



Pharmacognostical and Phytochemical Analysis of *Vanya Haridra* (*Curcuma Aromatica Salisb.*) Rhizomes

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Abstract:

Vanya Haridra (*Curcuma aromatic Salisb.*) also known as *Aranya Haridra* in Sanskrit, very important plant in use since long decade in traditional medicine in India. It is known as wild turmeric in English/cochin turmeric, *van haldi* in hindi, *anakuva* in Malayalam, *kasturi manjal* in tamil. It is mentioned as *kusthaghna*, *arshaghna* properties in *Bhavaprakas Nighantu*, Throughout the India, specially found in Mysore, Cochin and Bengal etc , rootstock large of palemately branched, sessile annulated biennial tubers yellow and aromatic inside, hairy and masked with circular disk. It is camphoraceous and jambula like fragrant odour. On these ground, this work was attempt to establish pharmacognostical and preliminary phytochemical standard of rhizomes, pharmacognostical parameters using like macromorphology, microcopy, physio-chemical constant and phytochemical analysis were done using standard methodology. It showed presence of carbohydrate, alkaloids, amino acids, protein, saponin, glycosides, flavonoids, steroids and tannins in aqueous, ethanol and petroleum ether extract.

Keywords: *Vanya Haridra* (*Curcuma aromatica Salisb.*), Pharmacognostical, Phytochemicals in aqueous, ethanol and petroleum Ether extracts.

Introduction

Curcuma aromatica Salisb is a medicinally important species belonging to the family Zingiberaceae. It is occurring wild throughout India and cultivated chiefly in West Bengal and Travancore. The plant is commonly known as Junglee Haldi (wild turmeric). The historical evidence of *Vanya Haridra* is not found in Vedic period, Samhita period, Ancient *Nighantu* period, except *Bhavaprakash Nighantu* and modern text. *Bhavamishra* described in *Bhavaprakash Nighantu* of *Vanya Haridra* about Vernacular Name, Morphological brief description, Properties and therapeutic used.¹

Taxonomical classification of *Vanya Haridra*^{2,3}

Division- Spermatophyta

Sub-division- Angiospermae

Class- Monocotyledonae

Series- Epigynae

Family- Zingiberaceae

Genus- Curcuma

Species - aromatica Salisb

Latin Name – *Curcuma aromatica Salisb.*

Vernacular Name:

Sans - *Aranya Haridra.*

Beng - Van Halodi, Van Halud

Hindi- Jangali Haladi, Van Haridra.

Mar- Vedi-Haldi, Amba Haldi.

Malayalam - Anakuva. Tamil-Kasturi manjal.

Tel - Kattu manal.

Eng - Wild Turmeric, Cochin Turmeric.

Botanical description:

Distribution- throughout the India, specially found in Mysore, Cochin and Bengal etc. Characters- Root stock large of paleately branched, sessile annulate beinnial tubers yellow and aromatic inside, hairy and masked with circular disk. It a camphoraceous and jambula like fragrant odour. Leaves-38-60 by 10-20 cm, oblong lanceolate, green often variegated above pubescent beneath, base deltoid. Flower- flowering stem appearing with or the leafing stem, flowers fragrant, shorter than the bracts, in spikes 15-30 cm. long. Calyx- 8 mm long, irregularly 3 lobed. Corolla tube 2.5 cm long, the upper half funnel shaped, lobess pale rose-coloured, Lip yellow.^{4,5}

Chemical constituents-Rhizomes yield 6.1% essential oil (Chopra et al, 1980). Essential oil contains α - and β -curcumene, d-camphene and p-methoxy cinnamic acid. The colouring matter is curcumin. Numerous sesquiterpenoids of germacrone and guaiane skeletons have been identified recently (Husain et al, 1992). *C. aromatica* rhizomes contain ar-curcumene (18.6%), beta curcumene (25.5%) and xanthorhizol (25.7%) (Zwaving and Bos, 1992). The active constituents of oil are curcumol and curdione (Asolkar et al, 1992). Structures of sesquiterpenes were studied by Kuroyanagi et al (1990). Its rhizome is a rich source of volatile oil, which consists of several major anti-tumor ingredients including demethoxycurcumin, β -elemene, curcumol, curdione, etc. It is used as anti-venom for Indian cobra, used as tonic, to treat digestive problems^{6,7}

Material and Methods:

Collection of Plant Materials-*Vanya harirda* plants were collected from Palakkad district, kerala, in the month of February 2017. Specimens were dried by keeping them between the folds of old newspapers. It is necessary to change these papers at regular intervals, until the plants are well dried. The dried specimens were pasted on the herbarium sheets of standard size with proper labeling. The authentication of plants material collected for study was done at Herbarium section, Botany department, University of Tripura, Suryamaninagar-799022, Tripura State, with Authentification Accession No.1664 as *Curcuma aromatic salisb.* and belong to family Zingiberaceae by Dr.B. K. Datta (Professor of Botany). After identifying the plant, for study purpose rhizomes of *Vanya Haridra* is washed with running water and kept for drying under shade. The procured dried parts were powdered, labeled, packed and subjected for organoleptic and other analytic studies.

Pharmacognostic Study:

Pharmacognostic study was carried on the basis of Morphological characters such as colour, odour, taste, size, fracture etc

Physiochemical Parameters:

Determination of moisture content⁸

Moisture content was determined by placing weighed sample of 5gm of drug in oven at 105° for 5 hours, and calculate weight of sample for every 30 minute, until the weight of the sample were constant, no variation of weight are recorded. This sample was allowed to cool at room temperature in desiccators for 1 hour before weighing.

Determination of Ash Value⁹

Total Ash Value- Weighed accurately 2 g of the air-dried drug (rhizome) in a silica dish and incinerated at a temperature not exceeding 450° until free from carbon. Then cool and weighed. Percentage of ash value was calculated on the the basis of airdried drug.

Acid Insoluble Ash- Boiled the ash with 25 ml of 2M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible, washed with hot water, ignited, cooled in a desiccator and weighed. Calculated the percentage of acid-insoluble ash on the air dried drug basis.

Water Soluble Ash- Boiled the ash for 5 minutes with 25 ml of water, collected the insoluble matter in a Gooch crucible, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Percentage of water soluble ash was calculated with reference to the air dried drug.

Extractive value:¹⁰

Water Soluble Extractive Value: Macerate 5 gm of the air dried drug, coarsely powdered of Curcuma aromatic Salisb, with 100 ml of water of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Alcohol Soluble Extractive value: Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Petroleum Ether Soluble Extractive value (Fixed Oil Content)-⁴Transfer a suitably weighed quantity (depending on the fixed oil content) of the air dried, crushed drug to an extraction thimble, extract with *Solvent* (petroleum ether, b.p. 40° to 60°) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filter the extract quantitatively into a tared evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105° to constant weight. Calculate the percentage of Petroleum Ether-soluble extractive with reference to the air-dried drug.

Preliminary Phytochemical Screening¹¹

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Tests for Carbohydrates

Molisch's Test-2 ml of test Solution was taken in a test tube and 2 ml of the Molisch's reagent(Dissolve 10gm of α -naphthol in 100ml of 95% alcohol) was added and shaken carefully and then about 1ml. of conc. H_2SO_4 is poured from side of the test tube and allowed to stand for one 1 minute. A Purple colour ring at the junction of the two layers if formed indicated the presence of Carbohydrate.

Test for reducing sugars:

Benedict's test -It is used for reducing sugars and composed of mainly Copper sulphate and sodium hydroxide. Mix equal volume of Benedict's reagent and test solution in test tube. Heat in boiling water bath

for 5 minutes. Solution appears green, yellow, orange, red or brown colour depending on amount of reducing sugar present in test solution.

Fehling solution test-It is generally used for reducing sugars and composed of two solutions, which are mixed in situ. Fehling solution A composed of 0.5% of copper sulphate whereas Fehling solution B composed of Sodium Potassium Tartarate. Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each), boil for 1 minute. Add 2 ml of aqueous solution of drug followed by boiling for 5-10 minutes on water bath. Formation of reddish brown coloured precipitate due to formation of Cuprous oxide indicates presence of reducing sugar.

Tests for Alkaloids

Mayer's reagent test- 2 ml of test Solution was taken in a test tube and 2 ml of the Mayer's reagent (Potassium Mercury Iodide solution) was added. A White or Pale Yellow precipitate if formed indicated presence of Alkaloids except with Alkaloids of the Purine groups and few others.

Dragondroff's reagent test- 2 ml of test Solution was taken in a test tube and 2 ml of the Dragon Droff's reagent (Mixture of Potassium Iodide and Bismuth sub nitrate solution) was added. An orange precipitate if formed indicated presence of Alkaloids.

Wagner's test- Drug solution + few drops of Wagner's reagent (dilute Iodine solution), formulation of reddish-brown precipitate.

Hager's Test- A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with Hager's reagent, an orange yellow precipitate was obtained which indicates the presence of alkaloids.

Test for Amino acids

Ninhydrin test -The Ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecules, it gives characteristic deep blue or pale yellow colour due to formation of complex between two ninhydrin molecule and nitrogen of free amino acid.

Test for proteins

Xanthoprotein test- A small quantity of test sample was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. Development of yellow colour indicates the presence of proteins.

Millon's test- The Millon reagent is a solution of mercuric and mercurous ions in nitric and nitrous acids. Take 1 ml of sample solution in a test tube and add few drops of Millons reagent. White precipitate is produced, which turns red after heating for 5 minutes on water bath.

Biuret test (General test)- Take 1 ml of sample solution in a test tube and add 2 ml of 10% NaOH solution followed by few drops of lead acetate solution. Shake the solution and boil on water bath for few minutes it produces black precipitate in presence of sulphur containing amino acids.

Tests for Glycosides

Borntragar's Test- 1 ml of Benzene and 0.5 ml of dilute ammonia solution was added to the extract and was observed for the formation of reddish pink colour.

Test for Phenolic Compound

The extract was taken in water and warmed; to this 2 ml of ferric chloride solution was added and observed for the formation of green and blue colour.

Test for Saponin

Foam test-About 1 ml of Aqueous Extract was diluted by distilled Water up to 10 ml and shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of froth indicates presence of Saponin.

Test for Flavonoids

Shinods test- A small quantity of test sample was dissolved in 5 ml ethanol (95%v/v) and reacted with few drops of concentrated hydrochloric acid and 0.5 gm of magnesium metal. Appearance of pink, crimson or magenta colour within a minute or two indicate the presence of flavonoids.

Test for Steriods

Salkowaskireaction- Few mg of extract was taken in 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added from the side of test tube. The test tube was shaken for few minutes. The development of red colour indicates the presence of sterols.

Test for Tannins

FecI₃ -A 5 percent solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Appearance of dark green or deep blue colour indicates the presence of tannins.

Lead acetate - A 10 percent w/v solution of basic lead acetate in distilled water was added to the test filtrate. Development of precipitate indicates the presence of tannins.

Pot. Dichromate - A solution of potassium dichromate was added to the filtrate. Appearance of dark colour indicates the presence of tannins.

Thin layer Chromatography:¹²

Chromatography plates-T.L.C. plate coated with 0.25 mm layer of silica gel 60 F₂₅₄ with fluorescent indicator, (Mercks) were used.

Activation of pre-coated Silica gel G60 F₂₅₄

Dried in hot oven at 105⁰ C for one to two hour.

Preparation of mobile solution- Toluene: Ethyl acetate (9:1)

Results and Discussion:

Pharmacognostic Study:

Macroscopical examination of fresh rhizome-Size, shape & structure-Rhizomes are 6.5 – 7 cm in length and 1.5- 2 cm in diameter, less lateral branches, palmately attached and root tubers present.

Colour-Light yellow inside.

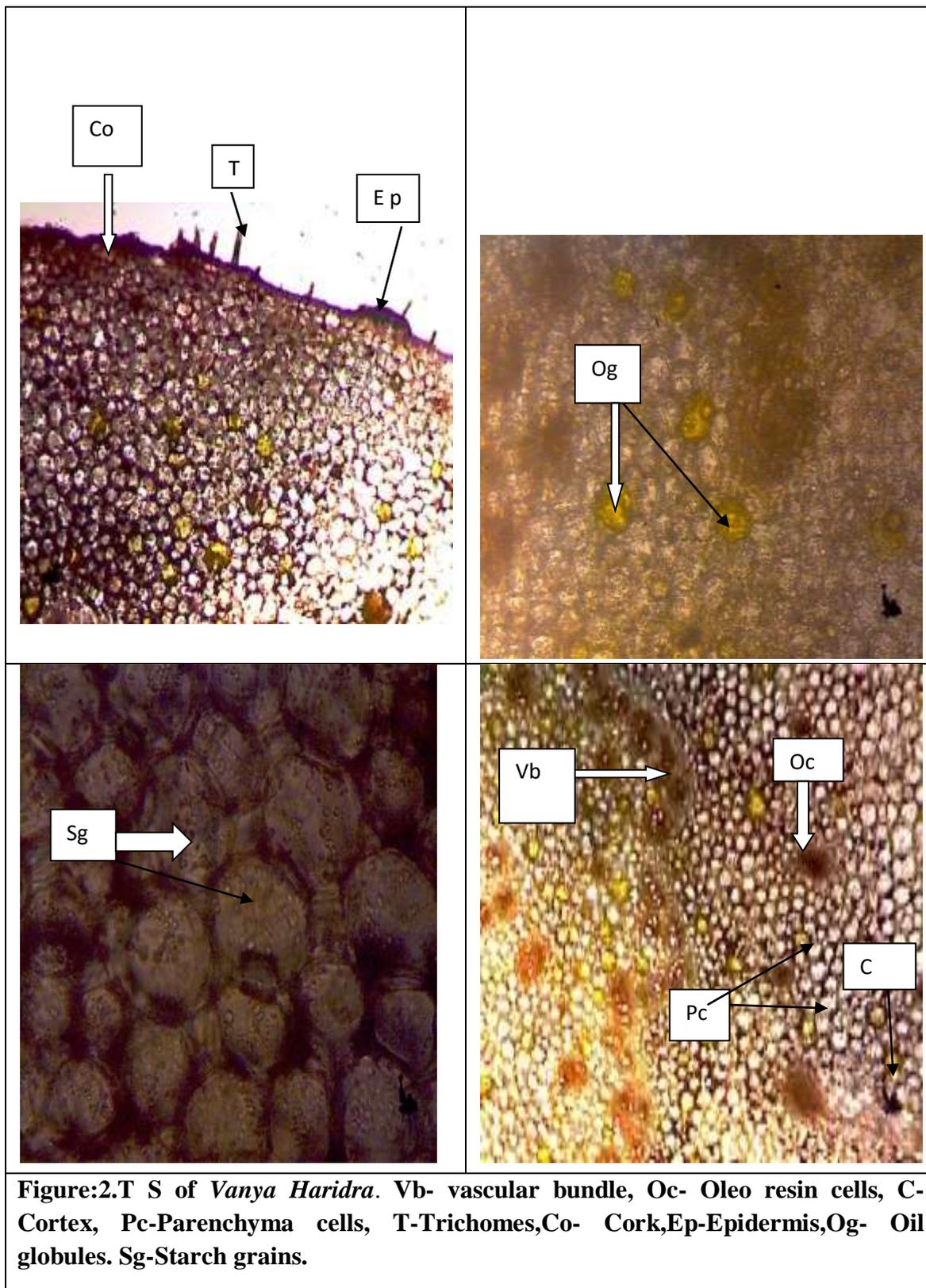
Odor-Sweet camphooraceous.

Taste- Pungent.

Touch-Slightly rough external surface with big round scar, hard & heavy.



Figure:1. fresh rhizomes of *Curcuma aromatica* Salisb.

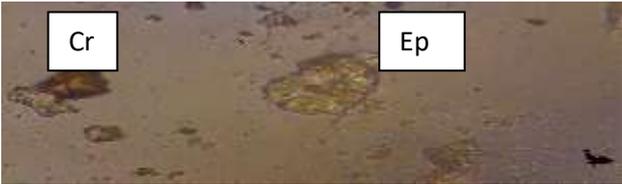


Microscopic characters fresh rhizome of *Vanya Haridra*

Epidermal hairs: 1-2 celled, branched, thick walled, lignified. Periderm - 3-5 layered. Outer cortex-Lesser number of scattered collateral vascular bundles, starch grains present. Inner cortex- Amphicribal vascular bundles arranged in patches. Vascular elements-Trachieds with spiral and scalary and vessels with reticulate thickening, bundle sheath absent, fibre present very few. Oil cells- Present.

Powder microscopic study of Vanya Haridra:

In Powder microscopylignin, Cork, crystalloids, Starchgrains, Mucilage, lignified fibres, epidermal cells, were also seen in powder microscopy.

		
Lignin. Stain: Safranin	Mucilage. Stain: Methylene blue	Crystalloids. Stain: Eosin
		
Starch grains. Stain- Iodine.	Cr-Cork. Ep. Epidermis. Stain: Iodine	
Figure: 3. Powder microscopic characteristic of Vanya Haridra		

Physicochemical parameters:

In this study moisture content, ash values (total ash, acid insoluble ash and water soluble ash), extractive value (water soluble extractive value, alcohol soluble extractive value and Petroleum Ether Soluble Extractive value) were determined below-

Moisture Content 4.35 % w/w

Total Ash value- 3.114 % w/w

Acid Insoluble Ash 0.807 % w/w

Water Soluble Ash 0.0411 % w/w

Water Soluble Extractive Value 1.648 % w/w

Alcohol Soluble Extractive Value 8.954 % w/w

Petroleum Ether Soluble Extractive value 1.044 % w/w

Phytochemical analysis:

Phytochemical are nutritive plant chemicals that have protective or disease preventive properties. A plant cell produces two types of metabolites primary metabolites involved directly in growth and metabolism (carbohydrates, lipids and proteins etc), and secondary metabolites not involved in metabolic activity (alkaloids, phenolics, sterols etc) but act as defense chemicals. The Preliminary Phytochemical Investigations of Aqueous, Ethanolic and Petroleum Ether extract of rhizome Curcuma aromatica Salisb were performed which reveals the presence of Carbohydrates, Alkaloids, Amino acids, Saponin, Glycosides, Flavonoids, Steroids and Tannins. The results of the screening were expressed in Table no.1

Table: 1. showing Phytochemical analysis

Carbohydrate test

Name of test	Aquous extract	Ethanol Extract	Petroleum Ether extract
Malish test	+ve	+ve	+ve
Benedict test	+ve	+ve	-ve
Feling test	+ve	+ve	+ve
Barfoad test	+ve	+ve	+ve

Alkaloids

Dragan drof	+ve	+ve	+ve
Wagner's test	+ve	-ve	-ve
Mayer's test	+ve	+ve	+ve
Hager's test	+ve	+ve	-ve

Amino acids

Ninhydrine	-ve	-ve	-ve
Xanthoprotic	+ve	+ve	-ve

Protein

Millon' test	-ve	-ve	-ve
Biuret test	-ve	-ve	-ve

Saponin

Foam test	-ve	-ve	+ve
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Glycosides

Killar kilini test	+ve	+ve	+ve
Borntrager's test	+ve	+ve	+ve

Phenolic compound

Phenolic test	-ve	-ve	-ve
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Flavonoids

Shinod's test	+ve	+ve	+ve
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Steroids

Salkowaski test	+ve	+ve	+ve
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Tannins

Fecl ₃ test	+ve	+ve	-ve
Lead acetade test	+ve	+ve	-ve
Pot.dichromate test	+ve	+ve	-ve

Thin layer chromatography:

Thin layer Chromatography is a tool for separation and identification of chemical constituents present in the herbs or chemical mixtures with mobile solution of Toluene: Ethyl acetate 9:1 ratio. Ethanolic extract of rhizomes of *Vanya Haridra* R_f values 0.08, 0.2, 0.25, 0.31, 1.03 in day light. R_f values 0.15, 0.23, 0.24, 0.25, 0.31, 0.42, 0.52, 0.65 in U.V (366nm) rays. R_f values 0.15, 0.23, 0.24, 0.25, 0.52, 0.61, 0.86, 0.91, 0.98. in Iodine Vapor.

Table:2. TLC Ethanol extract of rhizomes *Vanya Haridra*

Visualisation in day light	Visualisation in U.V (366nm) rays	Visualisation in Iodine Vapour
		
Rf value: 0.08, 0.2, 0.25, 0.31, 1.03	Rf value: 0.15, 0.23, 0.24, 0.25, 0.31, 0.42, 0.52, 0.65	Rf value: 0.15, 0.23, 0.24, 0.25, 0.52, 0.61, 0.86, 0.91, 0.98.

Conclusion:

The Phytochemical screening confirmed the presence of various phytochemical constituents such as Carbohydrates, Alkaloids, Amino acids, Saponin, Glycosides, Flavonoids, Steroids and Tannins. Different Physicochemical parameters such as Total Ash, Acid Insoluble Ash, Water Soluble Ash, Water soluble extract, Alcohol Soluble extract, Petroleum Ether extract and Loss on drying value was observed. These values can be useful to detect adulteration. All studied standardization parameters like Pharmacognostic study, Phytochemical screening and Physicochemical parameters provide the knowledge in the identification authentication of rhizome of *Curcuma aromatica* Salisb.

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