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Standardization, Phytochemical Screening and TLC Profiling of *Polygonum Glabrum*

Raja S*[1], Ramya I[1]

1.GITAM Institute of Pharmacy, GITAM University, Visakhapatnam- Andhra Pradesh.

ABSTRACT

Standardization of medicinal plants is an important step as it determines the identity and quality of a plant. The aim of present study was to standardize *Polygonum glabrum* by evaluating physicochemical parameters. Successive extraction, phytochemical screening and thin layer chromatography analysis were studied to detect the presence of various phytoconstituents. Physicochemical parameters such as loss on drying, total ash, acid insoluble ash, water soluble extractive and alcohol soluble extractive values were found to be 06.7±1.19%w/w, 15.52±0.28%w/w, 4.65±0.02%w/w, 20.05±1.07%w/w and 13.66±0.20%w/w, respectively. In phytochemical screening, petroleum ether extract revealed the presence of steroids, tannins and saponins whereas chloroform extract confirmed the presence of alkaloids, flavonoids and carbohydrates. Ethylacetate extract showed the presence of phenolic compounds, steroids, proteins and glycosides. Further, major constituents like alkaloids, sugars, phenols, proteins, steroids, saponins, tannins and flavonoids were present in methanol extract. In addition, thin layer chromatography analysis supports the presence of terpenoids, alkaloids and flavonoids in petroleum ether, chloroform and methanol extracts, respectively. It can be concluded that the standardization parameters investigated in this study could assist in safe, accurately collection, handling of crude materials, ensure efficacy and stability of ended product. Further, the major active constituents were identified in different extracts of *Polygonum glabrum*

Keywords: *Polygonum glabrum*, Physicochemical analysis, Standardization, Thin layer chromatography

*Corresponding Author Email: sraja61@gmail.com
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INTRODUCTION

With increase in global use of herbal drugs, several questions pertaining to efficacy and safety of the herbal medicines are raised in drug market. Hence priority is given to standardize the drugs to have uniform efficacy and safety measures[1]. Standardization of herbal medicine is the process of providing a set of standards that ensures scrutinizing of various parameters like morphological, microscopical, physical and chemical properties of plants. It also includes carrying out qualitative and quantitative tests which assures of quality, efficacy, safety and reproducibility[2]. Standardisation also quantifies one or more of the active constituents of the plant material by an optimised analytical method such as HPTLC, HPLC or GCMS. Factors which emphasis the need for standardization are (i) adulteration with other medicines, (ii) incorrect dosing, (iii) low quality products and (iv) a false idea that all herbs are safe to use. *Polygonum glabrum*(Figure 1) which is commonly called as dense flower knotweed is a semi aquatic perennial plant. It belongs to the family polygonaceae and genus *Polygonum*[3]. It is an annual herb which is spread in moist habitats like river banks, canals, tank beds etc. *Polygonum glabrum* has branched stems with swollen nodes. Rooting is seen in the nodes[4]. Leaves are generally lanceolate or oblong lanceolate in shape with an entire margin, acute base and spiral arrangement. Flowers are white or pinkish with parted petals. The fruits are lens shaped and angular. Triangular achenes with perianth are seen. Seeds have numerous endosperms and a curved embryo. They are glossy and dark brown in colour[5].

Figure 1: Courtesy -www.pybio.org.

Polygonum glabrum holds a variety of traditional applications. Traditionally, *Polygonum glabrum* is used as a cardiotonic[6], astringent, anthelmentic and in the treatment of rheumatism[7]. Pharmacological activities such as antinephrotoxic, antidepressant, antiinflammatory, antipyretic, antithrombolytic and anticytotoxic activities were demonstrated by various invivo and invitro methods.[8,9] One of the limitations in acceptance of herbal drug is a lack of standard quality control profile. Hence, the aim of present work

was to standardize whole plant of *Polygonum glabrum* by evaluating its physicochemical parameters. In addition, identification of various phytoconstituents were performed by preliminary phytochemical screening and thin layer chromatography studies.

MATERIALS AND METHOD

Collection of plant material

The plant material was collected from Tirupati (Andhra Pradesh) and further identified, confirmed & authenticated by Dr. Madavchetty, Professor, Botany department, Sri Venkateswara University, Tirupati. The plant was preserved in the Herbarium of GITAM Institute of Pharmacy, GITAM University, for future reference (Voucher specimen No - 1916).

Processing of plant material

The collected *Polygonum glabrum* whole plant was washed thoroughly under tap water. The plant was cut in to small pieces and air-dried under shade (at room temperature) for two months to avoid direct loss of phytoconstituents from sunlight. Next the shade dried materials were powdered using pulverizer and sieved up to 80 meshes. It was then homogenized to fine powder and stored in air-tight container for furthers analysis.

Organoleptic parameters

This parameter was done by observing the plant with naked eyes and a note on color, appearance and odour were noted.

Physico chemical constants

This analysis include the determination of pH value at 1% and 10% solution, ash value, loss on drying, solubility and extractive values. These were carried out as per guidelines of WHO[10,11].

Determination of pH range

The pH of different formulations in 1% w/v (1g:100ml) and 10% w/v (10g:100ml) of water soluble portions of whole plant powder of *Polygonum glabrum* were determined using standard simple glass electrode pH meter.

Determination of loss on drying

About 5.0g of whole plant powder of the *Polygonum glabrum* was placed in a previously dried and tared flat weighing bottle. For estimation of loss on drying, the sample was dried in an oven at 100°C–105°C until 2 consecutive weighings did not differ by more than 5 mg.

Then, it was cooled in a desiccator for 30 minutes, and weighed without delay. The loss of weight was calculated as the content of in mg/ g of air -dried material.

Determination of total ash

Four grams of the powdered material was accurately weighed and placed in a previously ignited and tared silica crucible. The material was spread in an even layer and ignited by gradually increasing the heat to a temperature not exceeding 450°C, until it was white, indicating the absence of carbon. The material was cooled in a desiccator and weighed. The content of total ash was calculated in mg/g of air-dried material.

Determination of water-soluble ash

A small amount of ash was obtained by igniting the plant material. It was taken in a crucible and twenty five ml of water was added to it. It was covered with a watch glass and boiled gently for 5 minutes. Insoluble matter was collected on an ash less filter paper and washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C in a muffle furnace. The residue was allowed to cool in desiccators for 30 minutes, and then weighed without delay. The weight of the residue was subtracted in mg from the weight of total ash. Water soluble ash content was calculated as mg/ g of air-dried material.

Determination of acid insoluble ash

To the ash obtained by igniting the plant material, twenty five ml of hydrochloric acid was added and covered with a watch-glass and boiled gently for 5minutes. The watch-glass was rinsed with 5ml of hot water and it was added to the crucible. The insoluble matter was collected on an ash less filter paper. The filter paper containing the insoluble matter was transferred to the original crucible, ignited in a crucible at a temperature not exceeding 450°C in a muffle furnace. The residue was allowed to cool in a suitable desiccator for 30 minutes, and then weighed without delay. Acid insoluble ash content was calculated as mg/g of air dried material.

Determination of water soluble extractive

Five gm of air dried *Polygonum glabrum*, coarsely powdered was macerated with 100 ml of chloroform water in closed flask for 24 hrs, shaking frequently during six hours and allowed to stand for eighteen hours. It was filtered rapidly. 25ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated as mg/g with reference to air dried drug.

Determination of alcohol soluble extractive

Five gm of *Polygonum glabrum*, coarsely powdered was macerated with 100 ml of alcohol in closed flask for 24 hrs, shaking frequently during six hours and allowed to stand for eighteen hours. It was filtered rapidly taking precaution against loss of alcohol. 25ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 105°C and weighed. The percentage of alcohol soluble extractive was calculated as mg/g with reference to air dried drug.

Extraction

Depending on polarity, *Polygonum glabrum* plant was extracted successively with different solvents like petroleum ether, chloroform, ethylacetate and methanol in a soxhlet extractor for 72 hrs in batches of 500g each. Every time, the marc was dried before extracting with the next solvent. The excess solvents were removed from all the extracts by vacuum rotary flash evaporator and hot plate and finally stored in desiccators for phytochemical analysis.

Phytochemical screening

The preliminary phytochemical screening of the petroleum ether, chloroform, ethylacetate and methanol extracts of whole plant powder of *Polygonum glabrum* was carried out using standard laboratory procedures, to detect the presence of different secondary metabolites (phytochemical constituents) such as alkaloids, flavonoids, saponins, glycosides, tannins, phenols, terpenoids, steroids, proteins[12,13].

Determination of alkaloids

Mayer's test: 1.3 ml of extract was taken in a test tube and 0.2 ml of dilute hydrochloric acid was added.To this 0.1 ml of Mayer's reagent (Potassium Mercuric Iodide) was added. Formation of yellow colored precipitate gives positive test for alkaloid.

Wagner's test: To 2 ml of extract .2 ml of dilute hydrochloric acid and 0.1 ml of Wagner's reagent. (Iodine in Potassium Iodide) was added. Formation of reddish brown precipitate indicated the positive response for alkaloid.

Determination of tannins

About 2ml of the aqueous extract was stirred with 2ml of distilled water and few drops of $FeCl_3$ Solution were added. Formation of green precipitate indicated the presence of tannins.

Determination of saponins

5 ml of aqueous extract was shaken vigorously with 5ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Determination of flavonoids

To 1 ml of extract, magnesium ribbons were added. To this conc hydrochloric acid was added. The formation of a reddish pink colour was taken as a positive test for flavonoids.

Determination of phenols

To the extract, 5 mL of distilled water was added. To this, 3ml of 10% lead acetate were added. A bulky white precipitate indicated the presence of phenol compounds.

Determination of terpenoids

2 ml of the organic extract was dissolved in 2ml of chloroform and evaporated to dryness.2 ml of concentrated sulphuric acid was added and heated for about 2 min. Development of a greyish colour indicated the presence of terpenoids.

Determination of glycosides

Borntrager's test: Few ml of dil.sulphuric acid added to the extract. It was Boiled and filtered. The filtrate was treated with with ether or chloroform. Then organic layer was separated to which ammonia was added, pink red color was produced in organic layer.

Determination of carbohydrates

To the extract a small amount of Molisch's reagent (α-naphthol dissolved in ethanol) was added in a test tube. Concentrated sulfuric acid was slowly added down the sides of the test tube, to form a layer. A positive reaction is indicated by appearance of a purple ring at the interface between the acid and test layers indicated that presence of carbohydrates.

Determination of proteins

Biuret test: 1 ml of 40% NaOH mixed with 2 drops of 1%copper sulphate to the extract, a violet color indicated the presence of proteins.

Determination of steroids

I. 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it. A red color formed in lower chloroform layer indicated the presence of steroids.

II. 1 ml of the organic extract was dissolved in 1 ml of chloroform and treated with sulphuric acid and acetic acid. A green color formed indicated the presence of steroids.

Thin layer chromatography

Different mobile phases were tried for developing a TLC system. Taking into account the chemical nature of the constituents, the solvent system that showed maximum separation was selected as an ideal mobile phase for the study.

Preparation of TLC plates

TLC plates were prepared by using Silica Gel-GF254 as adsorbent. 15gm silica gel-G was mixed with 30ml of distilled water (1:2) to make slurry. The slurry was immediately poured into the plates. Plates were then air dried and activated for 30 minutes at a temperature of 110°C before use. Using a micropipette, about 10µl of different extracts were individually loaded over the plates and air dried. The plates were developed in different solvent systems such as hexane : ethyl acetate (7.2:2.9); toluene: ethyl acetate: diethyl amine (7:2:1); chloroform: benzene (1:1); acetone: water: conc ammonia (9:7:.3); ethyl acetate: formic acid: glacial acetic acid: water (10:1.1:1.1:2.7) etc. The chromatograms were observed under visible light, UV light, iodine vapours and certain spraying reagents were used.

RESULTS AND DISCUSSION

Organoleptic evaluation

The appearance colour, taste, and odour of whole plant of *Polygonum glabrum* course powder were noted which provided first hand information. The powder appeared to be brown in color with a characteristic odour. The taste was found to be bitter.

Physicochemical investigation

Different physicochemical constants were determined three times and the average values were recorded. The pH value of crude drug indicates whether the drug will be absorbed in intestine or stomach. pH of 1% w/v formulation solution and pH of 10% w/v formulation solution were found to be 6.3±0.04%w/w and 5.5±0.02%w/w respectively. Loss on drying represents the loss of volatile substances along with the water. The loss on drying value for *Polygonum glabrum* was found to be 6.7±1.19%w/w. Determination of ash value gives us a rough outline for detecting the nature of the material, added to the crude drug for adulteration purpose. The total ash, water soluble ash and acid insoluble ash values were found to be 15.52±0.28%w/w, 1.19±0.33%w/w and 4.65±0.02%w/w respectively. Results of extractive value determination show that water soluble extractive value (20.05±1.07%w/w) was more than ethanol soluble extractive value (13.66±0.20%w/w). The values of physico chemical constants of whole plant of *Polygonum glabrum* are given in Table 1.

Table 1: Physicochemical parameters of whole plant of *Polygonum glabrum*

Parameters	Values
pH of 1% w/v formulation solution	6.3±0.04
pH of 10% w/v formulation solution	5.5±0.02
Loss on drying	6.7±1.19%W/W
Total ash value	15. 52±0.28%W/W
Water soluble ash	1.19±0.33%W/W

Acid insoluble ash	4.65±0.02%W/W
Water soluble extractive value	20.05±1.07%W/W
Ethanol soluble extractive value	13.66±0.20%W/W

Determination of solvent extractive value

The air dried powder of *Polygonum glabrum* plant was extracted successively with different solvents. The average yield obtained in successive extraction with petroleum ether, chloroform, ethyl acetate and methanol was found to be 3.1% w/w, 4.2%w/w, 2.4%w/w and 6.5%w/w respectively. The average yield is given in Table 2.

Table 2: **Successive extraction of *Polygonum glabrum***

Solvent	Extractive yield(% w/w)	Appearance
Petroleum ether	3.1	Greenish brown
Chloroform	4.2	Greenish brown
Ethyl acetate	2.4	Brownish black
Methanol	6.5	Brownish mass

Phytochemical screening

The preliminary phytochemical screening of *Polygonum glabrum* revealed the presence of steroids, terpenoids, tanins and saponins in petroleum ether extract. Chloroform extract revealed the presence of alkaloids, flavonoids and carbohydrates. Ethylacetate extract showed the presence of phenolic compounds, steroids, proteins and glycosides. Methanol extract showed the presence of alkaloids, sugars, phenols, proteins, steroids, saponins, tannins and flavonoids. The preliminary phytochemical screening for various functional groups was mentioned in Table 3.

Table 3: Phytochemical screening of whole plant of *Polygonum glabrum*

s.no	Phytoconstituents	Tests	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract
1	Alkaloids	Mayers test	-	+	-	+
		Wagners test	-	+	-	+
2	Glycosides	Borntrager's test	-	-	+	-
3	Saponins	Foam test	+	-	-	+
4	Phenolic compounds	Lead acetate test	-	-	+	+
5	Tannins	Ferric chloride tests	+	-	-	+
6	Steroids	Libermann Buchard test	+	-	-	+
		Salkowski test	+	-	+	-
7	Terpenoids	Tin & thionyl chloride test	+	-	-	-
8	Flavonoids	Shinoda test	-	+	-	+

		Alkaline test	-	+	-	+
9	carbohydrates	Benedicts test	-	+	-	+
10	Proteins	Biuret test	-	-	+	+

(+) presence, (–) absence

Thin layer chromatography

Thin layer chromatography analysis of *Polygonum glabrum* petroleum ether extract showed the presence of terpenoids with R_f value of 0.57 and 0.73 in solvent system of hexane:ethyl acetate (7.2:2.9) and benzene:chloroform (1:1), respectively. Vanillin in sulphuric acid was used as the spraying reagent. Appearance of blue and purple colour spot indicated the presence of terpenoids in petroleum ether extract. Similarly, chloroform extract showed the presence of alkaloids with R_f value of 0.79 and 0.87 in solvent system of acetone: water: conc ammonia (9:7:0.3) and toluene: ethyl acetate: diethyl amine (7:2:1) respectively. Dragondroff reagent was sprayed for the detection of alkaloids. Appearance of brown and orange colour spot indicated the presence of alkaloids in chloroform extract. It was also observed that the methanol extract of *Polygonum glabrum* showed the presence of flavonoids with R_f value of 0.82 in ethyl acetate: formic acid: glacial acetic acid: water (10:1.1:1.1:2.7) as solvent system. A second spot with R_f value of 0.90 was detected with a solvent system of toluene: ethyl acetate: diethyl amine (7:2:1). Appearance of yellow and orange colour spot indicated the presence of flavonoids in methanol extract. R_f values of terpenoids, alkaloids, flavonoids identified from various extracts of *Polygonum glabrum* are tabulated in Table 4,5 &6 .

Table 4: **Terpenoids: TLC studies for petroleum ether extract of *Polygonum glabrum***

Solvent system	**Spraying reagent**	**Color of spot**	**R_f value**	**Inference**
Hexane : ethyl acetate (7.2:2.9)	Vanilin sulphuric acid	Blue	0.57	Presence of terpenoids
Benzene : chloroform(1:1)	Vanilin sulphuric acid	Purple	0.73	Presenceof terpenoids

Table 5.**Alkaloids: TLC studies for chloroform extract of *Polygonum glabrum.***

Solvent system	**Spraying reagent**	**Color of spot**	**R_f value**	**Inference**
Acetone:water:conc ammonia (9:0.7:0.3)	Dragondroff reagent	Brown	0.79	Presence of alkaloids
Toluene :ethyl acetate:diethyl amine(7:2:1)	Dragondroff reagent	orange spot	0.87	Presenceof alkaloids

Table 6:**Flavonoids: TLC studies for methanol extract of *Polygonum glabrum***

Solvent system	**Spraying reagent**	**Color of spot**	**R_f value**	**Inference**
Ethyl acetate:fornmic acid: glacial	Iodine	Yellow	0.82	Presence of

acetic acid: water (10:1.1:1.1:2.7)	vapours			flavonoids
Chloroform: acetone: formic acid (7.5:1.65:.85)	Iodine vapours.	Orange color	0.90	Presenceof flavonoids

DISCUSSION

The physicochemical parameters of whole plant powder of *Polygonum glabrum* were estimated based on the methods recommended by World Health Organization (WHO). The pH of 1% w/v and 10% w/v solutions were found to be 06.3 ± 0.04 and 05.5± 0.02 respectively. It works on the principle that drug in the opposite pH are unionized and absorbed rapidly from the stomach/ intestine accordingly. Percent weight loss on drying or moisture content value was found to be 06.7±1.19%w/w. Generally less moisture content helps in keeping the crude drug dry without detoriation from bacterial, fungal or yeast growth during storage. Ash value is generally used to determine the inorganic substances present in the drug. The ash values like total ash; water soluble ash and acid insoluble ash were found to be 15.52 ± 0.28%w/w, 01.19 ± 0.33%w/w, and 04.65 ± 0.02%w/w respectively. Ash values helps to find out quality, authenticity and purity of crude drug and also these values are important quantitative standards. The solubility percentage of *Polygonum glabrum* in aqueous extraction was found to be higher (20.05±1.07%w/w) than ethanolic extraction (13.66±0.205%w/w). These extractive values are significant as they help in the identification of chemical components present in the crude drug. The average yield during successive extraction of *Polygonum glabrum* with four different solvents including petroleum ether, chloroform, ethyl acetate and methanol was found to be 3.1%w/w, 4.2%w/w, 2.4%w/w and 6.5%w/w. The preliminary phytochemical screening of *Polygonum glabrum* showed the presence of steroids, terpenoids, tanins and saponins in petroleum ether extract. Besides, chloroform extract revealed the presence of alkaloids, flavonoids and carbohydrates. Furthermore, ethylacetate extract showed the presence of phenolic compounds, steroids, proteins and glycosides. However, it was methanol extract which showed the presence of more phtoconstituents like alkaloids, sugars, phenols, proteins, steroids, saponins, tannins, flavonoids etc. than the rest of other extracts. It is apparent that glycosides are present only in ethylacetate extract and terpenoids are present in petroleum ether extract alone. As seen in Table 4, 5 and 6 all the extracts were subjected to thin layer chromatography by using different solvent systems. TLC of petroleum ether extract showed the presence of terpenoids with R_f vaue of 0.57 and 0.73. Similarly, chloroform and methanol extracts revealed the presence of alkaloids (R_f values of 0.79 and 0.87) and flavonoids (R_f values of 0.82 and

0.90), respectively. From TLC results it is evident that the terpenoids detected were weakly polar in nature and by using solvents of low polarity as mobile phase they could be separated. In TLC of alkaloids, addition of base such as ammonia and diethyl amine (short chain amines) to the mobile phase led to better selectivity of separation. Similarly flavonoids detected from TLC analysis may be highly methylated flavones or flavonols as they require nonpolar solvents for their separation. TLC profiling of all extracts gave satisfactory results that indicates the presence of number of phytoconstituents.

CONCLUSION

The present work was undertaken to lay down the standards which could be valuable for establishing the validity of *Polygonum glabrum*. The organoleptic characters and physicochemical parameters were found to be within the prescribed limits as per WHO. The quality control study was supported by phytochemical and thin layer chromatography analysis which confirmed the presence of various phytoconstituents such as terpenoids, alkaloids and flavonoids in petroleum ether, chloroform and methanol extracts. The important diagnostic features of *Polygonum glabrum* established in this study may be useful to conduct various isolation and pharmacological activities in this plant.

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