IJBPAS, September, 2026, 15(9): 4181-4190

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

CALOTROPIS GIGANTEA AND HIBISCUS ROSA SINENSIS: A BRIEF STUDY ON PHYTOCHEMICAL ANALYSIS

GOUD SK*[1], KANNOJIA P[2], MISHRA P[3] AND PANDEY JC[4]

1: Bareilly International University, Bareilly-243006, Uttar Pradesh, India

2: BIU College of Pharmacy, Bareilly International University, Bareilly-243006, Uttar Pradesh, India

3: Rohilkhand College of Pharmacy, Bareilly International University, Bareilly-243006, Uttar Pradesh, India

4: Keshlata College of Pharmacy, Bareilly International University, Bareilly-243006, Uttar Pradesh, India

***Corresponding Author: Mr. Santosh Kumar Goud: E Mail: santosh.goud81@gmail.com**

Received 30th Sept. 2025; Revised 20th Oct. 2025; Accepted 9th March 2026; Available online 1st Sept. 2026

https://doi.org/10.31032/IJBPAS/2026/15.9.10441

ABSTRACT

The extracts were thought to be insoluble in water but soluble in an organic solvent. After measurement, the yield percentage was determined to be 5.56% w/w. The resulting *Hibiscus rosa sinensis* petroleum ether extract had an astringent texture, was light to dark green in color, and was oily with a vague smell. The extracts were thought to be insoluble in water but soluble in an organic solvent. The percentage yield was measured and set at 6.23% w/w. A straightforward chemical test was used to screen the crude petroleum ether extract of *Calotropis gigantea* and *Hibiscus rosa sinensis* for the presence of alkaloids, flavonoids, glycosides, carbohydrates, proteins, starches, amino acids, steroids, tannins, and saponins.

Keywords: Alopecia, Methodology, Phytochemical screening

1. INTRODUCTION

Calotropis gigantea belonging to the family Apocynaceae is a well-known medicinal herb commonly known as milkweed or crown flower weed has been used in Ayurveda, Unani, Siddha system of medicine for long year **[1]**. This plant is latex bearing plant and when the tissue is injured then latex is released. Plant latex is the combination of tannins, alkaloids, sugar, starch, resins, protein and gum **[2]**. *Hibiscus rosa-sinensis*, commonly known as the shoe flower, thrives in tropical and subtropical regions across South China, Asia, Africa, and America, adapting well to diverse environments. Its therapeutic potential is attributed to a range of bioactive compounds Anthocyanins, saponins, quercetin, kaempferol, and anthraquinones are found in various plant parts.

2. METHODOLOGY

Fresh leaves were collected from the Calotropis procera and *Hibiscus rosa sinensis* plant growing in the medicinal garden of K. S. Saket P.G. College, Ayodhya-224123, Uttar Pradesh, India. The fresh leaf was then studied for pharmacognostic evaluation, including examination of morphological and some preliminary phytochemical evaluation. Fresh leaves of Calotropis spp. Linn. and Hibiscus rosa-sinensis Linn. were subjected to color, odor and taste, determination of shape, size, surface characteristics and appearance etc. Descriptive features were matched with those included in standard anatomical books **[3-14]**.

2.1 EXTRACTION

The selection and the collection of plant material are important in making efficient phyto constituent isolation. The disease free and healthy plants only selected for the plant extraction which is protected from weeds and insect. The numerous factors involved in the collection of the plant materials. NRCS (Natural resources conservation service) Plant Materials Program created the guidelines for the collection of plant material, which includes the collection of seed and vegetative collections. This guideline explains the relevant collection time, collection techniques, processing and storage of plant materials **[15]**.

i) Drying of Plant Materials: The drying process is important for the extraction of plant materials, the fresh plant materials are having the active enzymes which is produces the active constituents intermediates and metabolic reactions in the plant materials, so that the drying is important for the pre-extraction preparation of plant materials. Drying the plant in air dry process under the shade in the dark room because the overheat can losses the volatile substances from plant

materials and some of the light sensitive constituents may losses in light condition.

ii) Grinding and Size Reduction: Grinding and size reduction is the essential for the Soxhlet extraction process because smaller the particle size greater the surface area of the powdered particles. Large surface area improves the contact of the powdered particles with the solvent used for extraction, and hence efficient extraction takes place. The drug material grinding by the electric mixer grinder **[16, 17]**.

iii) Size Separation/Sieving: The size separation is important for efficient Soxhlet extraction. Uniform powdered particle size provides maximum extraction as the solvent can pass uniformly through the powdered particles packed in the thimble. The very fine powder and very coarse powders are not suitable for effective extraction. The very fine powder may produce the beds during extraction and very coarse powders delay the extraction process. The size separation is done by the sieving method and the determination of particle size by the sieving method, microscopic method etc. Sieve no. 44 was used for size separation of coarse powder of *Calotropis gigantea* and *Hibiscus rosa sinensis*.

iv) Selection of Solvent for Soxhlet Extraction: The selection of the solvent for Soxhlet extraction is based on the phyto constituent isolation process. The solvent should be easy to remove and inert. Normally the solvent selection is based on the increasing polarity order like the order of acetone, petroleum ether, ethyl acetate, chloroform, methanol, ethanol and water. The petroleum ether is commonly used for the extraction of the steroids and fixed oils, and also used for the removal of the chlorophyll from the leaf powder; some of the researchers uses petroleum ether for defatting of the plant material. After defatting, main solvent like alcohol or aqueous extraction was performed. Some of the plant materials the defatting is essential because the waxy substances produce the emulsification process with the solvent and interferes the extraction process. Ethanol is the semi polar solvent which can extract many of the phytoconstituents and water is the polar solvent which is cheap solvent and nontoxic. Numbers of polar constituents are isolated by water and which is also suitable for the animal studies and human studies. Petroleum ether was using for extraction of *Calotropis gigantea* and *Hibiscus rosa sinensis* leaves **[18, 19]**.

v) Preparation of Extracts: The leaves section of the selected plants was chosen to evaluate their activity, and this piece was subjected to the extraction procedure. Raw

leaves were collected from the surrounding areas. Fresh leaves were washed in tup water, rinsed in distilled water, and air dried for an hour. They were pounded into a powder with a mortar and pestle and kept at room temperature until used. The dried, powdered of leaves were Soxhlet extracted with petroleum ether (60-80 °C), and the extract was weighed under decreased pressure following solvent removal. For both plants, the percentage yield of the extract was recorded **[20, 21]**.

2.2 PHYTOCHEMICAL SCREENING

The extracts obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, proteins and amino acids, saponins and phytosterols using reported methods **[22-26]**.

1. Alkaloids:

a. Dragondorff's test: To the extract add Dragondorff's reagent, reddish brown precipitate indicates the presence of alkaloids.

b. Mayer's test: To the extract add Mayer's reagent, cream colored precipitate indicates the presence of alkaloids.

c. Wagner's test: To the extract add Wagner's reagent, reddish brown precipitate indicates the presence of alkaloids.

d. Hager's test: To the extract add Hager's reagent, yellow precipitate indicates presence of alkaloids

e. Tannic acid test: To the extract add tannic acid solution, buff colored precipitate indicates presence of alkaloids.

2. Amino acids:

a. Millon's test: To the extract add about 2 ml of Millon's reagent, white precipitate indicates the presence of amino acids.

b. Ninhydrin test: To the extract add Ninhydrin solution, boil, violet color indicates the presence of amino acids.

3. Carbohydrates:

a. Molisch's test: To the extract add few drops of alcoholic a-napthol, then add few drops of concentrated sulphuric acid through sides of test tube; purple to violet ring appears at the junction.

b. Barfoed's test: 1 ml extract is heated with 1 ml of Barfoed's reagent, if red cupric oxide is formed, monosaccharide is present. Disaccharides on prolong heating (about 10 min) may cause reduction, owing to partial hydrolysis to monosaccharides.

c. Selwinoff's test (Test for ketones): To the extract add crystals of resorcinol and equal volume of concentrated hydrochloric acid and heat on water bath, rose color is produced.

d. Test for Pentoses: To the extract add equal volume of hydrochloric acid containing a

small amount of phloroglucinol and heat, red color is produced.

4. Flavonoids:

a. Shinoda test: To the extract add few magnesium turnings and concentrated hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

b. Alkaline reagent test: To the extract add few drops of sodium hydroxide solution, intense yellow color is formed which turns to colorless on addition of few drops of dilute acid indicates presence of flavonoids.

c. Zinc hydrochloride test: To the extract add a mixture of zinc dust and concentrated hydrochloric acid. It gives red color after few minutes.

5. Glycosides

Test A: Extract 200 mg of drug with 5 ml of dilute sulphuric acid by warming on water bath. Filter it. Then neutralize the acid extract with 5% solution of sodium hydroxide. Add 0.1 ml of Fehling's solution A and B until it becomes alkaline (test with pH paper) and heat on water bath for 2 minutes. Note the quantity of red precipitate formed and compare with that of formed in Test B.

Test B: Extract 200 mg of drug using 5 ml water instead of sulphuric acid. After boiling add equal amount of water as used for sodium hydroxide in the above test. Add 0.1 ml Fehling's A and B until alkaline (test with pH paper) and heat on water bath for 2 minutes. Note the quantity of red precipitate formed. Compare the quantity of precipitate formed in Test B with that of formed in Test A. If the precipitate in Test A is greater than in Test B then glycoside may be present. Since Test B represents the amount of free reducing sugar already present in the crude drug, whereas Test A represents free reducing sugar plus those related-on acid hydrolysis of any glycoside in the crude drug.

i. Anthraquinone glycosides

a) Borntrager's test: Boil the test material with 1 ml of sulphuric acid in a test tube for 5 minutes. Filter while hot. Cool the filtrate and shake with equal volume of dichloromethane or chloroform. Separate the lower layer of dichloromethane or chloroform and shake it with half of its volume of ammonia. A rose pink to red color is produced in the ammonical layer.

b) Modified Borntrager's test: Boil the test material with 2 ml of sulphuric acid. Treat it with 2 ml of 5% aqueous ferric chloride solution (freshly prepared) for 5 minutes, shake it with equal volume of chloroform and continue the test as above. As some plants contain anthraceneaglyconein reduced form, if ferric chloride is used during extraction;

oxidation to anthraquinones takes place, which shows response to Borntrager's test.

ii. Cardiac glycosides:

a) Kedde's test: Extract the drug with chloroform, evaporate to dryness. Add one drop of 90% alcohol and 2 drops of 2 % 3, 5-dinitro benzoic acid in 90% alcohol. Make alkaline with 20% sodium hydroxide solution, purple color is produced. The color reaction with 3, 5-dinito benzoic acid depends on the presence of a, ß- unsaturated lactones in the aglycone.

b) Killer- Killiani test: Extract the drug with chloroform and evaporate it to dryness. Add 0.4 ml of glacial acetic acid containing trace amount of ferric chloride. Transfer to a small test tube; add carefully 0.5 ml of concentrated sulphuric acid by the side of test tube. Acetic acid layer shows
blue color.

c) Raymond's test: Treat the extract with hot methanolic alkali, violet color is produced.

d) Legal's test: Treat the extract with pyridine and alkaline sodium nitroprusside solution, blood red color appears.

e) Baljet's test: Treat the extract with picric acid or sodium picrate, orange color is produced.

iii. Coumarin glycosides:

Place a small amount of sample in test tube and cover the test tube with a filter paper moistened with dilute sodium hydroxide solution. Place the covered test tube on water bath for several minutes. Remove the paper and expose it with ultraviolet (UV) light, the paper shows green fluorescence.

iv. Saponin glycosides:

a. Froth formation test: Place 2 ml solution of drug in water in a test tube, shake well, stable froth (foam) is formed.

b. Haemolysis test: Add 0.2 ml of extract to 0.2 ml of blood in normal saline and mix well. Centrifuge and note the red supernatant compare with control tube containing 0.2 ml of 10% blood in normal saline diluted with 0.2 ml of normal saline.

6. Phenolic compounds (Tannins):

a. Ferric chloride test: Treat the extract with ferric chloride solution, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

b. Phenazone test: Add about 0.5 gm of sodium acid phosphate to 5 ml of extract warm it and filter. To the filtrate add 2 % phenazone solution, bulky precipitate is formed, which is often colored.

c. Gelatin test: To the extract add 1 % gelatin solution containing 10% sodium chloride. Precipitate is formed.

7. Proteins:

a. Biuret test: To the extract (2 ml) add Biuret reagent (2 ml), violet color indicates presence of proteins.

b. Xanthoproteic test: To the 5 ml of extract, add 1 ml of concentrated nitric acid and boil, yellow precipitate is formed. After cooling it, add 40 % sodium hydroxide solution, orange color is formed.

8. Steroids and Triterpenoids:

a. Libermann-Burchard test: Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid from the side of test tube, brown ring is formed at the junction. The layer turns green which shows presence of steroids and formation of deep red color indicates presence of triterpenoids.

b. Salkowski test: Treat the extract with few drops of concentrated sulphuric acid red color at lower layer indicates the presence of steroids and formation of yellow colored lower layer indicates presence of triterpenoids.

c. Sulfur powder test: Add small amount of sulfur powder to the extract, it sinks at the bottom.

3. RESULTS AND DISCUSSION

3.1 Evaluation of *Calotropis gigantea* extract:

The petroleum ether extract obtained was dark green in colour, greasy with an indistinct odour and astringent in texture. The extracts were soluble in an organic solvent and were considered in water to be insoluble. The percentage yield was measured and set at 5.56% w/w.

The *Hibiscus rosa sinensis* petroleum ether extract obtained was light to dark green in colour, greasy with an indistinct odour and astringent in texture. The extracts were soluble in an organic solvent and were considered in water to be insoluble. The percentage yield was measured and set at 6.23% w/w.

3.2 PHYTOCHEMICAL ANALYSIS

The crude petroleum ether extract of *Calotropis gigantea* and *Hibiscus rosa sinensis* were screened the present alkaloids, flavonoids, glycoside, carbohydrate, protein, starch, amino acid, steroid, tannins and saponins using the simple chemical test as reported in a standard reference book.

Table 1: Observation of *Calotropis gigantea* petroleum ether extract

Sr. No.	Extract study	Observation
1.	Plant parts	Leaves
2.	Extraction process	Soxhlet
3.	Solvent	Petroleum ether
4.	Colour	Dark green
5.	Texture	Greasy
6.	Odour	Indistinct
7.	Soluble	Water and alcohol
8.	Insoluble	Acid
9.	Practical yield	5.56 gm
10.	Percentage yield	5.56% w/w

Table 2: Observation of *Hibiscus rosa sinensis* petroleum ether extract

Sr. No.	Extract study	Observation
1.	Plant parts	Leaves
2.	Extraction process	Soxhlet
3.	Solvent	Petroleum ether
4.	Colour	Dark green
5.	Texture	Greasy
6.	Odour	Indistinct
7.	Soluble	Water and alcohol
8.	Insoluble	Acid
9.	Practical yield	6.23 gm
10.	Percentage yield	6.23% w/w

Table 3: Phytochemical Screening of petroleum extract of *C. gigantea* and *H. rosa sinensis*

Sr. No.	Class of Drug	Chemical test		C. gigantea	*H. rosa sinensis*
1.	Alkaloids	Dragendroff test		Positive	Positive
		Hager's test		Negative	Positive
		Mayer's test		Positive	Negative
		Wagner's test		Positive	Negative
		Tannic acid test		Negative	Negative
2.	Amino Acid	Millon's test		Positive	Positive
		Ninhydrin test		Positive	Positive
3.	Carbohydrate	Molisch		Positive	Positive
		Barfoed's test		Negative	Negative
		Selwinoff's test		Negative	Negative
		Test for Pentoses		Negative	Negative
4.	Flavonoid	Shinoda test		Positive	Positive
		Alkaline reagent test		Positive	Positive
		Zinc hydrochloride test		Negative	Positive
5.	Glycoside	Test A		Positive	Positive
		Test B		Positive	Negative
		i) Anthraquinone glycosides	a) Borntrager's test	Negative	Negative
			b) Modified Borntrager's test	Positive	Negative
		ii) Cardiac glycosides	a) Kedde's test	Negative	Negative
			b) Killer- Killiani test	Negative	Negative
			c) Raymond's test	Positive	Negative
			d) Legal's test	Negative	Positive
			e) Baljet's test	Negative	Positive
		iii) Coumarin glycosides	-	Positive	Positive
		iv) Saponin glycosides	-	Positive	Negative
6.	Phenolic compounds (Tannins)	Ferric chloride test		Positive	Positive
		Phenazone test		Negative	Positive
		Gelatin test		Positive	Negative
7.	Proteins	Biuret test		Negative	Positive
		Xanthoproteic test		Negative	Positive
8.	Terpenoid and steroid	Libermann-Burchard test		Positive	Positive
		Salkowski Test		Positive	Negative
		Sulfur powder test		Negative	Negative

CONCLUSION

The petroleum ether extract of *Calotropis gigantea* was dark green, oily, with an unclear odor and astringent texture, yielding 5.56% w/w. *Hibiscus rosa sinensis* extract was light to dark green, oily, astringent, and had a vague smell, with a yield of 6.23% w/w. Both extracts were considered insoluble in water

but soluble in organic solvents. A chemical test screened the petroleum ether extract of *Calotropis gigantea* and *Hibiscus rosa sinensis* for alkaloids, flavonoids, glycosides, and other compounds.

REFERENCES

[1] Singh U, Wadhwani AM, Johri BM. Dictionary of Economic Plants of India. New Delhi: Indian council of Agricultural Research; 1996:38-39.

[2] Aarti C. A review on pharmacological and biological properties of Calotropis gigantea. International Journal of Recent Scientific Research. 2014;5(4):716-719.

[3] Gilhar A, Etzioni A, Paus R. Alopecia areata. N Engl J Med. 2012 Apr 19; 366 (16):1515-25.

[4] Habib MR, Karim MR. Antimicrobial and Cytotoxic Activity of Di-(2-ethylhexyl) Phthalate and Anhydrosophoradiol3-acetate Isolated from *Calotropis gigantea* (Linn.) Flower. Korean Society of Mycology. 2009;37(1):31–6.

[5] Evans WC. Trease and Evans Pharmacognosy. Saunders an Imprint of Elsevier; 2005. pp. 41-7.

[6] Alikhan I, Khanum A. Medicinal and Aromatic Plants of India. Ukaaz Publication; 2005.pp. 133–4.

[7] Raghubir R, Rasik M, Gupta AJ. Healing potential of *Calotropis procera* on dermal wounds in guinea pigs. J Ethnopharmacol. 1999; 68:261–6.

[8] Johanson. Plant Microtechnique. New York: Mc Graw Hill Book Co; 1940. p. 323.

[9] Sass JR. Elements of Botenical Microtechnique. New York: Mc Graw Hill Book Co; 1940. p. 275.

[10] Easu K. Anatomy of Seed Plants. New York: John Wiley and Sons; 1979. p. 550.

[11] Fahn A. Plant Antomy. Oxford, England: Pergamous Press; 1974. p. 611.

[12] Chase CR, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. Am Pharm Assoc. 1949; 38:324–31.

[13] The Indian Pharmacopoeia. New Delhi: Govt. of India Publication; 1996. Anonymous.

[14] Corner EJH. The Seeds of Dicotyledons. London: Cambridge University Press; 1976.

[15] Carol, J. P., Jignesh, H. P., Msyuree, A. P. and Anar, J. P. 2012. A comprehensive review on plant *Calotropis gigantea*. Int. J. of Institutional Pharmacy and Life Sciences, 2(2): 463-470.

[16] Dhivya, R. and Manimegalai, K. 2013. Preliminary Phytochemical Screening and GCMS profiling of ethanolic flower extract of *Calotropis gigantea* Linn. J. of

Pharmacognosy and Phytochemistry, 2(3): 28-32.

[17] Habib, M., R. and Karim, M. R. 2009. Antimicrobial and cytotoxic activity of Di- (2ethyl hexyl) Phthalate and anhydrosophoradiol- 3-acetate isolated from *Calotropis gigantea* (Linn.) flower. Mycobiology, 37(1): 31- 36.

[18] Kirtikar, K. R. and Basu, V. D. 1994. Indian Medicinal Plants, 3(2): 1606-1609.

[19] Rahman, Md. S., Moly, N. N. and Hossen, Md. J. 2013. Antimicrobial, cytotoxic and antioxidant activity of the exudates of *Calotropis gigantea*. Int. J. of Pharmaceutical Sciences and Res., 4(2): 745-753.

[20] Shirsat, M. K., Mahatama, O. P., Pal, U. S., Bais, S. K. and Dwivedi, J. 2013. GC-MS analysis of *Calotropis gigantea* Linn whole plant chloroform extract. J. of Pharmaceutical and Biosciences, 1: 26-29.

[21] Trease, G. E. and Evans, W. C. 1989. A Textbook of Pharmacognosy. Bacilliere tinal Ltd., London, 13th edition

[22] Kumari, O. S., Baburao, N. and Reddy, K. V. 2015. Phyto-chemical analysis and antimicrobial activity of *Hibiscus rosa sinensis*. World Journal of Pharmacy and Pharmaceutical Sciences. 4(5): 766-771.

[23] Raaman, N., 2006, Phytochemical techniques. New India Publishing Agency, New Delhi: 19, 21, 22.

[24] Tiwari, U., Yadav, P. and Nigam, D. 2015. Study on phytochemical screening and antibacterial potential of methanolic flower and leaf extracts of *Hibiscus rosa sinensis*. International Journal of Innovative and Applied Research. 3(6): 9- 14.

[25] Udo, I. J., Ben, M. G., Etuk, C. U. and Tiomthy, A. I. 2016. Phytochemical, proximate and antibacterial properties of *Hibiscus rosa-sinensis* L. leaf. Journal of Medicinal Plants Studies. 4(5): 193-195.

[26] Jyoti V. Vastrad and Shameembanu A. Byadgi. 2018. Phytochemical Screening and Antibacterial Activity of *Hibiscus rosa - sinensis* Leaf Extracts. Int. J. Curr. Microbiol. App. Sci. 7(03): 3329-3337.