IJPSR (2023), Volume 14, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 November 2022; received in revised form, 09 January 2023; accepted, 01 May 2023; published 01 July 2023

PHYTOPHARMACOGNOSTICAL STUDY OF AERIAL PARTS OF *HOUTTUYNIA CORDATA* THUNB.

Archana Modi * [1] and Mamata Shah [2]

Department of Pharmacognosy [1], Sardar Patel College of Pharmacy, Vidhyanagar-vadtal Road, Bakrol - 388315, Gujarat, India.
Department of Pharmacognosy [2], L. M. College of Pharmacy, Ahmedabad - 380009, Gujarat, India.

Keywords:

Houttuynia cordata Thunb. HPTLC, Phytopharmacognostic, Quercitrin

Correspondence to Author:
Mrs. Archana Modi

Research Scholar,
Department of Pharmacognosy,
Sardar Patel College of Pharmacy,
Vidhyanagar-vadtal Road, Bakrol - 388315, Gujarat, India.

E-mail: amodi87@yahoo.com

ABSTRACT: Natural products are becoming increasingly important as alternative medicines and a source of pharmacotherapeutics either directly or as raw materials from which more or less complex chemical structures proven biological activity are isolated. *Houttuynia cordata* Thunb. is one of the important drugs, which is traditionally used as anti-inflammatory, diuretic, anti-pyretic and detoxicant. In Indo-china, the entire herb is considered cooling, resolvent and emmenagogue. In the present study, the pharmacognostic and phytochemical evaluation of aerial parts of *Houttuynia cordata* Thunb. (F: Saururaceae) was performed as it is helpful for the standardization and authentication of medicinal plants. The microscopical study showed the presence of blunt-ended multicellular trichomes, lignified fibres, spiral xylem vessels, pitted xylem vessels, striated cuticles, fragments of multicellular trichomes with pointed ends, and cluster crystals of calcium oxalate. The phytochemical analysis indicated the presence of carbohydrates, alkaloids, flavonoids, tannins, steroids, phenolics, *etc*. The results of the TLC and HPTLC study of plant extract showed the presence of the flavonoids like quercitrin in the aerial parts of *Houttuynia cordata* Thunb.

INTRODUCTION: Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(7).3533-38

This article can be accessed online on www.ijpsr.com

DOI link: http://doi.org/10.13040/IJPSR.0975-8232.14(7).3533-38

To have global acceptance, research is also necessary to give consistent quality of the desired actives by estimating the concentration in terms of purity, assay or potency. Analytical methods are developed and validated by instruments such as HPLC, HPTLC and GC *etc* [2].

A single species of a creeping herb, viz. *Houttuynia cordata* Thunb. (F: Saururaceae) is found in India it is a perennial herb having leaves that are alternate, large and broad; flowers bisexual, regular in a long dense spike, perianth zero, stamens of six or fewer in number, hypogynous or united with the pistil, carpels three to four either separate or united into a three to four celled ovary, ovules two to several presents in parietal placentation, stigmas as many as the carpels; fruits follicles or lobed berries. It is

closely related and anatomically similar to the Piperaceae family. Vascular bundles are widely spaced, and secretory cells are present, often abundant in ground tissue. Cluster crystals are common. The active principles of this family are flavonoids, benzamides, monoterpenes, and sesquiterpenes [3, 4, 5, 6] and [7]. It is distributed in Tropical Himalayas from Panjab to Sikkim, up to 5000 ft also in Assam and Khasi hills extending to China and Japan. The plant is used in traditional Chinese herbal medicine as detoxicant, anti-inflammatory, antipyretic and diuretic agent. The plant is used along with the leaves of *Polygonum chinense* Linn. As a folk medicine to cure uterus swelling [8, 9, 10, 11, 12, 13]. The present study investigates the development of its pharmacognostic and phytochemical parameters.

MATERIALS AND METHODS:

Collection and Authentification: Dried sample of aerial parts of *Houttuynia cordata* Thunb. was collected from Assam. Taxonomist of Veer Narmad South Gujarat University did the authentification No. LM-HC 54.

Stems and leaves were separated from the dried plant material for pharmacognostical studies, and dried leaves and stems were kept separately in a mixture of alcohol, glycerine and water (50:50:10) for ten days and then used. The aerial parts of plant powdered to 60# separately, stored in airtight containers and used for phytochemical and pharmacological studies.

Macroscopical and Microscopical Study:

Macroscopical Study: The aerial parts of *Houttuynia cordata* Thunb. were studied and identified by comparing their morphological characters as mentioned in the literature [8, 10, 13].

Microscopical Study: Powdered material of Aerial parts *Houttuynia cordata* Thunb was observed under microscope in 10 x resolution and 45x and identified by comparing their microscopical characters as mentioned in the literature.

Physico-chemical Parameters: The powder of aerial parts of *Houttuynia cordata* Thunb was used or the physico-chemical studies of the powdered drug, such as determination of the ash values and extractive values, were performed according to the WHO guidelines [14, 15].

Determination of Ash Value: Ash values of aerial parts of *Houttuynia cordata* Thunb. were determined by the following method:

Determination of Total Ash: 2 g of accurately weighed powder was incinerated in a crucible at a temperature of 500-600°C in a muffle furnace until carbon-free ash was obtained. It was then cooled, weighed and percentage of ash was calculated with reference to the air-dried drug.

Determination of Acid Insoluble Ash: The ash obtained above boiled for 5 min. with 25 ml of 70 g/l hydrochloric acid and filtered using an ash less filter paper to collect insoluble matter. The ash obtained, had washed with hot water and filter paper burnt to a constant weight in a muffle furnace. The percentage of acid-insoluble ash with reference to the air-dried powered drug (40#) was calculated.

Determination of Water-soluble Ash: Total ash was boiled for 5 min. with 25 ml of water and insoluble matter collected on an ash-less filter paper, washed with hot water, ignited for 15 min. at a temperature not exceeding 450 °C in a muffle furnace. The difference in weight of ash and the weight of water-insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash with reference to the air-dried powered drug (40#) was calculated.

Determination of Extractive Values: Extractive values of powder of aerial parts of *Houttuynia cordata* Thunb. Were determined by the following methods:

Determination of Alcohol Soluble Extractive: 4 g of the air-dried powdered material (40#) of each plant macerated with 100 ml of alcohol in a closed flask for 24 h, frequently shaking at an interval of 6 h. Then allowed it to stand for 18 h and filtered rapidly to prevent any loss during evaporation. 25 ml of filtrate evaporated to dryness in a porcelain dish and dried at 105°C to a constant weight. The percentage of alcohol-soluble extractives was calculated with reference to the air-dried drug.

Determination of Water-soluble Extractive: 4 g of the air-dried powdered material was soaked in 100 ml of water in a closed flask for 1h with frequent shakings. It was then boiled gently for 1 h

on water bath; cooled, weighed, and readjusted the weight. 25 ml of the filtrate evaporated to dryness in a porcelain dish and dried at 105°C to a constant weight. The percentage of water-soluble extractive calculated with reference to the air-dried powered drug (40#).

Phytochemical Screening: The powder (60#) of aerial parts of *Houttuynia cordata* Thunb. was subjected to chemical tests to check the presence of various phytoconstituents like, alkaloids [16], flavonoids [17, 18], saponins [19, 20], Carbohydrates [21], steroids and triterpenoids [22, 23], Tannins [24, 25], phenolics [26, 27], coumarins [28, 29] and anthraquinone glycosides [30] using standard procedures.

Estimation of Flavonoid Content (by $AlCl_3$ Method) [31]:

Preparation of Extracts: 1g of air-dried powder of aerial parts of *H. cordata* Thunb. was extracted with 25 ml of 95% ethanol by maceration for 24 h and filtered. The final volume of the filtrate was adjusted to 25 ml using Ethanol.

Method: To 0.5 ml of the ethanolic extract, 1.5 ml of 95% Ethanol and 0.1 ml of 10% $AlCl_3$ was added After 30 min., the absorbance read at 415 nm. Results were expressed in g/100g of dry matter with respect to Rutin serves as a standard.

TLC and HPTLC Study of Plant Extract for Estimation of Quercitrin: Based on earlier reports on quercitrin's presence in aerial parts of *H. cordata* Thunb. TLC was developed using Ethyl acetate: Toluene: Glacial acetic acid: Formic acid (4: 2: 1: 0.2) as mobile phase and Silica Gel G60 F254, as a stationary phase. Derivatization was done by using 1% ethanolic solution of $AlCl_3$ and heating at 110 °C for 10 min.

Estimation of Quercitrin of Alcoholic Extract of *Houttuynia cordata* Thunb:

Chemicals and Instruments: Precoated silica gel G60 F254 plate (methanol washed) (E. Merck) Ethyl acetate, Toluene, Formic acid, Acetic acid (AR grade) Standard quercitrin.

Camag Linomat V (semiautomatic spotting device) Hamilton 100µl HPTLC syringe Camag twin-trough chamber Camag TLC Scanner 3, Camag WINCATS integration software Camag Reprostar-3.

Standard Preparation and Calibration Curve of Quercitrin: Accurately weighed 3 mg of quercitrin (Laboratory repository) dissolved in 1 ml of methanol in a volumetric flask (3 mg/ml). A solution of 3 mg/ml of was prepared in methanol. Graded concentration of solution of standard quercitrin (3 mg/ml) in 2, 4, 6, 8, 10 µl volume were spotted on precoated TLC silica gel 60G F254 plate (E. Merck) with Camag Linomat V automatic spotter. The concentration of quercitrin was 6, 12, 18, 24, 30 µg/spot respectively. The plate was developed in Camag twin-trough chamber presaturated with mobile phase Ethyl acetate: Toluene: Glacial Acetic acid: Formic acid (4: 2: 1: 0.2). After development the plate was dried and derivatized with 1% Ethanolic solution of AlCl3 and heated at 110°C for 10 min. The plate was scanned at 254 nm and 366 nm, data of peak area of each quercitrin spot was recorded. A calibration curve was obtained by plotting mean peak area vs. concentration of each peak corresponding to the respective spot.

Preparation of Extract: Powdered plant material (5 gm) was exhaustively extracted with methanol (5X20 ml). 10 mg of the methanolic residue was washed with chloroform until green colour chlorophyll disappears. The residue left after washing dissolved in 5ml of ethyl acetate to obtain methanolic fraction. 10, 20 and 30 µl of this was spotted on the plate and after developing in the mobile phase Ethyl acetate: Toluene: Glacial Acetic acid: Formic acid (4: 2: 1: 0.2) was scanned at 254 nm and 366 nm. The later one was scanned after derivatized with 1% Ethanolic solution of $AlCl_3$ using the Camag Scanner 3. Peak area noted and concentration of quercitrin in the sample was calculated using standard curve.

Experimental Conditions: The plates were prewashed by methanol and activated at 110 ºC for 5 min prior to chromatography. The standard solutions of Quercitrin and the alcoholic extract of aerial part of *Houttuynia cordata* Thunb were spotted using Camag Linomat V sample applicator. HPTLC Fingerprinting was performed as follows.

Stationary Phase: TLC Aluminium sheets pre-coated with silica gel G60 F254, thickness 0.2mm, (20 x 20cm) (E Merck).

Mobile Phase: Ethyl acetate: Toluene: Glacial Acetic acid: Formic acid (4: 2: 1: 0.2)

Chamber Saturation: 30 min.

Temperature: 25 ± 2°C

Slit Dimension: 6 × 0.45 mm.

Chamber: Camag flat bottom and twin–trough developing chamber.

Separation Technique: Ascending

Migration Distance: 7.5 cm

Detection: Scanned at 254 nm and 366 nm after derivatized with 1% Ethanolic solution of $AlCl_3$

RESULTS:

Macroscopical and Microscopical Study:

Macroscopy of the Selected Plant: Fig. 1 Leaf is simple, alternate, petiolate and broadly ovate to cordate 5 nerved. Surface is glabrous and gland dotted. Petioles are 1-2 in. long and winged. Stipule is present. Stems are angular, 1.5-2.4 in. long. They are occasionally branched.

The outer surface is rough, yellowish brown with clearly visible longitudinal ridges Odour is not characteristic and taste is bitter. Dark brownish green coloured powder with characteristic odour and bitter taste.

FIG. 1: *H. CORDATA* THUNB., HERB WITH STEMS AND FLOWER

Powder Study: Fig. 2 Dark brownish green coloured powder with characteristic odour and bitter taste. It shows blunt-ended multicellular trichome and anomocytic stomata in surface view **Fig. 2A**, lignified fibres **Fig. 2B**, spiral xylem vessels **Fig. 2C**, pitted xylem vessels **Fig. 2D** striated cuticle **Fig. 2F** and anther head **Fig. 2J**. Other characters like fragments of multicellular trichomes with pointed end **Fig. 2f** and cluster crystals of calcium oxalate are scattered as such throughout or embedded to the parenchymatous cells **Fig. 2G**. Secretory canal in surface view **Fig. 2H** and fragments of anther **Fig. 2I** were also seen in powder study.

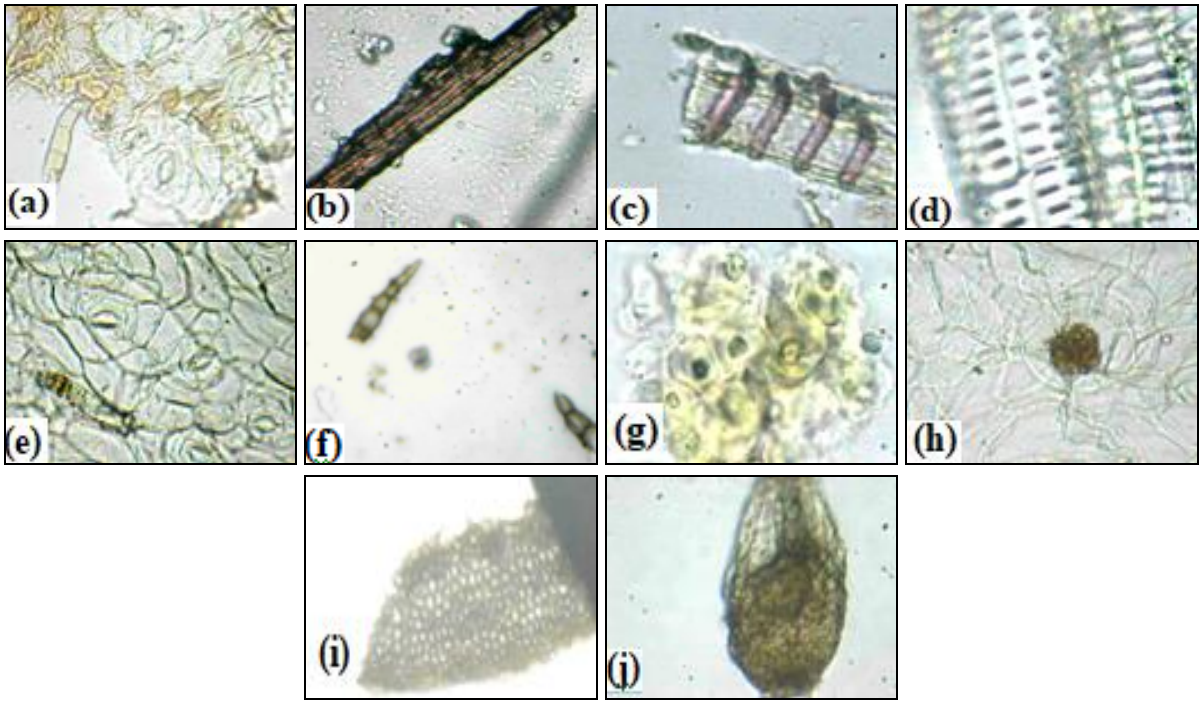

FIG. 2: (A) RANANCULACEOUS STOMATA AND BLUNT ENDED TRICHOME(10X), (B) GROUP OF LIGNIFIED XYLEM FIBRES(10X), (C) SPIRAL VESSEL(45X), (D) PITTED XYLEM VESSEL(45X), (E) STRIATED CUTICLE(10X), (F) MULTICELLULAR TRICHOME WITH POINTED END(4X), (G) CLUSTER CRYSTALS OF CA- OXALATE(45X), (H) SECRETORY CANAL IN SURFACE VIEW(10X) (I) FRAGMENT OF ANTHER(10X) (J) ANTHER HEAD(10X)

TABLE 1: PHYSICO-CHEMICAL PARAMETERS

Sr. no.	Physicochemical parameters	Aerial parts of h. Cordata thunb. %w/w
1	**Ash Values**	
	Total ash	8.65 ± 0.288
	Acid insoluble ash	8.01 ± 0.176
	Water-soluble ash	3.41 ± 0.0850
2	**Extractive values**	
	Alcohol soluble extractive	5.33 ± 0.462
	Water soluble extractive	15.86 ± 0.611

Phytochemical Screening: The powder of aerial parts of *Houttuynia cordata* Thunb. was subjected to chemical tests to check the presence of various phytoconstituents like, alkaloids, flavonoids, saponins, carbohydrates, steroids, triterpenoids, tannins, phenolics, coumarins, and anthraquinones.

The results of the phytochemical screening are described in **Table 2.**

TABLE 2: PHYTO-CHEMICAL SCREENING

Phytoconstituents	Aerial parts of *H. cordata* Thunb.
Carbohydrates	+
Anthraquinone Glycosides	-
Steroids	+
Saponin Glycosides	-
Coumarins	-
Flavonoids	+
Tannins	+
Phenolics	+
Alkaloid	+

Where, (+) = present, (-) = absent.

TABLE 3: ESTIMATION OF FLAVONOID CONTENT

S. no.	Constituents	Extract % w/w
1.	Flavonoids	2.74-3.85

TABLE 4: ESTIMATION OF QUERCITRIN IN H. CORDATA THUNB.

Sample	Mean peak area (n=4)	Average amount of quercitrin (µg/spot)	Average %w/w of quercitrin ± SD.	% C.V.
H. cordata	10,903	21	0.8 ± 0.021	4.3

TABLE 5: CALIBRATION DATA OF STANDARD QUERCITRIN CONCENTRATION VS. MEAN PEAK AREA

Sr. no.	Concentration of quercitrin (µg/spot)	Mean peak area ± S.D. (n=5)	% C.V.
1	6	3627.6 ± 63.53	1.46
2	12	7277.4 ± 62.16	1.76
3	18	10197.5 ± 68.71	2.74
4	24	12934.9 ± 89.66	3.03
5	30	13536.6 ± 72.11	2.39

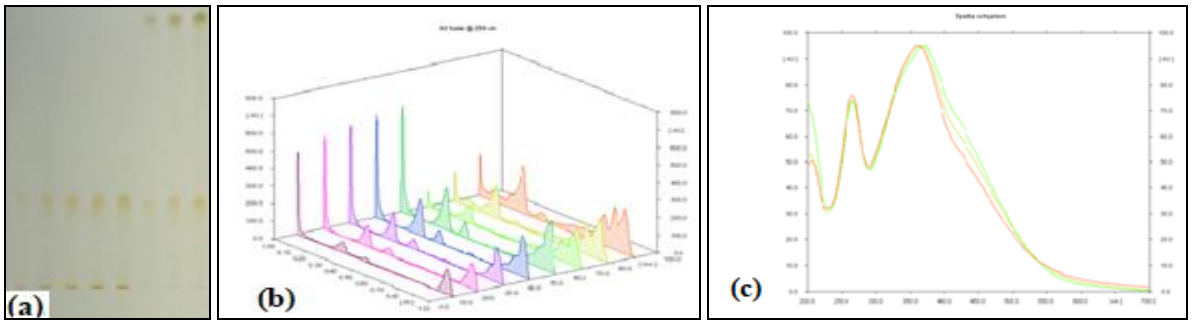

FIG. 3: (A) HPTLC CHROMATOGRAM OF QUERCITRIN SCANNED AT 254nm, (B). DENSITOMETRIC CHROMATOGRAM AT 254nm, (C). OVERLAY U.V. SPECTRA

CONCLUSION: In the present study, the phytopharmacognostical evaluation of the aerial parts of *H. cordata* Thunb. was performed. The pharmacognostical study of medicinal plants is very important as it gives the parameters for the standardization and authentication of the medicinal plant. Organoleptic evaluation and macroscopical description are the simplest and quickest methods to establish the identity and quality of a medicinal plant. The microscopical study of any plant part can be used to identify the distinguishing characteristics of the plant. These parameters can be used to identify and prevent adulteration and substitution of medicinal plants [32]. In the present study the organoleptic and the macroscopical and microscopical characters of the tubers of *H. cordata* Thunb. were studied. The physicochemical analysis of the aerial parts of *H. cordata* Thunb. was performed where the parameters such as ash values and extractive values were performed. Ash

values are used to determine the quality and purity of crude drug. It indicates the presence of various impurities like carbonate, oxalate and silicate. The water-soluble ash is used to estimate the amount of inorganic compounds present in drugs. The acid insoluble ash consists mainly silica and indicate contamination with earthy material. Estimating extractive values determines the amount of active constituents in a given amount of plant material when extracted with a particular solvent [32]. Moreover, the phytochemical analysis of the aerial parts of *H. cordata* Thunb. They indicated the presence of carbohydrates, alkaloids, flavonoids, steroids, triterpenoids, *etc*. The results of the HPTLC fingerprinting confirmed the presence of the flavonoids like Quercitrin. The phyto-pharmacognostical study of aerial parts of *H. cordata* Thunb. would help carry out further research and explore its therapeutic potential.

ACKNOWLEDGEMENTS: Authors are thankful to the Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad, Gujarat, India.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Patwardhan B, Vaidya ADB and Chorghade M: Ayurveda and natural products drug dis-covery. Current Science 2004; 86(6): 789-99.
2. Handa SS, Katiyar CK and Sood OP: Herbal drugs-Perspective in the New Millenium, Ranbaxy science foundation, New Delhi, India 2006: (15): 45.
3. Bailey LH: The Standard cyclopedia of Horticulture the Mcmillian Company, Ne-wyork. 1959; 1: 117.
4. Mukherjee SK: College Botany, New central book agency, Kolkata, India 2002; 3:122.
5. Metcalf CR and Chalk L: Anatomy of the dicotyledons: leaves, stems and wood in relation to taxonomy with notes on economic uses oxford at the clarenion press 1950; 2: 1127-1128.
6. Bailey LH: The Standard cyclopedia of Horticulture. The Mcmillian Company Ne-wyork 1959; 1: 23.
7. Nishiya H: Chem Pharm Bull 1988; (36): 1902.
8. Anonymous: *Houttuynia cordata* Thunb. The Wealth of India: Raw Materials, Council of Scientific and Industrial Research New Delhi 1959; 5: 123.
9. Kanjilal UN, Das A, Kanjilal PC and DE RN: Flora of Assam. A von Book Company Delhi 1982; 4: 30-31.
10. Brahmananda P and Ananta B: Wild edible palnt of Assam Geetakhi Printers & Pub-lishers, Zoo-Road Tiniali, Guwahati 2007; 119-120.
11. Bailey LH: The Standard Cyclopedia of Horticulture. The Mcmillian Company, Ne-wyork 1959; 2: 1611.
12. Chopra RN and Verma BS: Supplement to glossary of Indian Medicinal plant, New Delhi CSIR 1956; 41.
13. Duthie JF: Flora of the Upper Gangetic Plain Bishen Singh, Mahendra Pal Singh. De-haradun Plambaginaceae to Juneaceae 1973; (2-3): 45-46.
14. Anonymous WHO Guidelines 1st ed. Delhi, A.I.T.B.S. Publishers and distributors 2002; 40-43.
15. Khandelwal KR: Practical Pharmacognosy. Technique and Experiments. 9th ed. Nirali Prakashan 2008; 65.
16. Geissman A: Modern Methods of Plant Analysis. Vol III. Peach K and Tracy MV eds. Heidelberg, Berlin, Springer Verlag 1955; 471.
17. List PH and Horhammer L: Hager Hand buch der pharmazeutischem praxis. Berlin, Springer Verlag Band 1967; 1: 256.
18. Geissman A: Morden methods of plant analysis. Peach K, Tracey MV, editors. Sprin-ger Verlang Vol.3. Berlin, Gottingen, Heidelberg 1955; 473.
19. Fishcher R: Praktikum der Pharmakognosic. 3rd ed. Berlin, Springer Verlag 1952; 362.
20. Evans WC and Evans D: Trease and Evan's Pharmacognosy. 15th Ed London, WB Saunders Company Ltd 2002; 193.
21. Wilson JA and Merill HB: Analysis of Leather and Material used in making it, 1st ed. New York, The Mcgraw Hill Book Co. Inc 1931; 290-293.
22. Freudenberg K and Weinger K: The Chemistry of Flavonoid Compounds. Geissman A eds. Oxford, Pregamon Press 1962; 211.
23. Robinson T: The Organic Constituents of Higher Plants, their Chemistry and Interre-lationships. Minneapalis 15 Minn., Burgers publishing company 1964; 64.
24. Clerk JD, Descamps A and Vander Meersch E: Colorimetric Method for Determining Tannin. Bulletin Assocaition Anciens etud. Brass, University Louvain, 1947; 43: 68-76.
25. Harborne JB: Phytochemical Methods. 2nd ed. London, Champan & Hall Ltd 1973; 42.
26. Feigl F: Identification of individual organic compound. In: Spot Tests in Organic Analysis, 4th ed. London, Elsevier Publishing Company 1956; 237.
27. Feigle F: Identification of individual organic compound In: Spot Tests in Organic Analysis. 4th ed. London, Elsevier Publishing Company 1956; 419-421.
28. Kokate CK, Purohit AP and Gokhale SB: Pharmacognosy, 12th ed. Nirali Prakashan 1999; 145-155.
29. Anonymous: WHO Guidelines, 1st ed. Delhi, A.I.T.B.S. Publishers and Distributors 2002; 45-46.
30. List PH and Horhammer L: Hager Hand Buch der Pharmazeutischem Praxis. Berlin, Springer Verlag Band 1967; 1: 447.
31. Baharam T, Gressier B, Trotin F, Brunet C, Dine T and Pinkash M: Oxygen spicies scavenging activity of phenolic exctracts from Hawthornfresh plant organsand pharma preparations. Drug Res 1996; 46: 1086-89.
32. Chanda S: Importance of pharmacognostic study of medicinal plants: An overview. JPP 2014; 2(5).

How to cite this article:
Modi A and Shah M: Phytopharmacognostical study of aerial parts of *Houttuynia cordata* Thunb. Int J Pharm Sci & Res 2023; 14(7): 3533-38. doi: 10.13040/IJPSR.0975-8232.14(7).3533-38.