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Research Article

Pharmacognostical Standardization and Phytochemical Profiling In Biophytum Reinwardtii (Zucc.) Klotzsch. (Oxalidaceae)

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Abstract: 'Biophytum is an important medicinal herb used both in traditional systems of medicine as well as in Ayurvedha. The present investigation is concerned with the pharmacognostic standardization and phytochemical screening of a very common species of Biophytum, B. reinwardtii. A morphological study of the plant was conducted. The whole plant was shade dried and ground to fine powder and subjected to organoleptic, fluorescent and physicochemical analysis. The plant powder was subjected to Soxhlet extraction using methanol. Phytochemical screening (Qualitative, Quantitative, Thin layer chromatography) of the methanol extract of the plant sample was done according to the standard biochemical procedures. Anti-inflammatory activity of the plant extract was tested using inhibition of albumin denaturation assay. The organoleptic analysis revealed the characteristic colour, taste, odour and nature of powder of B. reinwardtii. In fluorescence analysis, on treatment with different solvents, colour changes could be noticed in the plant powder. The results of the physicochemical analysis provide an important parameter in detecting adulteration or improper handling of drugs. Qualitative and quantitative analysis confirmed the presence of many important phytochemicals in the plant. The presence of steroids was detected by thin layer chromatography. Bioactivity study indicated the anti-inflammatory potential of the plant comparable to that of the standard aspirin.

Keywords: Biophytum reinwardtii, Pharmacognostic, Steroids, Anti-inflammatory, thin layer chromatography

Introduction

The practice of traditional medicine is based on hundreds of years of belief and observations, which predate the development of the modern medicine (Hussain and Sheikh, 2008). In India, it is said that the recipes used in the traditional medicine have been handed down from the forefathers usually by oration (Lalithrani et al., 2011). Though acceptable to a certain extent in developing countries, people in developed countries have many apprehensions. Due to the lack of stringent quality control, it is not well accepted by the modern system of medicine. In addition, it is also known that the trade of crude drugs has always remained in the hands of unskilled persons. Research on traditional medicinal plants is neither systematic nor complete. A key obstacle that has hindered the acceptance of alternative medicine in the developed countries is the lack of proper documentation of research work on traditional medicine (Dahanukar et al., 2000). All these ultimately cause the collection of premature or incorrect drug leading to adulteration or substitution.

Pharmacognosy is the study of the structural, physical, chemical and sensory characters of crude drugs of animal, plant or mineral origin (Trease and Evans, 2011). According to WHO (1992), the macroscopic and microscopic descriptions of a plant material forms the initial step towards establishing the plant's identity. Thus, pharmacognostic studies help in the identification and authentication of the plant material.

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Phytochemicals are secondary metabolites produced by the plants for their protection, repair processes and survival in the natural environment. The determination of the phytochemical profile of a plant provides evidence for the major classes of compounds in that plant. The secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, Saponins and steroids produced naturally in plants are also known to possess curative properties. Experimental validation shows that the bioactive components could be localized in specific plant parts like roots, stems, barks, leaves, flowers or seeds. Thus, the determination of the phytochemical profile of plants/plant parts is of great significance to human health (Sreeshma and Bindu, 2017).

The genus Biophytum (Oxalidaceae) contains herbaceous annuals or perennials and about 80 species are reported to be distributed in the tropical and subtropical regions of the world (Lourteig, 1987).The genus is reported to be represented by 17 species in India (Manna, 1987) and nine species in Kerala (Sasidharan, 2004).The medicinal value of species of Biophytum is quite evident from the fact that these plants have been used in traditional folk medicine for a very long time. Biophytum reinwardtii (Zucc.) Klotzsch is an annual herb seen during the rainy season throughout the warm parts of India. It is also distributed in Indo-Malaysia and China. The leaves and roots of B.reinwardtii are used to treat common fever. The plant is reported to possess tonic and stimulant properties, used for chest complaints, convulsions, cramps, inflammatory tumours and its ash for stomach ache. The whole plant is dried, powdered and given internally to cattle to stop excess salivation. The whole plant is used as sedative, for removing dandruff and as hair tonic. In Bihar state of India, the leaves and roots are given for insomnia, fever, gonorrhea and lithiasis.

Even though, Biophytum is used widely in Ayurvedic formulations, but information on the chemical constitution is very limited and therefore at present, a chemo-taxonomic appreciation is difficult. The only species which has been studied in detail is Biophytum sensitivum. The plant, B. sensitivum has been reported to possess various pharmacological activities (Puri and Baral, 1998). The medicinal properties attributed to B. sensitivum are due to the presence of flavonoids, especially a biflavonoid, amentoflavone.

Materials and Methods

Plant Material

The plant selected for the study, Biophytum reinwardtii (Zucc.) Klotzsch were collected from Pandalam, Pathanamthitta. Fresh plant of Biophytum reinwardtii is collected in polythene bags. The collected material was washed under running tap water to remove adhered dirt. It was then rinsed with distilled water, blotted and dried in shade. The shade dried specimen were powdered in a mixer and sieved with a fine mesh sieve. The powder was then used for the organoleptic study and solvent extraction. Dried and powdered plant material was subjected to extraction by soxhlet apparatus using solvent methanol. The extract was concentrated under reduced pressure and preserved in refrigerator until further use.

Morphological Study

The morphology of the species of Biophytum was studied using taxonomic kit based on qualitative and quantitative characters. The observations were recorded (Gamble, 1935.)

Organoleptic Study

The plant powder characteristics like the color, odour, taste and nature were evaluated.

Fluorescence Analysis

The crude drug powder was treated as such with eight different reagents/ solvents and kept for 10 minutes for the analysis. Each solution was loaded on an activated thin gel layered slide and the fluorescence under normal light, short UV (100nm) and long UV (320nm) was observed (Chase and Pratt, 1949).

Physicochemical Characterization

Loss on drying, foaming index, swelling index, foreign matter and pH were determined according to the official methods and guidelines on quality control for

medicinal plant materials (Indian Pharmacopoeia, 1996; Iqbal et al., 2010).

Phytochemical Screening

Different phytochemical constituents were tested using standard biochemical procedures (Harborne, 1973).

Thin Layer Chromatography

Methanol extract of the whole portion of the plant was concentrated and the residue was chromatogramed by TLC using the solvent system Toluene: Ethyl acetate (8:2). The components separated were detected by means of their RF value that is unique for the component in the particular solvent system.

Anti-Inflammatory Activity

The in vitro assessment of anti-inflammatory activity was done by means of albumin denaturation assay (Mizushima and Kobayashi, 1968). The reaction mixture consisted of test extract and 1% aqueous solution of bovine albumin fraction. The pH of the reaction mixture was adjusted using small amounts of 1N HCl. The sample extracts were incubated at 37oC for 20 min and then heated at 51oC for 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. Aspirin was used as the standard. Percent inhibition of protein denaturation was calculated.

Results

Morphological Study

An account of the morphological characters of Biophytum reinwardtii is given below.

Table 1: Morphological characters of *Biophytum reinwardtii*

CHARACTER	DESCRIPTION
Habit	Annual or perennial herb but looks like a miniature tree.
Stem	Woody, long, slender, erect and hairy.
Leaf	Abruptly pinnate, crowded at the ends of stem.
Root	Tap root system.
Leaflet	Opposite, oblique, compound, oblong, 14- 20 pairs, main nerves slender and straight.
Inflorescence	Umbel
Flower	Dimorphous, peduncle long terminal, yellow, pedicellate arising in umbels of 3-7 flowers.
Sepal	Sepals 5, green
Petal	Petals 5, yellow, connate with salver – shaped corolla.
Androecium	Stamens 10, 5 inner ones longer, filamentous, free.
Gynoecium	Ovary 5- celled styles 5, stigmas notched or bi- fid, Parietal placentation.
Fruit	Capsule and ellipsoid.
Seed	Ovoid, loculicidally dehiscing into 5 valves, transversely striate.
Flowering	October- December.

Fluorescence Analysis

The dry powder was subjected to fluorescence analysis with different reagents in normal light, short UV and long UV. The colour changes are summarized (Table 2).

Table 2: Fluorescent analysis of *Biophytum reinwardtii*

Powder+ Reagent	Visible (400-800 nm)	U.V short (254 nm)	U. V long (366 nm)
Powder as such	Brown green	Black	Green
Powder + 1N NAOH in H_2O	Black	Black	Dark green
Powder+1N HCl	Black	Black	Dark green

Powder+50% HNO_3	Black	Black	Dark green
Powder+ Iodine solution	Dark black	Black	Dark green
Powder+ Acetic acid	Yellow green	Black	Dark green
Powder+5% $FeCl_3$	Brown	Black	Bright green
Powder+ Con. H_2So_4	Brown	Black	Bright green
Powder+ Con. HCl	Brown	Black	Bright green
Powder+ 95% Alcohol	Yellow brown	Black	Dark green

Physicochemical Characterization

A total of five physico-chemical parameters were evaluated in Biophytum reinwardtii (Table 3). The moisture content was reported to be in low amounts in the plant. Foaming index was found to be more than 100 units and no considerable swelling was observed. The amount of foreign matter was low in Biophytum.

Table 3: Physicochemical characters of *Biophytum reinwardtii*

PARAMETERS	VALUES
Foreign matter	Nil
Loss on drying	8.50% ± 0.02
Swelling index	Nil
Foaming index	>100ml
P^H	6.3

Phytochemical Screening

Most of the compounds were present in the extract. Coumarins, glycosides and mucilage were absent in the extract of the plant. The results of the phytochemical screening tests are provided (Table 4). The quantitative estimation of three phytochemicals was done in the methanol extract of Biophytum reinwardtii (Table 5) by standard procedures. The amount of steroids was the highest and that of alkaloids was low.

Table 4: Phytochemicals tested in *Biophytum reinwardtii*

Sl. No	Phytochemicals	Present / Absent
1	Tannins	+
2	Saponins	+
3	Flavonoids	+
4	Alkaloids	+
5	Terpenoids	+
6	Phlobatannins	+
7	Glycosides	-
8	Simple phenolics	+
9	Coumarins	-
10	Quinones	+
11	Acids	+
12	Flavonols	+
13	Lignin	+
14	Steroids	+
15	Gums and mucilage	-

Table 5: Quantitative estimation of *Biophytum reinwardtii*

Phytochemicals	Amount (mg/g)
Alkaloids	0.42 ± 0.6
Steroids	2.9 ± 0.2
Phenols	0.342 ± 0.1

Thin Layer Chromatography

The methanol extract was chromatogramed over silica gel using the solvent system Toluene: Ethyl acetate (8:2) for the detection of phytochemicals. The plates with spots showed light green colour indicative of the presence of steroids. Two bands could be identified with RF values 0.55 and 0.52 in B. reinwardtii. The RF value obtained corresponded to steroids. But this requires further confirmation by subjecting the extract to chromatographic and spectroscopic techniques.

Bioactivity Study - Anti-Inflammatory Activity

Protein degradation is considered to be the cause of inflammation and this assay reflects the ability of the extracts to block protein denaturation. The methanol extract of B. reinwardtii was effective in inhibiting heat induced albumin denaturation (Table 6). The IC50 value by probity analysis of the methanol extract of B. reinwardtii was 18.94µg/ml which was comparable to the standard aspirin (IC50 value 17.21µg/ml).

Table 6: Anti-inflammatory assay in *Biophytum reinwardtii*

Concentration of extract	Aspirin(percentage of inhibition)	Test extract (Percentage of inhibition)
20µg/ml	55%	53%
40µg/ml	76%	72%
60µg/ml	90%	86%
80µg/ml	97%	92%

Discussion

Plant identification and documentation is an essential part of any phytochemical study. Biophytum reinwardtii was studied for the morphological characters. The plant showed several vegetative characters such as plant habit, nature of pubescence, inflorescence type, shape of petiole and pedicel, colour of the sepal and petal, shape of stigma and style, ovary placentation and color of the seed (Table 1).

Powder analysis in the present study revealed the different macroscopic characters. Fluorescent analysis of the drug powder with different reagents / solvents is an important pharmacognostic tool in checking adulterants (Trease and Evans, 2011). The pharmaceutical and neutraceutical industries are now a day's confronted with adulteration and cheating. The establishment of salient features of the plant correctly is inevitable in this field. On treatment with different reagents/ solvents color changes could be noticed in the plant in fluorescent analysis (Table 2).

The physico-chemical parameters play an important role in determining the purity and quality of a plant sample. The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs (Mukherjee, 2002; Saha et al, 2011).The moisture content of the plant in the present study was low (<14%) indicating that it could discourage bacterial, fungal or yeast growth . A drug containing excess water leads to the activation of enzymes and will lead to the proliferation of living organisms. Foaming index is a significant characteristic for the presence of saponins in the crude drug. It was found to be more than 100 units, which suggests the presence of saponins in the plant powder. Swelling index value was low for the plant which may probably be due to the absence of mucilage. Medicinal plant materials should be entirely free from visible signs of contamination by moulds or insects, and other animal contamination, including animal excreta (Batra etal., 2012; Saha and Swati, 2012a; Ramasubramaniraja, 2011). In the present study, small amount of foreign matter could be observed in Biophytum reinwardtii probably because the samples were collected mostly from and around the college campus. According to WHO, the permissible limit is not more than 2% (Saha and Swati, 2012b; Hamid et al, 2016). The pH of the 5% aqueous solution of the plant indicated the acidic nature of the drug powder which may be due to the presence of acidic salts (Table 3).

The phytochemical profile of plants reveals their compound composition. The presence/ absence of specific compounds are determined by specific colored products produced in reactions on the addition of specific chemicals (Zahara et al., 2019). The methanolic extract of Biophytum reinwardtii was subjected to a preliminary phytochemical screening. Tannins, flavanols, lignin, saponins, simple phenolics, terpenoids, phlobatannins, flavonoids, alkaloids, steroids and quinones were present in the methanolic extracts of B. reinwardtii (Table 4).

Isolation and separation of bioactive compounds is necessary for further analysis. Accordingly, chemical screening of crude extracts is performed first to allow localization and targeted isolation of new or useful types of constituents with potential activities (Meena et al., 2011). The methanolic extract of B. reinwardtii was subjected to thin layer

chromatography. The analysis showed the presence of two bands at RF values of 0.55 and 0.52. These values were found to correspond to steroid compounds.

Protein denaturation is a process in which proteins lose their secondary and tertiary structure upon the application of extraneous agents, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat (Venkateswarlu et al., 2011; Swati and Saha, 2012). Most biological proteins lose their biological function when denatured. Denaturation of proteins is the cause of inflammation and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins in vivo (Anbarari and Vidya, 2015; Opie, 1962). Therefore, development of anti-inflammatory drugs that could prevent protein denaturation could be of interest. The results of the present study showed that the test extract of Biophytum reinwardtii was effective in inhibiting heat induced albumin denaturations at different concentrations (Table. 6).

Conclusion

The present study deals with the pharmacognostical characterization and phytochemical screening of the plant Biophytum reinwardtii. The scientific investigation of traditional herbal remedies may provide valuable data for the development of alternative drugs and therapeutic strategies. A detailed morphological characterization was attempted and several vegetative and floral characters were recorded. Pharmacognostic studies can serve as a basis for proper identification and investigation of a plant. The present organoleptic analysis will be useful in the proper identification, collection and investigation of the plant. Fluorescent analysis was conducted in the plant powder which provided a valuable clue in establishing the identity of the plant powder. Since the different reagents /solvents give characteristic fluorescent patterns, they are considered important pharmacognostic tools in checking adulteration. The physico-chemical investigation of crude drugs plays a crucial role in detecting adulteration. This data will serve as a standard for the quality control of any drug preparation containing these plants in future. The phytochemical screening detected the presence of many useful phytocompounds in the methanol extract of Biophytum reinwardtii. In the present study, qualitative and quantitative tests showed significant indication about the presence of metabolites which contribute to the pharmacological activity. The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the plant. The presence of some of these compounds has also been confirmed to have antimicrobial, anti-inflammatory, antioxidant as well as anti-cancer properties. The thin layer chromatography of methanol extract revealed the presence of steroids. The RF values estimated corresponded to that of steroid compounds. The in vitro anti-inflammatory activity of the methanol extract of Biophytum reinwardtii was comparable with standard aspirin. The findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of anti-inflammatory agents from the plant.

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