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PHYTOCHEMICAL ANALYSIS AND PHARMACOGNOSTICAL STANDARDIZATION OF STEM OF *CAYRATIA TRIFOLIA* (LINN.) DOMIN.

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ABSTRACT

Cayratia trifolia (Linn.) Domin (Vitaceae) is a perennial climber, commonly known as fox grape in English, Amalbel, Ramchana in Hindi and Amlavetash in Sanskrit, found in India, Asia and Australia. The infusion of seeds along with extract of tubers is used traditionally for the treatment of diabetes. Whole plant is used as diuretic, in tremors and splenopathy. It is reported to possess antiviral, antibacterial, antiprotozoal, hypoglycaemic, anticancer and diuretic activity etc. The present study was carried out to establish the pharmacognostical studies, physico-chemical parameters along with preliminary phytochemical screening of petroleum ether, chloroform, methanolic and aqueous extracts of *Cayratia trifolia* (Linn.) Domin. The macroscopical and microscopical characters were studied. The transverse section (T.S.) of stem indicated the arrangement of various cells in cork, cortex, phelloderm and pith region. The histochemical color reaction of T.S with different chemical reagents and preliminary phytochemical screening of various extracts revealed the presence of carbohydrate, flavonoids sterols, phenolic & tannins compounds. The physico-chemical parameters such as total, acid insoluble, water insoluble and sulphated ash (6.85, 0.47, 5.45 and 4.45%w/w respectively), loss on drying (8.17 %w/w) extractive values and fluorescence analysis of extracts and powder treated with different chemical reagents were studied under ordinary light, short and long UV lights. The foaming and swelling index were also studied. These studies will be helpful in developing standards for quality, purity and sample identification of this plant.

Keywords:

Cayratia trifolia,
Vitaceae,
Pharmacognostical,
Physico-chemical parameters

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INTRODUCTION: *Cayratia trifolia* (Linn.) Domin (Vitaceae) is a perennial climber, commonly known as fox grape in English, Amalbel, Ramchana in Hindi and Amlavetash in Sanskrit, found in India, Asia and Australia¹. The plant is found in hilly regions as well as the hotter part of India from Jammu and Rajasthan to Assam. The plant have trifoliated leaves with (2-3cm) long petioles and ovate to oblong-ovate leaflets. Flowers are small greenish white and brown in colour.

Fruits are fleshy, juicy, spherical, about 1 cm in diameter of dark purple or black color. The roots of the plant is used as poultice on boils. Infusion of seeds along with extract of tubers is traditionally given orally to diabetic patients to check sugar level of blood. Whole plant is used as diuretic, in tumors, neuralgia and splenopathy. The paste of tubers is applied on the affected part in the treatment of snake bite. It is reported to possess antiviral, antibacterial, antiprotozoal, hypoglycaemic, anticancer and diuretic

activity etc ². For the standardization and quality assurance purpose, the following three attributes must be verified: authenticity, purity and assays. Hence, in this work we make an attempt for the standardization of *Cayratia trifolia* (Linn.) Domin stem by carrying out its pharmacognostical studies, physico-chemical parameters and preliminary phytochemical screening.

MATERIALS AND METHODS:

Plant Material: The stem of *Cayratia trifolia* (Linn.) Domin was collected from kurukshetra, Haryana in the month of October 2010 and authenticated by Dr. H.B. Singh, Head Raw Material Herbarium & Museum, New Delhi vide Ref. NISCAIR/RHMD/Consult-2010-11/1667/265. A voucher specimen has been retained in Department of Pharmaceutical Science, Guru Jambheshwar University of Science & Technology, Hisar. The plant material (1kg) was air-dried at room temperature (30-40°C) and then powdered to pass through a sieve of 1mm and further subjected to various studies.

Chemical and Reagent: All the chemical and solvents used for the study were of analytical grade and all methods were taken from official methods.

Macroscopical Characters: The fresh and dried stem were studied for their macroscopical characters such as colour, odour, taste, shape, size and texture.

Microscopical Characters: Thin transverse sections of the stem were cut using microtome (WES WOX Model, MT-1090 A), stained with phloroglucinol and hydrochloric acid and observed under compound microscope. Photomicrographs of the sections were captured with the help of motic photomicroscope provided with motic image plus 2.0 software ⁴.

Histochemical Colour Reactions: The histochemical colour reactions on the transverse section of the stem of *Cayratia trifolia* were performed according to standard procedures reported ⁶⁻⁸. The colour tests were performed for the identification of the major cell components.

Physicochemical Parameters: The physicochemical parameters such as percentage of total ash, acid-insoluble, water soluble and sulphated ash, loss on drying, extractive values, foaming index, swelling index,

fluorescence analysis were determined according to official methods for quality control of medicinal plant ¹⁰⁻¹¹.

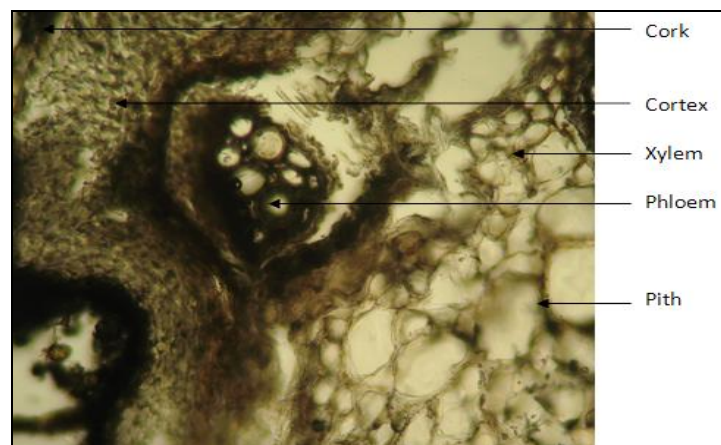
Fluorescence Analysis: Fluorescence characters of powdered plant material with different chemical reagents were determined under ordinary and ultraviolet light. 1 mg of the sample was taken in a glass slide and treated with various reagents for the presence of their fluorescence characters under ultraviolet lamp. Fluorescence analysis was carried out according to methods of Kokoski ¹⁴. These plant material was subjected to fluorescence analysis in visible/ daylight and UV light (254nm & 365nm).

Preliminary Phytochemical Screening: The preliminary photochemical screening was carried out on extracts obtained after successively extraction with petroleum ether, chloroform, methanol and aqueous solvents. The dried extracts were treated for the presence or absence of phytoconstituents ¹².

RESULTS AND DISCUSSIONS:

Macroscopical Characters: The stems of *Cayratia trifolia* were green when fresh and dark brown in colour when dried. It was slightly scaly and curved in shape. The average stem size was 10-20 cm, with characteristic taste and odour. Outer surface was rough.

Microscopical Characters: The stem composed of cork cells on the outer side and composed of small size sclerenchymatous cells. The cortex is wide and has parenchymatous cells. Numbers of sclereids are widely distributed in the cortex region. Cortex also shows the presence of calcium oxalate crystals.



TRANSVERSE SECTION OF CAYRATIA TRIFOLIA STEM

Histochemical Colour Reaction Tests: Transverse sections of the stem when treated with various chemicals reagents for the tests of cell components showed change in colour as shown in **Table 1**.

TABLE 1: HISTOCHEMICAL COLOUR REACTIONS ON TRANSVERSE SECTION (STEM)

Reagents	Test for	Nature of colour change	Chemical constituents
Iodine solution	Carbohydrate	Brownish black	++
Acidic Ferric chloride	Tannins	Light brown	+
Liebermann Burchard	Terpenes	Yellowish black	++
Sulphuric acid	Sterol	Yellowish black	+
Millions's reagent	Proteins	Yellow	-
Vanillin HCl	Flavonoids	Pink	+

++ : more, + : less, - : not present

Physico-chemical Parameters: The various parameters such as total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying were established and shown in **Table 2**. The extractive values by successive extraction method and colour change of extracts, in visible and UV light are summarized in **Table 3**.

TABLE 2: ASH VALUES AND LOSS ON DRYING

Parameter	% w/w
Total ash	6.85%
Acid insoluble ash	0.47%
Water soluble ash	5.48%
Sulphated ash	4.45 %
Loss on drying	8.17%

TABLE 3: EXTRACTIVE VALUES AND COLOUR OF EXTRACTS UNDER DIFFERENT LIGHTS

Extract	Yield (%w/w)	Colour of extract		
		Ordinary light	UV light (254 nm)	UV light (365 nm)
Petroleum ether	9.45	Light Brown	Greenish brown	Greenish
Chloroform	12.75	Light brown	Greenish brown	Yellowish black
Methanol	16.88	Brown Black	Greenish Black	Black
Water	14.95	Brown Black	Greenish Black	Black

Fluorescence Analysis: Fluorescence analysis is the quick method for the resolution study of crude drug of doubtful specimen, when physical and chemical methods produce inadequate results. The plant

material may be identified from their adulterants on the basis of fluorescence nature. The powder of stem was treated with different chemical reagents and results are reported in **Table 4**.

TABLE 4: FLUORESCENCE ANALYSIS OF POWDERED STEM OF CAYRATIA TRIFOLIA (LINN.) DOMIN

Treatment	Colour observed under ordinary light	UV Light	
		254 nm	365 nm
Powder as such	Brown	Brown	Dark Brown
Powder + NaOH	Greenish Brown	Brownish	Brownish Black
Powder + Glacial acetic acid	Yellowish Brown	Greenish Brown	Yellowish black
Powder + HCl	Light Brown	Dark Brown	Greenish Black
Powder + HNO ₃	Brownish	Brownish black	Black
Powder + Iodine	Brownish	Brownish Black	Greenish Black
Powder + FeCl ₃	Brownish Blue	Greenish Brown	Black
Powder + H ₂ SO ₄	Brownish black	Blackish	Black
Powder + Methanol	Greenish Brown	Dark Brown	Black

Quantitative Studies: The other quantitative studies for foaming index and swelling index were performed. The results are tabulated in **Table 5**.

TABLE 5: QUANTITATIVE STUDIES OF SUAEDA MARITIMA STEM

Sr. No.	Estimation	Observations
1	Foaming index	> 100
2	Swelling index	2 mL

Preliminary Phytochemical Investigation: The successive extracts obtained were subjected to investigation for various phytoconstituents. It revealed the presence of different phytoconstituents, like carbohydrates, glycoside, phenolic & tannins, flavonoid, protein & amino acid and steroids in different extracts as in **Table 6**.

TABLE 6: PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF VARIOUS EXTRACTS

Test	Petroleum ether	Chloroform	Methanol	Water
Carbohydrate				
Alkaloid	—	—	+	++
Glycoside	—	—	---	---
Phenolic and	—	—	---	---
Tannin	—	+	+	+
Flavonoid	—	+	++	++
Saponin	—	—	—	—
Protein and	—	—	+	+
Amino acid	---	---	++	+
Steroids				

++ : more present, + : less present, — : not present

CONCLUSION: The scientists from past few decades are keen and sincere to evaluate many ethno medicinally used plants, due to their specific healing properties, desirable action, easy availability and less toxicity. The stem of *Cayratia trifolia* (Linn.) Domin is still used in treatment of various disorders by many populations. The pharmacognostical standardization on this plant gives the idea about identification, standardization and monograph of the plant. It is also important in long term study of plant to evaluate the medicinal and therapeutic action of this plant.

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