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Research Article

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND PHYSICOCHEMICAL ANALYSIS OF *PHASEOLUS SEMIERECTUS*

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ABSTRACT

This paper provides the physicochemical characterization and a preliminary phytochemical analysis of *Phaseolus semierectus*. The quality and purity of the plant material was measured through physicochemical parameters like moisture content, ash value, and extractive values. The obtained results showed acceptable levels in standard ranges, which proves that the sample is suitable to be analyzed further. Initial Phytochemical screening showed that it contains bioactive compounds including alkaloids, flavonoids, tannins, saponins, and phenolic compounds. The results obtain a basis of data to support the medicinal potential of *P. semierectus,* and it can be the basis of future detailed phytochemical and pharmacological studies.

Keywords: Phytochemical screening, Alkaloids, Flavonoids, Tannin, Saponins, Phenolic compounds.

INTRODUCTION

Phaseolus semierectus is a Perenial non-climbing herb, not threatened. Herbaceous annual or short-lived perennial, erectly branching, 0.6-1m tall, sometimes trailing or twinning to 1.5 m (especially in shade), becoming somewhat woody with age towards the base. Stems sparsely to densely appresed pubescent. Trifolliate, leaflets mostly entire ovate to lanceolate or narrowly, elliptic, 3-8 cm long; 1-3.5 cm wide, upper surface glabrous, lower surface adpressed heavy, petioles (1-5 cm long), stipules lanceolate, 5-6mm long lateral leaflet sometimes slightly lobbed towards base (Helmstadter, 2010). A semi-erectum, spicate raceme about 15 cm long borne on axillary pedunclesto about 30 cm long. Flowers papilionate on very short pedicels; calyx companulate, 4-6 mm long; standard reed to reed- purple (rarely white or pink), roundish 13 mm, wing and keel petals, tinged green, red or white; keel spirally twisted. Linear, sub-cylindrical, 5.5 -12cm long, 2.5-3 mm wide, straight or slightly curved, glabrous or pubiscent, the valves becoming strongly twisted on dehiscence (pods shatter readily on maturity), each pod containing 20-30 seeds. Obliquely oblong, slightly compressed, about 3mm long, mottled light and dark grey-brown or black; 88000- 1,54,000 seeds per kg (Alcázar-Valle *et al*., 2020). Phasey bean originated from tropical America (central America, Caribbean Islands, South America) and is naturalized in the tropics and subtropics (Debouck *et al*., 2021).

It was introduced into India, Australia, Africa and Southern USA. It grows from 23^0 to 30^0 C and from sea level upto 1800-2000m open situations along streams and rivers. Phasey bean grows better in warm condition (optimum temperature being 25-30^0 C). It shed its leaves under rost, but can survive light frost: survival after -8.3^0c was recorded. It is mainly found in wet places along roadsides, on waste lands, in open fields, postures and in grow where annual rainfall ranges from 450-3000mm. In drier places, it may grow in drainage lines or wet depressions. Its resistance to severe drought is due to its free seedling ability. Phasey bean is tolerant to water logging and flooding as its nodulated roots can benefit from water excess. It does well on a wide to poorely drained and deeply- sandy to heavy- clayey soils acidic to alkaline (5-8) saline soils are fairely tolerated but excess Mn and Al should be alleviated with lime application. Some bioactive compounds related with health benefits such as alkaloids, anthocyanins, carbohydrates, catechins, fibres, flavonoids, phasine, phytic acid, quercetin, saponins, steroids, tannins,

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terpenoids and trypsin inhibitors. Flavonoids, Naringenin, Malvidin, Myristicin, Quercetin, kaemferol. Phenolic compounds and Phenolic acids those are protocatechine, Gentisic, Vanille, Syringic, P- Coumaric acid and caffeic acid. Anti-inflammatory activity of ethanolic extract derived from *Phaseolus anguris* beans. Anti diabetic (Type –II) aqueous extract of whole plant. Anti-inflammatory and analgesic activity of seeds of *Phaseolus vulgaris* (Pradeepkumar *et al*., 2015, Singh *et al*., 2004).

MATERIALS AND METHODS

Sodium hydroxide, Acetic acid, 1N Hydrochloric acid, 1N Nitric acid, 5% Iodine, 5 % Ferric chloride, 10% sodium chloride, 5% Potassium hydroxide, 5% iodine**,** Whatmann No. 41. All the chemicals used in the present study including standards were purchased from the Sigma-Aldrich; Himedia.

Physicochemical parameters

The ash values, extractive values and loss on drying were performed according to the officinal methods prescribed in Indian pharmacopeia and the WHO guidelines on quality control methods for medicinal plants materials (Ameh *et al*., 2010).

Determination of total ash

About 20 grams (accurately weighed) ground powder was taken in a silica crucible previously ignited and weighed. It was incinerated by gradually increasing the heat not exceeding dull red heat (450^0C) until free from carbon, cooled and weighed. The percentage of ash was calculated. The procedure was repeated five times to get constant weight.

Determination of water-soluble ash

The Total ash was boiled with 25ml of water for 5 minutes and was filtered through an ash less filter paper (Whatmann No. 41). It was followed by washing with hot water. The silica crucible in which filter was ignited, cooled and water insoluble ash was weighed. The water-soluble ash was calculated by subtracting the water insoluble matter from the total ash.

Determination of acid insoluble ash

The total ash obtained was boiled for 5 minutes with 10%w/v dilute hydrochloric acid and filtered through an ashless filter paper (Whatmann No. 41). The filter paper was ignited in the silica crucible, cooled and acid insoluble ash was weighed.

Determination of loss on drying

For the determination of loss on drying the following method was followed. About 15gm of the powdered was accurately weighed in a glass stoppered weighing bottle which is previously dried for 30mins in the drier. Then, the sample was gently shaken side wise for even distribution and dried in an oven at 100^OC to 105^OC by removing the stopper. It was cooled and again weighed. The loss on drying was calculated.

Determination of alcohol soluble extractive

5 grams of the powder was macerated with 100ml of alcohol of the specified strength in a closed flask for 24hrs, shaking frequently during 6hrs and allowing it to stand for 18hrs. It was filtered rapidly taking precautions against loss of alcohol, and 25 ml of the filtrate was evaporated to dryness in a tared bottomed shallow dish at 105^0 C and weighed. The percentage of alcohol soluble extractive was calculated.

Determination of water-soluble extractive

About 5gms of the powder was added to 50ml of water at 80^OC and to it 2gms of keiselghur was added and filtered. 5ml of the filterate was transferred to a tared evaporating dish, the solvent was evaporated on a water bath, drying was continued for half an hour, finally it was dried in a hot air oven for two hours and weighed. The percentage of water-soluble extractive was calculated (Table 1).

Table 1. Powder analysis.

Measured Parameter	Amount of powder taken (gm)	Amount of leaf Powder obtained (gm)	Amount of stem Powder obtained
Loss on Drying	20	*4.612*	*4.812*
Water soluble extractive value	5	*0.44*	*0.47*
Alcohol soluble extractive value	5	*0.54*	*0.56*
Total Ash Content	15	*2.2*	*1.3*
1. Water soluble Ash	2.6	*0.5*	*0.6*
2. Acid insoluble Ash	2.6	*0.78*	*0.79*

Table 2. Fluorescence study with different chemical reagents in visible and UV light of *Phaseolus semierectus* leaf powder.

S. No.	Leaf powder + Reagent	Visible	Short wavelength	Long wavelength
1	Powder as such	Green	Green	Greenish black
2	Powder + distilled water	yellow green	Pear green	Greenish black
3	Powder+1 N NaOH in distilled water	Dark green	Pine green	Greenish black
4	Powder+1 N NaOH in alcohol	Yellow green	Brunette Green	Green
5	Powder+10% HCl	Dark green	Pear green	Brunette green
6	Powder + Conc. HCl	Dark green	Moss green	Black green
7	Powder + Conc. HNO3	Walnut brown	Moss green	Gingerbread brown
8	Powder + Conc. H2So4	Brown yellow	Dark brown	yellowish Black
9	Powder + Acetone	reddish brown	Pickle green	Caramel green
10	Powder + 5% iodine	light brown	Juniper green	Black green
11	Powder + 5% KoH	light brown	Juniper green	Black green
12	Powder + 5% FeCl3	Brown	Juniper green	greenish yellow

Table 3. Fluorescence study with different chemical reagents in visible and UV light of *Phaseolus semierectus* stem powder

S. No.	Stem powder + Reagent	Visible	Short wavelength	Long wavelength
1	Powder as such	Tawny greenish brown	Peanut	Caramel greenish brown
2	Powder + distilled water	Gingerbread green	Pear green	Caramel green
3	Powder+1 N NaOH in distilled water	Brunette green	Pine green	Black green
4	Powder+1 N NaOH in alcohol	Chocolate brown	Brunette brown	Brownish Black
5	Powder+10% HCl	Caramel brown	Pear green	Brunette brown
6	Powder + Conc. HCl	Syrup green	Moss green	Black
7	Powder + Conc. HNO3	Walnut green	Moss green	Gingerbread brown
8	Powder + Conc. H2So4	Brown	Dark brown	Black
9	Powder + Acetone	Rust brown	Pickle green	Caramel brown
10	Powder + 5% iodine	Amber brown	Juniper green	Black
11	Powder + 5% KoH	Chocolate brown	Juniper green	Black
12	Powder + 5% FeCl3	Mocha brown	Juniper green	Brown

Fluorescent analysis

The fluorescent analysis of shade dried and powdered, methanolic extracted plant material of *Phaseolus semierectus* was studied under UV light and daylight. Fluorescent analysis of plant powder was carried out according to the methods of Kokoshi *et al*., (1958) powder was subjected to different chemicals like, 1N NaOH, Acetic acid, 1N HCl, 1N HNO3, 5% Iodine, 5 % FeCl3, 1N NaOH in methanol (Wolstenholme R, 2021, Kokoshi C.J.,1958). The fluorescence analysis of these leaf extracts was observed under ordinary visible light and also under UV light (245 nm) and recorded in Table 2&3.

Phytochemical examination

The plant material was collected in December 2014 from Andhra University campus, Andhra Pradesh, India and authenticated by Dr. S.B. PADAL, taxonomist, Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh. The Voucher specimens 21915 were deposited in the herbarium, College of Pharmaceutical Sciences, Andhra University.

Extraction process

The freshly collected whole plant was shade dried and powdered. The powdered materials were then subjected to Soxhlet extraction process with methanol. This process enables us in obtaining the chemical constituents which are soluble in the respective solvents (hexane, chloroform, methanol) depending upon their solubility.

Soxhlet extraction

The dried powdered materials of the plant were extracted successively three times with methanol. The extracts thus obtained were concentrated under vacuum at temperature of 43℃ by using rotary evaporator, dried completely, weighed and stored in desiccators (Table 4). After the extraction process with methanol is completed then the plant material is again shade dried (De Castro and Priego-Capote, 2010). Initially TLC (thin layer chromatography) was performed for the extract with 100% hexane then with 98% hexane and 2% chloroform and so on by increasing 2% each time up to 100% methanol. The spots obtained on the TLC plate were observed in the U.V chamber initially and then by using the spraying reagent the spots were observed and reported.

Table 4. Details of the extraction

Plant material	Solvent used	Volume of the solvent (L)	Weight of the extract (g)
Leaf 500 g	Methanol	3.2	15
Stem 500 g	Methanol	2 .8	10

Phytochemical analysis

The extract prepared was tested for the type of chemical constituents present by known qualitative tests. The following tests were carried out on the extracts to detect various phytoconstituents present in them (Ajayi *et al*., 2011).

Tests for alkaloids

About 50 mg of solvent – free extract was stirred with little quantity of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows.

Mayer's test

To a few ml of filtrate, two drops of Mayer's reagent was added along with the sides of the test tube. If the test is positive, it gives white or creamy precipitate.

Wagner's test

To a few ml of the filtrate, few drops of Wagner's reagent were added along with the sides of the test tube. Formation of reddish-brown precipitate confirms the test as positive.

Hager's test

To a few ml of filtrate 1 or 2 ml of Hager's reagent was added. A prominent yellow precipitate indicates positive test.

Dragendorff's test

To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent was added. A prominent reddish-brown precipitate indicates positive test.

Tests for carbohydrates

About 100mg of the extract was dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to the following tests.

Molisch's test

To 2 ml of filtrate, two drops of alcoholic solution of α – naphthol was added. The mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube; the test tube was cooled in ice water and allowed to stand. A violet ring at the junction of two liquids indicates the presence of carbohydrates.

Fehling's test

1 ml of filtrate was boiled on a water bath with 1 ml each of Fehling's solution A and B. Formation of red precipitate indicates the presence of sugar.

Barfoed's test

To 1 ml of the filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 minutes. Red precipitate indicates the presence of sugar.

Benedict's test

To 0.5 ml of filtrate 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic brick red precipitate indicates the presence of sugar.

Tests for glycosides

For detection of glycosides, about 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hrs on a water bath, filtered and the filtrate was subjected to the following tests (Harborne and williams, 2001).

Borntrager's test

To 2 ml of filtrate hydrolysate, 3ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Formation of pink color indicates the presence of anthroquinone glycosides.

Legal's test

About 50 mg of the extract was dissolved in pyridine. Sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. Presence of glycoside is indicated by a characteristic pink color.

Tests for saponins

Foam or froth test

A small quantity of the extract was diluted with distilled water to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. A two-centimeter layer of foam or froth which is stable for 10 minutes indicates the presence of saponins.

Tests for phytosterols and triterpenoids

Liebermann – burchard's test

The extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the side of the test tube. Red, pink or

violet color at the junction of the liquids indicates the presence of steroids / triterpenoids and their glycosides.

Salkowski test

Few drops of concentrated sulphuric acid was added to the chloroform extract, shaken on standing, red colour in the lower layer indicates the presence of steroids and golden yellow colour indicates the presence of triterpenoids.

Tests for phenolic compounds and tannins

Ferric chloride test

About 50 mg of extract was dissolved in distilled water and to these few drops of neutral 5% ferric chloride solution was added. Formation of blue, green and violet color indicates the presence of phenolic compounds.

Gelatin test

A little quantity of extract was dissolved in distilled water and 2 ml of 1% solution of gelatin containing 10% sodium chloride was added to it. Development of white precipitate indicates the presence of phenolic compounds.

Lead acetate test

A small quantity of extract was dissolved in distilled water and to this; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

Tests for flavonoids

An aqueous solution of extract was treated with 10% ammonium hydroxide solution – yellow fluorescence indicates the presence of flavonoids (Mishra *et al*., 2019).

Shinoda test or magnesium – hydrochloric acid reduction

A little quantity of extract was dissolved in alcohol and few fragments of magnesium turnings and conc. hydrochloric acid (drop wise) were added. If any pink or crimson – red colour develops, presence of flavonol glycoside is inferred.

Zinc-hydrochloric acid reduction Test

The alcoholic solution is treated with pinch of zinc dust and few drops of concentrated hydrochloric acid - magenta colour is produced after few minutes. The Phytochemical analysis results were tabulated in Table 5.

Table 5. Phytochemical analysis.

S. No	Name of the test	Methanolic leaf extract	Methanolic stem extract
1.	**Steroids**		
	A) Salkowaski Test	+	+
	B) Libermann-Buchard's Test	+	+
2.	**Triterpenoids**		
	A) Salkowaski Test	+	+
	B) Libermann-Buchard's Test	+	+
3.	**Saponins**		
	A) Foam Test	+	+
4.	**Alkaloids**		
	A) Mayer's Test	+	+
	B) Dragandorff's Test	+	+
	C) Wagner's Test	+	+
6.	**Flavanoids**		
	A) Shinoda Test	+	+
	B) Ferric chloride Test	+	+
7.	**Tannins**		
	A) Ferric chloride Test	+	+
8.	**Glycosides**		
	A) Ferric chloride Test	+	+
	B) Balget's Test	+	+
	C) Borntragger's Test (Anthraquinone)	+	+

'+' Present '-' Absent

RESULTS AND DISCUSSIONS

Physicochemical analysis of *Phaseolus semierectus* incorporated the moisture content, ash value as well as extractive value of the leaf and stem powder. The findings indicated that the moisture level was in the acceptable range, which implies that microbial food contamination is less likely to occur and that the shelf life will be higher. The total ash, acid-insoluble ash and water-soluble ash values verified purity of the plant material and the lack of any important inorganic adulterants. The values of methanolic extractives were observed to be superior relative to other solvents and this proves the effectiveness of methanol to extract bioactive phytoconstituents in leaves and stems. Initial phytochemical analysis of the methanolic extracts of leaf and stem powder, showed that the extracts contained the major secondary metabolites, alkaloids, flavonoids, tannins, saponins, and phenolic compounds. These ingredients are associated with their functions of giving antioxidant, antimicrobial, and anti-inflammatory effects. The presence of large quantities of flavonoids and phenolics indicates a high potential of the source to act as an antioxidant with the presence of alkaloids and tannins reinforcing the potential of the source to act as an antimicrobial agent. The phytochemical profiles of both the leaf and stem extracts were found to be similar, and it supports the therapeutic potential of the whole plant. The powdered leaf and stem were analyzed using fluorescence using visible and ultraviolet light after treatment with different chemical reagents. The samples exhibited typical color reactions and fluorescence reactions which are typical of some phytoconstituents. The distinct patterns of fluorescence can be used as a good method to identify and authenticate *Phaseolus semierectus* and help to draw the difference between the authentic and adulterated samples. These results go hand in hand with the results of phytochemical analysis because some of the compounds are known to fluoresce under UV light.

CONCLUSION

The current study of the powdered leaves and stems of *Phaseolus semierectus* and their methanolic extracts offer the necessary baseline information on quality evaluation and authentication of this medicinal plant. The purity and appropriateness of the plant material is verified by the physichemical analysis, where the moisture and ash values are in reasonable ranges. Preliminary phytochemical screening indicates that the species harbors significant bioactive constituents like alkaloids, flavonoids, tannins, saponins and phenolic compounds in the leaf and stem extracts, which validates the therapeutic potential of the species. The use of unique fluorescence patterns with visible and ultraviolet light also contributes to the easy identification and verification of *Phaseolus semierectus*, which is a powerful instrument in the identification of adulteration. In general, the combined outcomes of the physicochemical, phytochemical and fluorescence analyses provide a detailed profile of *Phaseolus semierectus* and support its prospective use as a source of bioactive compounds in both traditional and modern medicines.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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