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Preliminary Phytochemical Screening of Leaf and Stem Extracts of *Canavalia rosea*

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Abstract

The coastal sand dunes (CSD) are exclusive ecosystems, plays a foremost role in defending the zone from erosion, and flood, also abundant in biotic and abiotic resources. The bioactive compounds and phytochemicals from these plants were stated to have copious pharmacological applications. The current research evaluates the comparative study of phytochemical analysis of leaf and stem extract of *Canavalia rosea*. The extraction is performed using different solvents namely ethanol, aqueous, chloroform, acetone and methanol. Preliminary analysis of the leaf and stem extract validates the presence of major bioactive compounds, namely terpenoids, saponins, flavonoids, alkaloids, phenolic groups, phlobatannins, cardiac glycosides, coumarin etc.

***Key words*:** *Canavalia rosea*, Qualitative phytochemical analysis, Leaf extract, Stem extract, Secondary metabolites

Since ancient era, traditional medicines were followed across the globe, for diagnosis of several chronic ailments. India has diverse traditional medical systems like Ayurveda, siddha, Unani and a vast class of ethnomedicine [1]. Plants act as foremost source of diet supplement, as well as medicinal source for diseases. The plants are used up as whole or specific parts of it, possibly in the form of powder, paste, juice extracts, decoction, for oral administration. The plants may be used alone or used in combinations (polyherbal formulation) [2].

The phytochemicals are widely distributed among terrestrial plant sources, vegetables, fruits, spices and herbs; marine sources like seaweeds, algae, sponges and coastal plants [3]. The secondary metabolite constituents existing in the herbs are apparent elements of diagnosis. Phytochemicals are classified into three major classes namely terpenoids, polyphenols and thiols. Polyphenols are divided further as phenolic acids, flavonoids and non-flavonoids such as tannin, lignans and curcumins. Allyl sulfides, glucosinolates and sulphur-free indoles form the thiol group [4].

The advantages of these phytonutrients comprise of anti-microbial, antioxidant, antibiotic, anti-inflammatory, antidiabetic, anti-cancer properties. There are numerous phytochemicals explored recently by researchers, which contributes significantly for the welfare of human kind, since they are beneficial in routine health improvement [3]. World Health Organization (WHO), reports that over 80% of the people of developing countries are depending on the traditional medicines from the plant extracts for their primary health needs. Use of these traditional medicines for the preparation of modern medical preparations is indispensable and thus 'Phytomedicines' are a link between the traditional and modern medicine.

The present research work utilizes *Canavalia rosea*, a leguminous, sand dune plant, commonly known as "beach bean or jack bean". Coastal Sand Dunes (CSD) is an exclusive ecosystem between ocean and land areas which protect the area, and also has ecological importance and socio-economic value [5-6]. Coastal dunes are natural structures that protect the coastal environment by absorbing the energy of wind, tides and waves [7]. The study involves preliminary phytochemical analysis of leaf and stem extract of *C. rosea* with different solvents.

Canavalia rosea

The genus name "*Canavalia"* is a Latin derivative of "Kanavali" (Malabar) means "forest climber" and "climbing herb" [8]. The species name "rosea" is from the Latin "roseaus", "rosy" which refers to pink flowers. The plant is also named as bay bean, seaside jack bean and beach bean in regional language.

Synonyms

- *Canavalia apiculata* (Piper),
- *Canavalia arenicola* (Piper),

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- *Canavalia obtusifolia* (Lam.),
- *Canavalia maritima* (Aubl.),
- *Dolichos emarginatus* (Jacq.),
- *Dolichos roseus* (Sw.), and
- *Dolichos maritimus* (Aubl.),
- (In Latin "maritimus" means ''belonging to the sea.'')

Taxonomic description [9]

Kingdom	:	Plantae
Subkingdom	:	Tracheophytas
Super phylum	:	Espermatophyta
Phylum	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Faboideae (Papilionoideae)
Subtribe	:	Diocleinae
Genus	:	*Canavalia*
Subgenus	:	*Canavalia*
Species	:	*rosea* (Sw.) DC

MATERIALS AND METHODS

Plant collection and identification

Canavalia rosea plant samples were collected from Cuddalore district. The collection location is 11° 33`44.5" N79 ° 45`25.7" E. Herbarium specimens have been sent to the Rapinet Herbarium of the Department of Botany, St. Joseph Autonomous College in Tiruchirappalli for identification. The specimen has been identified and confirmed to be *Canavalia rosea*. The sample registration number is 2016.

Plant drying

The samples were collected as a bundle and washed twice with deionized water. The various parts of the plant are isolated and dried separately in the shade. The leaves and stem were shade dried for about three to four weeks, and ground into a coarse powder and stored in an airtight container.

Extraction procedure

The bioactive complexes are retrieved from the plants, using several techniques namely maceration, supercritical fluid extraction, subcritical water extraction, and ultrasound-assisted extraction etc., The type of extraction procedure as well as the type of solvent used determines the quality of metabolites earned [10]. The yield of extraction relies on several factors like the chemical constituents of the extract, the solvent used, the particle size of the sample, etc. [11].

The polarity of the solvent was regarded as an essential criterion for the extraction of plant extracts. Solvents such as acetone, water, ethyl acetate, alcohols (ethanol, methanol and propanol) and their combinations could impact phenolic groups [12]. Similarly, methanol was found to be competent in retrieving low molecular weight polyphenols [13]. An experiment by Cowan **[**14], stated that flavonoids are extracted to a remarkable extent by acetone. Also, reports suggest that acetone and dimethylformamide could cause substantial exclusion of antioxidants [15]. Based on this cited literature knowledge, the extraction procedure is executed with five solvents namely ethanol, distilled water, chloroform, acetone and methanol [16].

Solvent extraction

10 grams of leaf and stem sample were weighed and dissolved in 100 ml of different solvents (distilled water, ethanol, chloroform, acetone and methanol). The sample mixture was heated at a temperature below the boiling point of each solvent. The solution containing leaf and stem extract were allowed to saturate for 24 hours at room temperature. The extracts were filtered using Whatmann filter paper No.1 filter paper. The filtered extract was used fresh for phytochemical analysis [17].

Qualitative analysis of primary and secondary metabolites

Phytochemical analysis involves the identification and validation of primary and secondary metabolites. The major primary metabolites include carbohydrates, lipids, proteins (amino acids) and fats (fatty acids). The common and promising secondary metabolites include alkaloids, terpenoids, phenolic compounds, flavonoids, tannins etc, which contribute to the bioactive proportion of herbal composition. The determination of primary and secondary metabolites was followed by the standard procedure [18].

Test for carbohydrates (Molisch's test)

To the Molisch's reagent, add few drops of concentrated sulphuric acid. The formation of purple ring or purple reddish ring between the sample and H_2SO_4, proves the presence of carbohydrates in the test sample.

Test for amino acids

Few drops of ninhydrin reagent (10 mg of ninhydrin in 200 ml of acetone) were added to 1 ml of the extract. The appearance of purple indicates the presence of amino acids.

Test for fatty acids

1 ml of extract is mixed with 5 ml of ether. The extract is evaporated on the filter paper and the filter paper is dried. Clear appearance indicates the presence of fatty oils.

Test for anthraquinones

To 5 ml of extract, add a few ml of concentrated H_2SO_4 and add 1 ml of diluted ammonia. The appearance of pink rose colour confirms the presence of anthraquinone.

Test for quinones

Alcoholic KOH was added to 1 ml of extract; the presence of red to blue indicates the presence of quinone.

Test for alkaloids

A few drops of Wagner's reagent, was added to approximately one millilitre of extract. A reddish-brown precipitate indicates the presence of alkaloids.

Test for glycosides

Mix 2 milliliters of the extract with approximately 0.4 millilitres of glacial acetic acid containing a trace of ferric chloride and 0.5 millilitres of glacial acetic acid. Add H_2SO_4; The presence of glycosides indicates blue colour formation.

Test for cardiac glycosides (Keller-Killani test)

Mix 5 ml of solvent extract with 2 ml of glacial acetic acid, add a drop of ferric chloride solution and then add 1 ml of concentrated sulfuric acid. The brown ring at the interface indicates the presence of deoxy sugar cardenoloides. A violet ring may appear below the brown ring, and the acetate layer may gradually form a green ring.

Test for phenol (Lead acetate test)

Add 3 ml of 10% lead acetate solution to 5 ml of extract and mix gently. The production of a large amount of white precipitate is positive for phenol.

Test for polyphenol

Add a few drops of 5% lead acetate solution to the 1 ml extract. The appearance of a yellow precipitate confirms the presence of polyphenols.

Test for tannins

A few drops of neutral 5% ferric chloride solution was added to 5 ml extract, resulting in a dark green color indicating the presence of tannins.

Test for flavonoids

When 1ml extract was added with 10% lead acetate, the yellow precipitate is formed, which is a positive inference of flavonoids.

Test for phytosterols

To the extract, 2 ml of acetic anhydride, and then 1 or 2 drops of concentrated sulfuric acid is added along the side. An array of colour-change indicates the presence of phytosterols.

Test for phlobatannins

The aqueous extract was boiled with dilute hydrochloric acid leads to the deposition of a reddish precipitate, validates the presence of phlobatannins.

Test for saponins

0.5mg of extract was stirred vigorously with a few milliliters of distilled water. The formation of frothing is positive for saponin.

Test for steroids

2ml of extract was added with 2ml of chloroform and 2ml of concentrated H_2SO_4. The formation of red colour and yellowish-green fluorescence, recognizes the presence of steroids.

Test for xanthoproteins

1ml of the extract was added with a few drops of nitric acid and ammonia. A reddish-brown precipitate indicates the presence of xanthoproteins.

Test for chalcones

2 ml of ammonium hydroxide was added to 0.5 g of the extract. The appearance of red colour indicates the presence of chalcones.

Test for terpenoids (Salkowski test)

3 ml of the extract was taken and mixed with 1 ml of chloroform and 1.5 ml of concentrated H_2SO_4 was added along the wall of the tube. The reddish-brown colour in the interface is considered to be positive for the presence of terpenoids.

Test for triterpenoids

1ml of chloroform was added to 10mg of extract and dissolved. 2 ml of concentrated H_2SO_4, then 1ml of acetic anhydride was added. The formation of reddish purple is positive for the presence of triterpenoids.

Test for anthocyanins

With 2ml of water extract, 2N HCl was added and continued by the addition of ammonia. The conversion of reddish pink color into blue-purple, indicates the presence of anthocyanins.

Test for Leucoanthocyanin

The extract was dissolved in water, and 5ml of isoamyl alcohol was added. The red appearance of the upper layer indicates the presence of colourless anthocyanins.

Test for coumarins

3 ml of 10% NaOH aqueous solution was added to 2 ml of extract. The yellow colour indicates the presence of coumarin.

Test for emodins

To 5 ml of extract, 2 ml of NH_3OH and 3 ml of benzene were added. The production of red colour indicates the presence of emodin.

RESULTS AND DISCUSSION

Canavalia rosea plant extracts (leaf and stem) were used for the study. Phytochemical analysis is performed with the extracts, using five different solvents namely, ethanol, distilled water, chloroform, acetone, and methanol.

Metabolites in leaf extract

The phytochemical screening of leaf extract, involves 3 tests for major primary metabolites namely carbohydrates, proteins and fatty acids, and 21 tests for secondary metabolites. (Table 1) represents the primary and secondary metabolites derived from the leaf extract. Carbohydrates are observed in all solvents. Anthraquinone, anthocyanin and leucoanthocyanin, chalcones and emodins are found to be absent in leaf extract. Cardiac glycosides are observed in all solvent of leaf extracts. Tannins and saponins are present in all solvents of leaf extract except chloroform extract. Phenol, polyphenol and fatty acids are found in only ethanol and aqueous extracts. Alkaloids, steroids and triterpenoids are found only in water, chloroform and methanol extracts. Flavonoids are recognized in water and methanolic extracts. Phytosterols are present in ethanol and methanol extracts. Phlobatannins and terpenoids are present in ethanol, chloroform and methanol extracts. Coumarin is present in water, chloroform and methanol extracts.

Metabolites in stem extract

(Table 2) displays the primary and secondary metabolites derived from the leaf extract. Carbohydrates, phenol, polyphenol, flavonoids, are present in stem extract. Proteins, quinone, glycosides, chalcones, anthocyanin, leucoanthocyanin and emodin are not observed in all solvents of stem extract. Fatty acids are observed in ethanol, water and methanol extracts. Anthraquinone is present only in methanol extract. Alkaloids are noted in all solvents except acetone. Cardiac glycosides and saponins are documented in all extracts other than chloroform. Tannins are absent in chloroform and acetone extracts, and noted in all other extracts. Phytosterols, terpenoids and triterpenoids are displayed in all extracts except distilled water. Phlobatannins are present in ethanol and methanol extracts only. Steroids are exposed in chloroform, acetone and methanol extracts. Xanthoproteins exists only in methanolic extract. All solvents exhibit coumarin presence except ethanol.

Table 1 Phytochemical analysis of leaf extract of *Canavalia rosea*

Name of the test	Ethanol	Water	Chloroform	Acetone	Methanol
Molisch's Test	+	-	+	-	+
Ninhydrin test	-	-	+	-	-
Fatty acid test	+	+	-	-	-
Anthraquinone	-	-	-	-	-
Quinone	-	-	-	-	-
Alkaloid	-	+	+	-	+
Glycosides	-	-	+	+	-
Cardiac glycosides	+	+	+	+	+
Phenol	+	+	-	-	-
Polyphenol	+	+	-	-	-
Tannins	+	+	-	+	+
Flavonoids	-	+	-	-	+
Phytosterols	+	-	-	-	+
Phlobatannins	+	-	+	-	+
Saponins	+	+	-	+	+
Steroids	-	+	-	+	+
Xanthoprotein	-	+	+	-	+
Chalcones	-	-	-	-	-
Terpenoids	+	-	+	-	+
Triterpenoids	+	-	+	+	+
Anthocyanins	-	-	-	-	-
Leucoanthocyanin	-	-	-	-	-
Coumarin	-	+	+	-	+
Emodin	-	-	-	-	-

(+) indicates present; (-) indicates absent

Table 2 Phytochemical analysis of stem extract of *Canavalia rosea*

Name of the test	Ethanol	Water	Chloroform	Acetone	Methanol
Molisch's Test	+	+	+	+	+
Ninhydrin test	_	-	-	-	-
Fatty acid test	+	+	-	-	+
Anthraquinone	-	-	-	-	+
Quinone	-	-	-	-	-
Alkaloid	+	+	+	-	+
Glycosides	-	-	-	-	-
Cardiac glycosides	+	+	-	+	+
Phenol	+	+	+	+	+
Polyphenol	+	+	+	+	+
Tannins	+	+	-	-	+
Flavonoids	+	+	+	+	+
Phytosterols	+	-	+	+	+
Phlobatannins	+	-	-	-	+
Saponins	+	+	-	+	+
Steroids	-	-	+	+	+
Xanthoprotein	-	-	-	-	+
Chalcones	-	-	-	-	-
Terpenoids	+	-	+	+	+
Triterpenoids	+	-	+	+	+
Anthocyanins	-	-	-	-	-
Leucoanthocyanin	-	-	-	-	-
Coumarin	-	+	+	+	+
Emodin	-	-	-	-	-

(+) indicates present; (-) indicates absent

The results of phytochemical screening affirm the presence of 7 major secondary metabolites namely tannins, phlobatannins, saponins, flavonoids, alkaloids, cardiac glycosides, and phenolics in crude leaf extract of *Canavalia rosea*, which correlates with earlier investigation done by researchers. Petroleum ether extract of the seeds was tested qualitatively for preliminary phytochemical screening confirms the presence of eight compounds such as, tannins, saponins, flavonoids, cardiac glycosides, terpenoids, phenols, coumarins and phlobatannins [19].

Also, results of phytochemical analysis for methanol extract of *Canavalia cathartica* showed the presence of phenols, terpenoids, tannins, steroids, flavonoids and glycosides [20]. Another report displays the presence of tannins, phlobatannins, saponins, flavonoids, alkaloids, cardiac glycosides, and phenolics in crude leaf extract of *Canavalia*

rosea correlate with the earlier studies performed by [21]. A study of GC-MS analysis reported, 15 compounds in leaf extract and 16 compounds in bark in ethanolic extracts of *Moringa concanensis* [22].

Alkaloids are applied in biomedical field as anaesthetics agent, and CNS inducer [23]. Glycosides, identified in plants, generally retain strong bitter taste. However, some plant extracts containing cyanogenic glycoside components, are used as flavouring material in pharmaceutical products [24]. Flavonoids, are the prominent group of polyphenols, contribute to the free radical scavenging property of the plants as per several research reports [25].

Phenolic groups play vital role in combating cancer, as they possess antioxidant property. The presence of phenolic groups in green tea, red-wine contribute to the anticancer activity. Saponins were found to possess therapeutic potential against hypolipidemic and anticancer activity. The bioactive metabolites like alkaloids, cardiac glycosides, flavonoids, phenolic groups, saponins and tannins, were recognized in the leaf and stem extracts of *Canavalia rosea.*

The plant has been validated with medicinal properties in earlier reports [26-28], this phytochemical screening procedure might facilitate the application of these extracts in pharmacological field with appropriate in-vivo experiments.

CONCLUSION

This research investigation is focused on performing phytochemical analysis in leaf and stem extract of *Canavalia rosea.* The experiment involves assessment of 3 major primary metabolites and 21 secondary metabolites. Quinones, chalcones and anthocyanins, leucoanthocyanin, emodin are not observed in both the extracts. Amongst the leaf and stem extracts, most of the active molecules like alkaloids, cardiac glycosides, tannins, flavonoids, phytosterols, phlobatannins, saponins, steroids, xanthoprotein, terpenoids, triterpenoids, are recognized in methanol extract. The presence of these versatile components could contribute appreciably for the medicinal value of this plant. The ethanolic extract comprises of 11 and 13 metabolites in leaf and stem extract respectively. The water extract showed a total of 11 and 10 metabolites in leaf and stem extract respectively. In both the leaf and stem extracts of chloroform, 10 compounds were found. The acetone extraction with leaf and stem includes 6 and 11 compounds respectively. The leaf methanolic extract comprises of 13 metabolites, whereas the stem methanolic extracts confirm the presence of 17 metabolites. Since the active metabolites are highly recognized, the methanolic extract could be explored further for research, by acquiring validated reports with quantitative phytochemical procedure and characterization studies.

Author contributions

Ramakrishnan Vasanthi: Conceptualization, Methodology, Literature search, Investigation, Data curation, Writing – original draft, Writing-review and editing.

Vadivel Balamurugan: Supervision, Investigation, Writing - review and editing.

Declaration of competing interest: The authors declare that there is no conflict of interest.

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