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Study Phytochemical Evaluation of *Cryptostegia grandiflora Linn Roxb.* Extract

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ABSTRACT

Now-a-days natural products are an integral part of human health care system, because there is popular concern over toxicity and resistance of modern drugs. India is one of the 12 leading biodiversity centers with presence of over 45,000 different plant species. As a result of rapid development of phytochemistry and pharmacological testing methods in recent years, new plant drugs are finding their way into medicine as purified phytochemical. There are various simple and cheap methods available for finding out about the various medicinal uses medicinal plants contain many bioactive compounds that can be used in the process of curing various human diseases to identify these compounds one can perform the phytochemical screening of various parts of any specific plant. Phytochemicals is generally used to describe plant compounds that are under research with unestablished effects on health. Cryptostegia grandiflora Linn Roxb. (Apocynaceae), commonly known as "Vilayti vakundi" is found all in dry area in India. The study includes preparation of different extracts by successive solvent extraction for detail analysis. Fluorescence analysis of different successive extract and powder were noted under UV light and normal ordinary light, which signifies there characteristics. Preliminary qualitative chemical test for different extracts showed presence of glycosides, flavonoids, fixed oil and fats, phenolic compounds, protein and amino acids, tannins, gum and mucilage and carbohydrates. Qualitative phytochemical analysis of methanol extracts of leaves of Cryptostegia grandiflora Linn Roxb showed the presence of cardiac and saponins glycosides, tannins, flavonoids, proteins.

Keywords: Cryptostegia grandiflora Linn Roxb, qualitative analysis, methanolic extract, flavonoids.

INTRODUCTION

Nature always stands as golden mark to amplify the outstanding phenomenon of symbiosis [1]. The Lord created medicines out of the earth and a wise man will not abhor them. Medicinal plants existing even before human being made their appearance on the earth. Men's existence on the earth has been made possible only because of the vital role played by the plant kingdom in sustaining life. It is therefore often said that wherever we are born we have around us useful herbs, shrubs and plants [2]. Practically every country develops its own medical system, which includes the ancient civilization of China, Egypt and India. Thus, the Indian Medical System-Ayurveda came into existence. The raw materials for Ayurvedic medicines were mostly obtained from plant sources in the form of crude drugs such as dried herbal powders or their extracts or mixture of products [3]. Also, Siddha, Unani and Tibetan are traditional health care systems have been flourishing for many centuries. Apart from these systems there is a rich heritage of ethno botanical usage of herbs by various colorful tribal communities in the country [4]. Now-a-days natural products are an integral part of human health care system, because there is popular concern over toxicity and resistance of modern drugs. India is one of the 12 leading biodiversity centers with presence of over 45,000 different plant species. As a result of rapid development of Phytochemistry and Pharmacological testing methods in recent years, new plant drugs are finding their way into medicine as purified phytochemical. WHO has emphasized the need to ensure the quality control of herbs and herbal formulations by using modern techniques? Several countries have herbal pharmacopoeias and lay down monographs to maintain their quality. Ayurvedic Pharmacopoeia of India recommends basic quality parameters for 80 common herbal drugs [5].

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. The phytochemical research based on ethno-pharmacological information is commonly measured an effective approach in the discovery of new anti-infective agents from higher plants. *Cryprtostegia grandiflora* Linn Roxb. is a stout, woody vine. Leaves are oblong-ovate, 6 to 10 centimiters, long, pointed at

the tip, rounded at base. Cymes are short. Sepales are, green, about 8 millimeters long. Corolla is pale purple, about 4 centemeters long, and often wider than it is long. Woody follicles are 10 to 12 centimeters long [6].

This plant species is also reported to possess various biological activities like antioxidant, anticancer, antianalgesic activity [16] which makes the plant a natural source of chemical compounds with medicinal value that can be used as a drug to cure diseases and making it a plant with greater commercial value [7]. The main objective of our research work was to analyze the presence or absence of different types of phytochemical constituents in two different parts i.e. leaves of medicinal plant. We had performed the comparative study of

Sample Collection

Fresh & healthy plant parts of *Cryptostegia grandiflora* Linn Roxb leaves (Fig. 1) were collected from Atpadi tal-Atpadi Dist-Sangali. Collected plant parts were examined and identified with the help of regional floras. Specimens were further confirmed with reference to Herbarium sheets available in the department of Botany. Krushna colleges of Science Karad.

Extraction:

The air-dried leaves of *Cryptostegia grandiflora* Linn.Roxb were reduced to coarse powder and around 300 gm of powder was subjected to successive solvent extraction using soxhlet apparatus with different solvents viz. petroleum ether (40-600C), chloroform, methanol.

Maceration was also carried out using chloroform water separately with 150 gm seed powder. After the effective extraction, the solvents were distilled off. The extract was then concentrated on water bath, percentage yields were recorded [11].

Chemical constituents of plant: [8]

Phytochemical studies of flowers yielded two cardenolides, oleandrigenin, and gitoxine, as well as two flavonoides, hypeersode and astrangalin ,and their aglycons and quercetin and aempferol. Latex of fresh unripe fruits yielded B- amyrin, leupol, a- amyrin,b-sitisterol. Hexane and ethyl acetate extracts yielded a mixture of phytosterols and triterpenoids, lanosterol, B-sitosterol, stigmasterol, campestrol, friedlin, lupeol, ursolic acid and B-amyrin.

Table No. 1: Classification of plant [9]

Kingdom	***Plantae***
Subkingdom	*Tracheobionta*
Superdivision	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Asteridae*
Order	*Gentianales*
Family	*Asclepiadaceae*
Genus	*Cryptostegia* R. Br.
Species	*Cryptostegia grandiflora* (Roxb. ex R. Br.) R. Br.

Physical Evaluation of Leaves of *Cryptostegia Grandiflora Linn*

Roxb*:*

The shade-dried leaves were subjected to size reduction to get coarse powder. Then subjected to standardization with different parameters which is prescribed in literature/ Pharmacopoeia.

1) Extractive Values:

The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents were used for determination of extractives. The solvent used for extraction is in a position to dissolve appreciable quantities of substances desired.

Extraction of Leaves:

Extraction for the phytochemical screening was performed by Soxhlet extraction.

Method of extraction of leaves *Cryptostegia grandiflora Linn* Roxb: Soxhlet extraction: [10]

Soxhlet apparatus was used for continuous extraction of the powdered crude drug. The material was packed in the apparatus and allowed to get extracted with hot solvent that continuously percolates from top to bottom. Condensed fresh solvent percolates every time through the powder and is the major advantage with this technique. Successive extraction was carried out depending on polarity of the solvent system. The plant material ideally, fresh plant tissues should be used for phytochemical analysis. Ethanol was all purpose solvent for preliminary extraction. This method was useful when working on gram scale. 50gm Powder was continuously extracted in soxhlet apparatus with range of solvents, starting in turn with petroleum ether, chloroform and then alcohol and ethyl acetate. The extract obtained was clarified by filtration through whatman filter paper 44 and then concentrated with vacuum or on rotary evaporator. Extract obtained was stored in air tight container in desiccators. Soxhlet extractor with one litter capacity was used for extraction. Powdered material was treated with pet ether for defatting. 300ml of Petroleum ether was then passed on the bed of powder for siphoning it once.

Mouth of the extractor was fitted to bulb type condenser and neck was packed with sealing wax. Heating was continued with continuous flow of water through the condenser. For all the extractions temperature was kept nearer to the boiling range of the respective solvent. Extraction cycle was observed continuously till completion of extraction. First petroleum ether was used for extraction of plant material for defatting of purpose. Then chloroform and finally methanol was used. Solvent was recovered by distillation and extract obtained was filtered through Whatmann filter paper 44. Extracts were concentrated on heating mental. Finally dried extracts were weighted and preserved in the air tight containers. Percent extracts were calculated.

Phytochemical Screening: [15]

Qualitative Phytochemical Analysis:

Preliminary phytochemical analysis was carried out for the extract as per standard methods described by Brain and Turner (1975) [12] and Evans (1996) [13].

Test for Carbohydrates:

Preparation of test solution:

The test solution was prepared by dissolving the test extract with water. Then it was hydrolyzed with 1 volume of 2N HCl and subjected to following chemical tests.

Chemical tests:

Molish test: To 2-3 ml aqueous extract, added few drops of α-naphthol solution in alcohol, shaken and added concentrated H_2SO_4 from sides of the test tube,then observed for violet ring at the junction of two liquids.

For Reducing Sugars:

a) Fehling's test: 1 ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Added equal volume of test solution. Heated in boiling water bath for 5-10 min. observed for a yellow, then brick red precipitate.

b) Benedict's test: Equal volume of Benedict's reagent and test solution in test tube were mixed. Heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.

Tests for Monosaccharide:

Barfoed's test: Equal volume of Barfoed's reagent and test solution were added. Heated for 1-2 min, in boiling water bath and cooled. Observed for red precipitate.

Tests for Hexose Sugars:

1) Cobalt-chloride test:

3 ml of test solution was mixed with 2ml cobalt chloride, boiled and cooled. Added FeCl3 drops on NaOH solution. Solution observed for greenish blue (glucose), purplish (Fructose) or upper layer greenish blue and lower layer purplish (Mixture of glucose and fructose).Test for Non-Reducing Polysaccharides (Starch):

a) Iodine test: Mix 3 ml. test solution and few drops of dilute Iodine solution. Blue colour appears; it disappears on boiling and reappears on cooling.

b) Tannic acid test for starch: With 20% tannic acid, test solution was observed for precipitate.

Tests for Proteins:

a) Biuret test (General test): To 3 ml test solution (T.S.), add 4% NaOH and few drops of 1% CuSO4 solution observed for violet or pink colour.

b) Millon's test (for proteins): Mixed 3 ml T.S. with 5 ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red colour.

c) Xanthoprotein test (For protein containing tyrosine or tryptophan): Mixed 3ml T.S. with 1 ml concentrated H2SO4 observed for white precipitate.

d) Test for protein containing sulphur: Mixed 5 ml T.S. with 2 ml 40% NaOH and 2 drops 10% lead acetate solution. Solution was boiled it turned black or brownish colour.

Tests for Amino Acids:

a) Ninhydrin test (General test): 3 ml T.S. and 3 drops 5% Ninhydrin solution were heated in boiling water bath for 10 min. observed for purple or bluish colour.

b) Test for Tyrosine: Heated 3 ml T.S. and 3 drops Million's reagent. Solution observed for dark red colour.

c) Test for Tryptophan: To 3 ml T.S. added few drops glycoxalic acid and concentrated H2SO4 observed for reddish violet ring at junction of the two layers.

d) Test for Cysteine: To 5 ml. T.S. add few drops of 40% sodium hydroxide and 10% lead acetate solution. Boil. Black ppt. of lead sulphate is formed.

Tests for Steroid:

Preparation of test extracts solution:

The extracts were refluxed separately with alcoholic solution of potassium hydroxide till complete saponification. The saponified extract was diluted with water and unsaponifiable matter was extracted with diethyl ether. The ether extract was evaporated and the residue (unsaponifiable matter) was subjected to the following test by dissolving the residue in chloroform.

a) Salkowski Reaction: Mixed 2 ml of extract, 2 ml chloroform and 2 ml concentrated H2SO4, Shake well, whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.

b) Liebermann-Burchard Reaction: Mixed 2ml extract with chloroform add 1-2 ml acetic anhydride and 2 drops concentration H2SO4 from the side of test tube observed for first red,then blue and finally green colour was observed.

c) Liebermann's reaction: Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H2SO4 observed for blue colour.

Tests for Flavonoids:

The flavonoids are all structurally derived from the parent substance called flavone. The flavonoids occur in the free form as well as bound to sugars as glycosides. For this reason, when analyzing flavonoids it is usually better to examine the flavonoids in hydrolyzed plant extracts.

Preparation of test solution:

i. To a small amount of extract added equal volume of 2M HCl and heated in a test tube for 30 to 40 min. at 100°C.

ii. The cooled extract was filtered, and extracted with ethyl acetate.

iii. Extract was concentrated to dryness, and used to test for flavonoids.

a) Shinoda test: To extract, add 5 ml 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink colour was observed. To small quantity of residue, acetate solution was added, observed for yellow coloured precipitate.

b) Mayers test: 2-3 ml filtrate with few drops Mayer's reagent observed for precipitate

3. Hager's test:

2-3 ml filtrate with few drops Hager's reagent observed Yellow precipitate

4. Wagner's test:

2-3 ml filtrate with few drops of Wagner's reagent observed reddish brown precipitate

Tests for Tannins and Phenolic Compounds:

To 2-3 ml of extract, add few drops of following reagents:

a) 5%FeCl3 solution: Deep blue-black color.

b) Lead acetate solution: White precipitate.

c) Gelatin solution:White precipitate.

d) Bromine water: Discoloration of bromine water.

e) Acetic acid solution: Red color solution

f) Dilute iodine solution: Transient red color.

g) Dilute HNO3: Reddish to yellow colour.

Chromatographic Studies:

Based on the results of preliminary phytochemical investigations the chromatographic studies were carried out. The methanolic and chloroform extracts were subjected to thin layer chromatography for the presence of phytoconstituents. In this technique, the Silica gel-GF254 (for TLC) was used as an adsorbent and plates were prepared by spreading technique [14].

TLC of Methanolic Extracts:

Stationary phase: Silica gel

Mobile Phase: Dichloromethane: methanol (9:1)

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RESULTS AND DISCUSSION

The present study was carried out on the *Cryptostegia grandiflora* Linn Roxb revealed that the presence of active phytochemical constituents. The macroscopic characteristics and standardization of leaves are mentioned in Table 2 and Table 3 respectively. The observation of nature, color, yield of extract process is mentioned in Table 4. The phytochemical active compounds of *Cryptostegia grandiflora* Linn Roxb *were* qualitatively analyzed from leaves and the results are mentioned in Table 5 and Table 6 respectively.

The present investigation shows that presence of contained alkaloids, flavonoids, steroids, saponins, tannins have various medicinal values such as anti-inflammatory, anti-diabetic and analgesic activities and for central nervous system activity.

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties.

Table No. 2: Macroscopic Characteristics of leaves of *Cryptostegua grandiflora* Linn Roxb

Sr. No.	Parameters	Observation of leaves
1	Colour	Greyish green
2	Odour	Odourless
3	Taste	Bitter
4	Size	1.2- 2.0 cm in diameter
5	Shape	Globular or oval
6	Surface	Smooth & shiny

Table No. 3: Standardization of leaves *Cryptostegia grandiflora* Linn Roxb

S. No.	Physical Contents	Result
1	**Extractive Values (% w/w)**	
	Petroleum ether soluble extractive	**6.40**
	Chloroform soluble extractive	**7.50**
	Methanol soluble extractive	**12.56**
2	**Loss on Drying (% w/w)**	**8.20**
3	**Ash Value (% w/w)**	
	Total Ash	**4.75**
	Acid Insoluble Ash	**0.95**
	Water Soluble Ash	**1.90**

Table No. 4: Observation of Nature, Colour and Yield of Extract

Sr. No.	Extracts	Nature of Extract	Colour	Weight (g) %	Yield (w/w)
1	Petroleum ether	Oily and Pungent	Greyish Green	15.80	6.40
2	Chloroform	Solid	Greyish Green	17.50	7.50
3	Methanol	Semi-solid	Greyish Green	26.50	12.56

Table No. 5: Chemical tests of *Cryptostegia grandiflora* Linn Roxb leaves extract

Sr. No.	Chemical tests	Observation	Inference		
			Pet ether extract	**Chloroform extract**	**Methanol**
1	**Test for steroids:** ***1)Salkowaski test:*** To 2ml extract add 2ml. of chloroform and 2ml. conc.H2SO4 shake well.	Chloroform layer appears red and acid layer shows Greenish yellow fluorescence.	Steroid present	Steroid present	Steroid present

2	***libermann burchard test:*** Mix 2ml.extract with chloroform .add 1- 2ml.acetic anhydride and 2 drops conc. H2SO4 from the side of test tube.	First red, then blue and finally green colour appears	Steroid present	Steroid present	Steroid present
3	**Test for cardiac glycoside** ***a)Baljet test:*** Test solution treated with sodium picrate.	It gives yellow to orange colour.	Cardiac glycosides absent.	Cardiac glycosides present.	Cardiac glycosides present.
	b)Killer-killani test: The test solution with few drops of glacial acetic acid in 2ml.of ferric chloride solution and conc.H2SO4 is added from side of test tube.	It shows separation between two layers, lower layer shows reddish brown and upper layer turns bluish green.	Deoxysugar present	Deoxysugar Present	Deoxysugar Present
3	**Test for saponin:** ***a)Foam test:*** Shake the drug extract or dry powder vigorously with water.	Persistent foam observed.	Saponin glycosides are Present	Saponin glycosides are Present	Saponin Glycosides are Present
4	**Test for carbohydrate:** ***a) Molisch test:*** To 2-3ml.extract add few drops of alpha Naphthol solution in alcohol. Shake and add conc.H2SO4 from side of test tube.	No Violet coloured ring formed at junction of two liquid.	Carbohydrates are absent.	Carbohydrates are absent.	Carbohydrates are absent.
	b) Benedict test: Test solution treated with Benedict reagent in boiling waterbath.	Test doesn't show reddish brown precipitate.	Reducing Sugar absent	Reducing sugar Absent	Reducing sugar Absent
5	**Test for alkaloids:** ***a) Mayer's test:*** Test solution treated with Mayer's reagent (potassiummercuric iodide)	Test doesn't gives cream coloured precipitate	Alkaloids are absent.	Alkaloids are absent.	Alkaloids are absent.
	b) Wagner's test: The solution is treated with Wagner's reagent. (iodine in potassium iodide)	No Brown precipitate	Alkaloids are absent.	Alkaloids are absent.	Alkaloids are absent.
	c) Hager's test: Test solution is treated with Hager's reagent.	No Yellow precipitate.	Alkaloids areabsent.	Alkaloids areabsent.	Alkaloids areabsent.
	d) Dragendorff's test: The test solution treated with dragendorff's reagent.(potassium bismuth iodide)	No reddish precipitate.	Alkaloids are absent.	Alkaloids are absent.	Alkaloids are absent.
6	**Tests for flavonoids:** ***a) Shinoda test:*** Test solution treated with 5ml 95% ethanol, few drops of conc.HCL and magnesium turnings.	Pink to magneta colour.	Flavonoids absent.	Flavonoids present.	Flavonoids present.
	b) To small quantity of residue add lead acetate solution.	Yellow coloured ppt formed.	Flavonoids absent.	Flavonoids present.	Flavonoids present.
7	**Test for tannins:** ***a) 5% ferric chloride solution:*** Test solution treated with few drops of ferricchloride solution.	White precipitate.	Tannins present.	Tannins present.	Tannins present.

Table No. 6: Results of Present Chemical Constituents of leaves of *Cryptostegia grandiflora* Linn Roxb leaves extract

Pet. ether extract	**Fats & Oil**
Chloroform extract	Protein, Cardiac Glycoside, Saponin Glycosides, and Flavonoids Tannins and phenolic compounds.
Methanol extract	Protein, Cardiac Glycoside, Saponin Glycosides, and Flavonoids Tannins and phenolic compounds.

Fig. 1: *Cryptostegia Grandiflora* Linn Roxb Plant

CONCLUSION

The present study suggests that qualitative phytochemical screening of crude extracts of *Cryptostegia grandiflora* linn Roxb supports the presence of bioactive compounds such as flavonoids, glycosides, phenolic compounds, fixed oil & fats, tannins, gum, mucilage in the medicinal plant and thus responsible for the antioxidant activities. The plant extract could be potential source of natural antioxidant that could have great importance as a therapeutic agent in degenerative disease. Further investigation of chemical constituents of *Cryptostegia grandiflora* linn Roxb and other poorly studied plants can be revealed. Therefore more of the researches and studies are required as the large untapped reservoir waiting to be investigated.

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