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Research Article

Evaluation of standardisation parameters and antioxidant activity of *Bergenia ligulata*

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ABSTRACT

Berginia ligulata is one of the prominent ayurvedic herb for acute and chronic urinary tract infection [UTI].The present study highlighted the botanical as well as phytochemical studies including parameters such as macroscopic, physiochemical, evaluation and preliminary phytochemical studies of roots. On physiochemical analysis it was found that the powder was soluble in methanol, ethanol and water. It was insoluble in petroleum ether. On phytochemical analysis of aqueous and chloroform extract, it contains alkaloids, Saponin and Flavonoids

Key words: Phytochemical studies, phytochemical analysis, macroscopic evaluation.

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Introduction

Berginia ligulata (Saxifragaceae) isknown as pashanbheda in ayurveda. `Pashanbheda is also known as "stonebreaker". It is a perennial herb found especially in the foothills of the Himalayas and the Khasi hills of Assam. It is distributed 8000 to 10000 ft above sea level at Himalayans. The stems are short and thick and leaves are fleshy, broadly obviate or elliptic, finely or sparsely denticulate or shallowly sinuate- dentate[1]. Flowers are white, rose or purple, in long cymose panicles. Rhizomes solid, barrel shaped, cylindrical, 1.5 – 3 cm long and 1-2 cm in diameter with small roots ; ridges, furrows and root scars distinct; transversely cut surface shows outer ring of brown colored cork, short middle cortex, vascular bundles and large central pith ; odour aromatic ; taste astringent[2]. Pashanbheda is one of the prominent ayurvedic herbs for acute and chronic urinary tract infection [UTI]. From the rhizomes of B. ligulata only bergenin and b-sitosterol have been isolated.The other constituents are tannic acid, Gallic acid, albumin, glucose, catechin-3- gallate and afzelechin[3]. It can be propagated by seeds and division of rhizomes. It is used as Uro litholytic, diuretic, anti-inflammatory, spasmogenic, anti-protozoal, anti-cancer, cardio toxic, CNS depressant, lowering of gastric output etc[4].

Material and method

The plant of Berginia ligulata is taken from an institute of the Forestry Research and Education. The powder of Berginia ligulata is weigh and is kept for maceration with different solvent like water, methanol, ethanol, chloroform, etc for 24 hours.

Moisture content

Purchase of drugs, which contain excess of moisture, is not only uneconomical but also at suitable temperature, moisture will activate the enzyme or facilitates growth of microbes, which leads to the decomposition. Some important physical and chemical methods used to determine the moisture content in drugs are:

Loss on drying

Loss in the weight of the sample is mainly due to the presence of water but to the presence of small amount of volatile materials. Contribute to the weight loss. LOD is determining by subjecting the crude material at 105 0c to constant weight and calculate the loss of weight[5].

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Extractive values

Crude drugs possess their biological activity due to the presence of active constituents, which are soluble in polar, semi polar and non polar solvents. Total soluble constituent of drug in particular solvent is called as extractive values. These values give idea about the extent of polar, semi polar and non polar compounds present in medicinal plant materials. Extractives values are studied by wet weight or dry weight basis by using maceration, percolation or continuous extraction using sox let apparatus.

- Water soluble extractive values for formulation.
- Alcohol soluble extractive values for formulation.
- Ether soluble extractive for formulation.

Ash value

When crude drugs are incinerated, they leave an inorganic salt know as ash. It varies within fairly wide limits and its study gives an idea about the quality and purity of the drug during evaluation. Various types of ash values determined are:

- Acid soluble ash value
- Acid insoluble ash value

Colour: Brown

Solubility: more soluble in water and insoluble or slightly soluble in organic solvent.

Detection of tannins
Calculation
Lead acetate Test: IU/ L=A/min × T.V × 10 S.V×
To 2-3ml of aqueous or alcoholic extract, add Absorptive × P
few drops of lead acetate solution, white precipitate are formed.

Detection of Flavonoids

a) Shinoda Test

To the alcoholic solution of extracts, a few fragments of magnesium ribbon and Conc. HCl was added. Appearance of magenta colour after few minutes indicates presence of Flavonoids.

Loss on drying: 4 %
Phytochemical Screening of Herbal Formulation

The Qualitative phytochemical analysis was carried out to find the presence of active chemical constituents such as alkaloids, glycosides, flavonoids and tannins according to the procedure[6]

Detection of Alkaloids

The small portions of extracts were treated with some drops of dil. HCl and filtered then subjected to test for alkaloids.

a)Dragendorff's Test: (14 gm of potassium iodide with 5.2 gm bismuth carbonate in 50 ml glacial acetic acid)

Extracts were treated with Dragendorff's reagent. Formation of orange brown precipitate indicates the presence of alkaloids.

Detection of glycosides

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

Detection of Saponin

Froth Test

Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of Saponin.

Organoleptic Evaluation

The macroscopic characters such as colour, size, odour, taste, nature, texture were studied for morphological investigation.

Antioxidant activity

Assay of perox idase activity

The assay was carried out b y the method of Addie and Goodman . The reaction mixture consisted of 3ml of buffered p yrogallol [0 . 05 M p yrogallol in 0 . 1 M phosphate buffer (pH 7 . 0)] and 0 . 5 ml of 1% H 2 O 2 . To this added 0 . 1 ml enzyme extract and O . D . change was measured at 430 nm for ever y 30 second for 2 minutes . The peroxides activit y

was calculated using an extinction coefficient of oxidized pyrogallol (4 . 5 liters/mol)[7] .

T.V = Total Rxn mixture
S.V = Sample volume
Absorptive = Mill molar Absorptive of NADH at
340 nm i.e. 6.22 × 103
P = Cuvette path length(1cm)

Assay of ascorbate oxidase activit y

Assay of ascorbate oxidase activit y was carried out according to the procedure of Vines and Oberbacher (20). The sample was homogenized [1: 5 (w/v)] with phosphate buffer (0 . 1 M/ pH 6 . 5) and centrifuged at 3000 g for 15 min at 50C . The supernatant obtained was used as enz yme source . To 3 . 0 ml of the substrate solution (8 . 8 mg ascorbic acid in 300 ml phosphate buffer, pH 5 . 6), 0 . 1 ml of the enz yme extract was added and the absorbance change at 265 nm was measured for ever y 30 seconds for a period of 5 minutes . One enz yme unit is equivalent to 0 . 01 O . D . change per min 8

Reagents

0.1M Phosphate / EDTA Buffer pH5.6
Substrate (0 . 005 M Ascorbic Acid)

Procedure

Into 10 mm quartz cells pipette the following at 250 C[8].

T a b l e : 1 T e s t s o l u t i o n

BUFFER (A)	2.0 ML	3.0 ML
SUBSTRATE (B)	0.1 ML	0.1 ML
EXTRACT (C)	0.1 ML	_
TOTAL	2.2 ML	3.1 ML

Results

Phytochemical Screening

Table:2 Phytochemical Screening of *Bergenia* ligulata

Constituents	**Water**	**Meth**	**Etha**	**Chloro**
Alkaloids	+	-	_	+
Carbohydrates	_	-	_	-
Saponin	+	+	+	+
Tannins	_	_	_	+
Flavonoids	+	+	_	-

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Table:3 Physiochemical analysis

Physiochemical Analysis	Results [in% w/w]
Loss on drying	0.00123 w/w
Total ash value	0.00678 w/w
Acid soluble ash value	0.0065 w/w
Acid insoluble ash value	0.0001 w/w
Water soluble extractive value	0.00151 w/w
Water insoluble extractive value	0.00256 w/w
Ethanol soluble extractive value	0.00155 w/w
Ethanol insoluble extractive value	0.00653 w/w
Methanol soluble extractive value	0.00180 w/w
Acetone soluble extractive value	0.0009 w/w
Petroleum ether soluble extractive value	0.0001 w/w

Antioxidant Activity

Calculation: Activity (µ/ml) = ΔA_{265} / min × 3.1 × dilution 13.386 × 0.1

Peroxides Activity: 1u/ml = 2.182 x 3.6 x 10^6 / 0.1 x 6.22 x 10^3 x 1 = 12.62 x 10^3

Ascorbic oxidase: Activity (µ/ml) = 2.8 x 3.1 x 20 / 13.386 x 0.1 = 173.6 / 1.338 = 129.74 (µ/ml)

Conclusion

The subject of herbal drug standardization is massively wide and deep. There is so much to know and so much seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function.

For the purpose of research work on standardization of herbal drug, a profound knowledge of the important herbs found in India and widely used in Ayurvedic formulation is most important.

Even when the chemical composition of a plant extract is known, the pharmacologically active moiety may not be. Environment, climate, and growth conditions influence composition, as does the specific part of the plant and its maturity. Monographs detailing standardization of active ingredients would improve the marketplace. Even if an herbal product is standardized to, for example, 4% of a constituent, the remaining 96% of ingredients is not standardized and may affect the product's solubility, bioavailability, stability, efficacy and toxicity. Just as controlled trials are necessary to establish safety and efficacy, manufacturing standards are required to ensure product quality.

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