showed the presence of trichomes, cork, cortex, pericylic fibres, xylem and phloem. Alcohol soluble extractive value was slightly higher than water soluble extractive values. Total ash, water soluble ash, acid-insoluble ash and sulphated ash were found to be 8.80, 5.26, 1.90 and 0.35% w/w respectively. Foreign matter, loss on drying, swelling index and foaming index were found to be nil, 8.50% w/w, nil, and less than 100 ml respectively. Microbial load, heavy metals and pesticide residues complied with the limits as described by WHO. Flavonoids, tannins, saponins, proteins and amino acids were present in the plant.

Keywords: *Biophytum sensitivum*, Heavy Metal Analysis, Microbial Load, Pesticide residue

1. INTRODUCTION

Herbal medicines have been major source of drugs in developing countries to meet health needs. There has been a sharp focus on interest in herbal

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medicines and their utilization in recent years (Anonymous, 2015a). The lack of standardization data on medicinally promising plants is major stumble block for developing them as medicine. World Health Organization has showed concern for herbal medicines to ensure their quality and safety by modern sophisticated techniques (Ariyanathan et al., 2010).

Biophytum sensitivum DC. (Family- Oxalidaceae) commonly known as 'Lajjalu' is traditionally used in the treatment of stomach ache, diabetes and asthma. It is distributed throughout the tropical regions of South Asia, Africa and Madagascar, the hotter parts of India, Nepal, Thailand, Malaysia, Indonesia and Sri Lanka. The plant has been traditionally used in the treatment of insomnia, convulsions, cramps, chest-complaints, inflammations, tumours and chronic skin diseases (Anonymous, 2015b; Kirtikar and Basu, 2003). The decoction of the root was recommended in fever, gonorrhoea and lithiasis. The leaves of the plant have been considered as traditional remedy of 'Madhumeha'.

B. sensitivum has been reported to contain flavonoids, polyphenolic compounds (Bucar et al., 1998), saponin, essential oil, polysaccharides and pectin (Yun-Lian and Wan-Yi, 2003), amentoflavone with minute amount of cupressuflavone (Ravishankara et al., 2003). A survey of literature reveals that the plant exhibits hypoglycaemic (Puri and Baral, 1998; Puri, 2006; Puri, 2001) immunomodulatory, chemoprotective (Guruvayoorappan and Kuttan, 2007), hypocholesterolemic (Puri, 2003), antioxidant (Guruvayoorappan et al., 2006), anti-inflammatory (Jachak et al., 1999) and antitumor activity (Bhaskar and Rajalakshmi, 2010). Establishment of pharmacognostic standards of traditional plant is prerequisite for its detailed phytochemical and pharmacological investigation. Thus, present studies were designed to establish pharmacognostic standards of *B. sensitivum*.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

The whole plant of *B. sensitivum* was collected from Tamilnadu (District, Thoothukudi) in January 2012. The identity of the plant was confirmed by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, NISCAIR, New-Delhi, (specimen No.-NISCAIR/RHMD/ Consult/2012-11/1528/126).

2.2 Solvents, Chemicals and Reagents

All the chemicals, reagents and solvents including petroleum ether (60–80ºC), chloroform, ethyl acetate, ethanol, 1-butanol, glacial acetic acid, acetone, phloroglucinol, glycerin, chloral hydrate, iodine, sodium hydroxide, sodium

chloride, potassium iodide, sodium thiosulphate, sulphuric acid, acetic anhydride, ammonia, diethyl ether, ninhydrin, magnesium turnings, picric acid, sodium nitroprusside, mercuric iodide, potassium bismuth iodide, disodium hydrogen phosphate, potassium dihydrogen phosphate, lead acetate, fehling's A, fehling's B solution, α-napthol, and pyridine (RFCL, Mumbai, India) of LR grade, were used in present investigations. Compound microscope, Soxhlet apparatus, vacuum rotary evaporator, glass slides, cover slips and watch glass were used for the study. Labomed ATC-200 microscope attached with Sony digital camera was used to take photomicrographs.

2.3 Preparation of the extracts

Dried coarsely powdered whole plant (1 kg) was subjected to exhaustive extraction successively with different solvents (5 L each) *viz.*, petroleum ether, chloroform, methanol and water. The crude extracts were filtered, concentrated using the rotary evaporator, weighed and percentage yields were recorded. The color and consistency of the extracts were also observed.

2.4 Macroscopic and Microscopic Evaluation

Various parts of the plant were macroscopically studied. The whole plant of *B. sensitivum* was boiled sufficiently with water until soft. Thin sections of roots and stems were cut by sharp blades, cleared with chloral hydrate solution and mounted in glycerin. Staining of sections was carried out with phloroglucinol and hydrochloric acid. Powder of the dried whole plant was also treated with chloral hydrate, phloroglucinol and hydrochloric acid, and iodine to study various diagnostic features (IP, 2007; Khandelwal, 2006; Kokate and Gokhale, 2008).

2.5 Fluorescence Analysis

Visible and ultraviolet light (U.V. short and U.V. long) were used to study fluorescence behavior of the powdered material and different extracts of plant (Chase and Pratt, 1949; Kokoshi et al., 1958).

2.6 Physicochemical Parameters and Phytochemical Evaluation

Foreign matter, moisture content, total ash, water soluble ash, acid insoluble ash, alcohol and water soluble extractive values were determined adopting standard procedures. All crude extracts of the plant were subjected to qualitative chemical tests to check the presence of different classes of phytoconstituents (Brain and Turner, 1975; Harborne, 1988; WHO, 1998).

2.7 Toxic Residue Determination

The toxic residues including pesticides residue, aflatoxin, heavy metals and microbial content were estimated in the whole plant powder as per W.H.O. guidelines (WHO, 1998). These studies were carried out at analytical laboratory of OSCAR Analytical Pvt. Ltd. Baddi, Solan (Certificate No. OAPL/19417/12DXII, dated 12/04/2012).

3. RESULTS AND DISCUSSION

3.1 Macroscopic and Microscopic Evaluation

The plant was 7.5 cm in height having an unbranched woody erect stem (Figure 1). Leaves grew at the top of the stem and were abruptly pinnate, sensitive and were made of 2-6 pairs of leaflets which are opposite, 1 cm in length. The flowers of the plant were yellow or white colored dimorphic with red streak in the centre of each of the five petals. Roots of the plant were brown in color. The transverse section of the roots showed the presence of root hair, cork, cortex, pericyclic region and vascular bundles followed by the pith in the centre. (Figure 2). Microscopy of stem showed the presence of trichomes, epidermis, cortex and xylem and phloem (Figure 3). Powder microscopy showed the presence of fibres, cork cells, trichomes, pitted vessels and vascular bundles (Figure 4).

The results of fluorescence analysis of the powdered drug after treatment with different reagents were shown in table 1. Various extracts of plants were observed in the day light and UV light, and colors were observed (Table 2).

Figure 1: Morphology of *Biophytum sensitivum* (A) whole plant and (B) Plant powder.

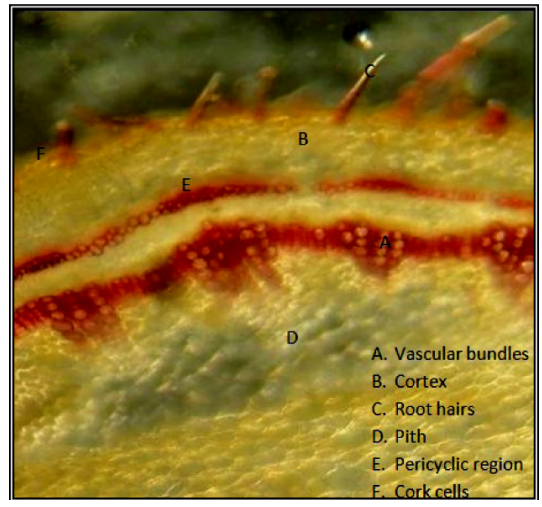

Figure 2: Transverse section of root of *Biophytum sensitivum.*

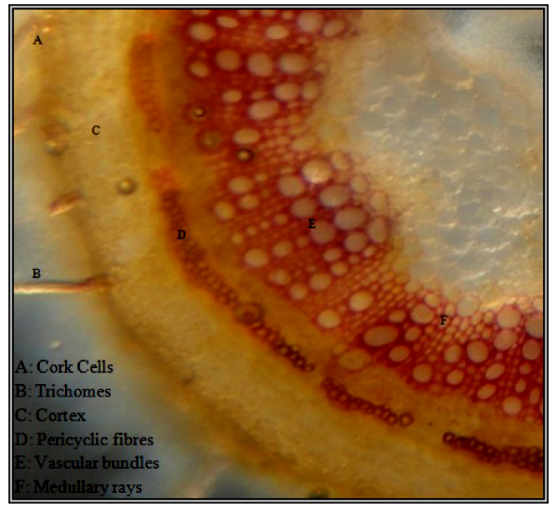

Figure 3: Transverse section of stem of *Biophytum sensitivum.*

3.2 Physicochemical Parameters and Phytochemical Evaluation

The purity of the plant can be judged by detecting adulteration using various standard physic-chemical parameters. Thus, various physicochemical parameters of *B. sensitivum* whole plant viz., foreign matter, moisture content, ash and extractive values, swelling index, foaming index and crude fibre

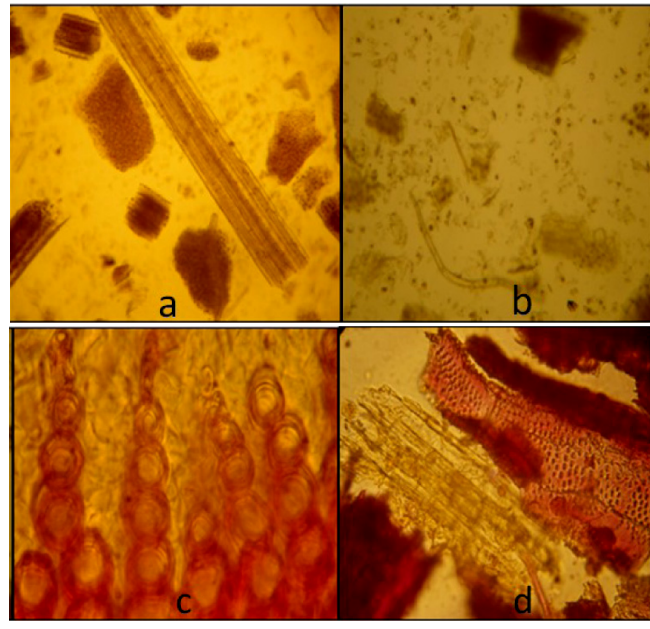

Figure 4: Powder Microscopy of whole plant of *B. sensitivum*. (a) Fibres and cork cells (b) Trichomes (c) Vascular bundles (d) Fibres and pitted vessels.

Table 1: Fluorescence of whole plant powder of *B. sensitivum* with different reagents.

S. No.	Treatment	Visible (400-800nm)	U.V. short (254 nm)	U.V. Long (366 nm)
1	Powder as such	Brownish Green	Black	Green
2	Powder + 1N NaOH in Methanol	Yellowish Brown	Black	Dark Green
3	Powder + 1N NaOH in Water	Brown	Black	Dark Green
4	Powder + 1N HCl	Brown	Black	Dark Green
5	Powder + 50% HNO_3	Brown	Black	Dark Green
6	Powder + 50% HCl	Brown	Black	Dark Green
7	Powder + Iodine sol	Dark Brown	Black	Dark Green
8	Powder + Picric acid	Yellowish Brown	Black	Bright Green
9	Powder + Acetic acid	Yellowish Green	Black	Green
10	Powder + 5% $FeCl_3$	Brown	Black	Bright Green
11	Powder + Conc. H_2SO_4	Brown	Black	Bright Green
12	Powder + Conc. HNO_3	Brown	Black	Dark Green
13	Powder + conc. HCl	Brown	Black	Bright Green
14	Powder + 95% alcohol	Yellowish Brown	Black	Dark Green

Table 2: Fluorescence of whole plant powder extracts of *B. Sensitivum.*

S. No.	Extract	Yield (% w/w)	Consistency	Colour of Extract		
				Visible light	U.V. short	U.V. Long
1.	Petroleum ether	7.09	Sticky solid	Green	Black	Brownish Black
2.	Chloroform	3.13	Solid	Green	Black	Brownish Green
3.	Methanol	21.33	Semisolid	Black	Bluish Black	Brownish Green
4.	Aqueous	16.02	Sticky semisolid	Dark Brown	Black	Yellowish Green

Table 3: Mean values for various physicochemical parameters of whole plant powder of *B. sensitivum.*

S. No.	Parameter	Values
1	Foreign Matter	NIL
2	Alcohol Soluble Extractive value	15.50 % w/w
3	Water soluble Extractive value	13.75 % w/w
4	Total ash	8.80 % w/w
5	Water soluble ash	5.26 % w/w
6	Acid-insoluble ash	1.90 % w/w
7	Sulphated ash	0.35 % w/w
8	Moisture content (Loss on drying)	8.50 % w/w
9	Swelling index	NIL
10	Foaming Index	Less than 100 ml
11	Crude fibre content	10.0

determination, were established, and the results have been shown in table 3. Phytochemical screening showed the presence of flavonoids, tannins, phenolic compounds, amino acids, proteins and saponin in *B. sensitivum*. The results are depicted in table 4.

Toxic residues like pesticide residue, heavy metals, aflatoxins and pathogens were completely absent in the whole plant powdered drug and total microbial, yeast and mould count were found to be within limits as described in WHO guidelines (Tables 5 and 6)**.**

Table 4: Preliminary Phytochemical screening of different extracts of *B. Sensitivum.*

Tests for constituents	Petroleum ether extract	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids	−	−	−	−
Carbohydrates	−	−	−	+
Flavonoids	−	+	++	++
Tannins and Phenolic compounds	−	−	+	+
Amino−acids	−	−	+	+
Proteins	−	−	+	+
Steroids/ Triterpenoids	−	−	−	−
Glycosides	−	−	−	−
Saponins	−	−	−	+
Fats and fixed oils	+	−	−	−

+ = Presence of constituent, − = Absence of constituent

Table 5: Estimation of heavy metals, mercury, arsenic, aflatoxins and pesticides residue in *B. sensitivum* whole plant powder.

Parameters	Observations	Limits (As prescribed by WHO)
Heavy metals-lead, cadmium;	Absent	NMT 1 ppm
Mercury	Absent	NMT 5ppm
Arsenic	Absent	NMT 3 ppm
Aflatoxins B_1	Absent	Should be absent
B_2	Absent	
G_1	Absent	
G_2	Absent	
Total	Absent	
Pesticides: Aldrin, Azinphos-methyl, Cypermethrin, Chlordane, Chlorfenviphos, Chlorpyrophos, Carbophenothion, Dimethoate, Diazinon, Dichlorvas, Dieldrin, DDT, Eldrin, Ethion, Endosulfan, Fenitrothion, Fensalfothion, Fonofos, Heptachlor, Cis & trans heptachlorepoxide, Hexachlorocyclohexane, Hexachlorobenzene, Heptachlor, Lindane, Malathion, Methidathion, Parathion, Permethrin, Phosalone, Pyrethrins, Pirimiphos-methyl.	Absent	Should be absent

Table 6: Microbial and pathogen content in in *B. sensitivum* whole plant powder.

Microbes	Observations	Limits (As prescribed by WHO)
Total microbial count	28 cfu/gm	NMT 1000 cfu/gm
Total yeast and mould count	Absent	NMT 100 cfu/gm
Salmonella typhymurium	Absent	Should be absent
Escherichia coli	Absent	Should be absent
Pseudomonas auroginosa	Absent	Should be absent
Staphylococcus aureus	Absent	Should be absent
Clostridium botulinum	Absent	Should be absent
Clostridium perfringens	Absent	Should be absent
Clostridium tetani	Absent	Should be absent

CONCLUSION

Non-availability of pharmacognostic standards for traditionally used plants is the major stumble block in investigating scientifically such medicinally promising plant drugs. Therefore, the plant should be properly authenticated using standard methods before phytochemical and pharmacological investigations. The WHO has recommended various physico-chemical methods for authentication of plant drugs. These parameters can help in detection of adulteration in plant drugs so these should be adopted to confirm the purity and quality of plant drug. Further, the presence of toxic residues (Heavy metals, arsenic, mercury, pathogens, aflatoxins and pesicides), which may have adverse effects on health of human being, must be tested in plant drugs. It is necessary that the raw material must comply the limits of such toxic residues as given in WHO guidelines. These studies are important for obtaining reproducible quality of plant drug.

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