



Phytopharmacognostical Evaluation of Leaf of *Cocculus Hirsutus*

Jethva Khushboo, Bhatt Dhara, Dhru Bhavita, Patel Sonal, Zaveri Maitreyi

K.B. Institute of Pharmaceutical Education and Research, Sector-23, GH-6, Gandhinagar, Gujarat, India.

Department of Pharmacognosy and Phytochemistry, Kadi Sarva Vishvavidyalaya, Sector-23, GH-6, Gandhinagar, Gujarat, India.

*Corresponding author's E-mail: khushi_198984@yahoo.com

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ABSTRACT

Ethanomedicinal plant *Cocculus hirsutus* belonging to the family *Menispermaceae* commonly known as Vevdi or vevati in Gujarat, India. Pharmacognostical evaluation including examination of morphological and microscopical characters, determination of quality control parameters such as ash values, extractive values, moisture content, and foreign matter were carried out. Phytochemical screening including qualitative chemical examinations was also carried out. Hence, the present attempt was undertaken to investigate the Phytopharmacognostical studies of leaf of *Cocculus hirsutus*. The study revealed the presence lamina and midrib regions. Surface of leaf consists of long and short unicellular trichomes. Anomocytic stomatas were found on lower epidermis only. Midrib exhibited crescent (semi-circular) shaped vascular bundle enclosed by sclerenchymatous bundle sheath. Phytochemical screening of the *Cocculus hirsutus* showed the presence of phytoconstituents like flavonoids, phenolics, saponins and steroids.

Keywords: *Cocculus hirsutus*, pharmacognostical screening, Phytochemical evaluation, vevdi

INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plant, animal and minerals have been the basis of the treatment of human disease. Today estimate that about 80% of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day.^{1,2}

The use of herbal medicine is becoming popular due to toxicity and side effects of allopathic medicines. This led to sudden increase in the number of herbal drug manufactures. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health³. Currently 80 % of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects. Plants are important sources of medicines and presently about 25 % of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources. Plants should be investigated to better understand their properties, safety and efficacy.

Ethanomedicinally, one of the important plants such as *Cocculus hirsutus* used in many diseases in tribal area. *Cocculus hirsutus* known as chilahinta in Ayurveda and

kattu kadi in Siddha system is important medicinal plant belonging to family *Menispermaceae* commonly known as Vevadi consists of 20 different species of mostly climber or shrubs, which are distributed all over tropics and subtropical regions⁴. In Sind, leaves are used in headache and neuralgic pains (Murray). The juice of the leaves, mixed with water, has the property of coagulation into a green jelly-like substance, which is taken internally, sweetened with sugar, as cure for gonorrhoea^{5,6}. The leaves and root of *Cocculus hirsutus* are used in tuberculosis⁷.

In Baluchistan the mucilage is used to cure spermatorrhoea, taken in milk; it is used for coughs and to put on to sore eyelids and to soften breasts. The leaf is used to treat polyuria, eczema, dysuria, abdominal disorders, rheumatoid arthritis, fevers, piles, syphilis, and disorders of blood and as an aphrodisiac, gonorrhoea, prurigo, impetigo, cough, ophthalmia, cephalalgia and neuralgia^{8,9}.

The present study was undertaken to evaluate the Phytopharmacognostical study of leaf of *Cocculus hirsutus*.

MATERIALS AND METHODS

Collection and authentication of plant material

Leaf of *Cocculus hirsutus* were collected from Rajkot district, Gujarat, India in the month of June 2013, when it fully grown with flowering. Total 1500 gm of leaves were collected to get 1000gm of dry powder. The procured material of *Cocculus hirsutus* was authenticated by taxonomist. Plant having Herbarium specimen number PH/013/001 was deposited at Pharmacognosy Department, K.B.I.P.E.R., Gandhinagar, Gujarat, India for

future reference. The leaves were dried under shade and reduced mechanically to moderate coarse powder. The coarse powders were analyzed for following Pharmacognostic parameters.

Pharmacognostical study of leaf of *Cocculus hirsutus*

Macroscopy

Morphological study of leaf of *Cocculus hirsutus* such as color, size, odor, taste, surface characteristic and fracture were examined.

Microscopy

For microscopical study free hand section of the leaf of *Cocculus hirsutus* were taken¹⁰, cleared with chloral hydrate solution with gentle warming. A drop of concentrated hydrochloric acid and phloroglucinol was used to detect the lignified cells in the cross sections and in the powder drugs. The cross sections and powder were mounted on slide in glycerin and studied under microscope. Photomicrographs were shot for histological observation (Labomed).¹¹

Powder study of leaf of *Cocculus hirsutus*

For powder study very little amount of powder of leaf of *Cocculus hirsutus* were taken on the glass slide. The lignified elements were visualized by staining the section with a drop of hydrochloric acid and phloroglucinol.

Quantitative Evaluation of the leaf powder

Physicochemical parameters of leaf of *Cocculus hirsutus*

The moisture content of the powdered material was determined by the loss on drying method. The total ash value, the acid insoluble ash value and the water soluble ash value were determined.^{12,13}

The extractive values (Petroleum ether, toluene, chloroform, ethyl acetate, n-Butanol, ethanol and water) of leaf powder were also determined. Average of three determinations for each procedure was calculated.

Preparation of extracts

20 gm of leaf powder of *Cocculus hirsutus* were taken to prepare its different extracts. Ethanolic and (70%) Hydro-alcoholic extracts were prepared by maceration of leaf powder of *Cocculus hirsutus* for 48 hours. Solvents were removed by Rota evaporator. Percentage yield were calculated.

Phytochemical screening of leaf of *Cocculus hirsutus*

The preliminary phytochemical screening¹⁴ of *Cocculus hirsutus* leaf extracts were carried out.

The freshly prepared leaf extracts of *Cocculus hirsutus* were quantitatively and qualitatively tested for the presence of chemical constituents (alkaloid, flavonoids, glycosides, steroids, terpanoids, carbohydrates, saponins, etc.) and these constituents were identified by characteristic color changes as per standard procedure.

Total Flavonoid content from the different extracts of leaf of *Cocculus hirsutus*

The total flavonoid content was determined using AlCl₃ method; 5 ml of 2 % Aluminium trichloride (AlCl₃) in methanol was mixed with the same volume of the extract solution of leaf of *Cocculus hirsutus* (0.4 mg/ml). Absorbance was measured at 415 nm using UV-VIS spectrophotometer after 10 min against methanol as a blank sample consisting of a 5 ml extract solution with 5 ml methanol without AlCl₃. The total flavonoid content was determined using a standard curve with quercetine (0-100 µg/ml). Quercetine was used as a standard. Total flavonoid content is expressed as mg of quercetine equivalents (CE)/g of extract¹⁵.

Total Phenolic content from the different extracts of leaf of *Cocculus hirsutus*

Total soluble phenolics of the fractions were determined with Folin Ciocalteu reagent using gallic acid as a standard, following the method. One ml of extract (500µg/ml) solution in a test tube was added to 0.2 ml of Folin Ciocalteu reagent (1:2 in distilled water) and after 20 min 2.0 ml of purified water and 1.0 ml of sodium carbonate (15 %) was added. Allowed to react for 30 m-in and then absorbance was measured at 765 nm. The concentration of total phenolic content was determined as a microgram of gallic acid equivalent¹⁶.

RESULTS

Morphology and Microscopical studies of leaf of *Cocculus hirsutus*

Macroscopic Characters

Morphological evaluation of Leaf of *Cocculus hirsutus* are green in color, odourless, mucilaginous when fresh, brittle and powdery on drying, with characteristic taste. Leaf of *Cocculus hirsutus* is dorsiventral, variable, and simple, shape is ovate- oblong or slightly lanceolate with truncate to cordate base, apex is mucronate, margins are entire or slightly wavy, lamina is hairy, venation is reticulate with 5-6 pairs of alternating lateral veins as shown in Figure 1 and 2. Organoleptic characters of the leaf powder are a pale green in color, course with characteristics taste and odour.

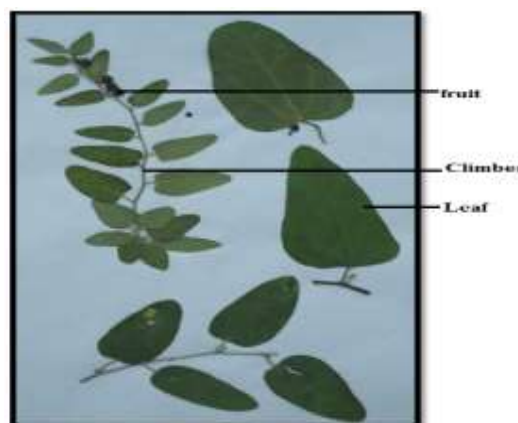


Figure 1: Climber and leaf of *Cocculus hirsutus*

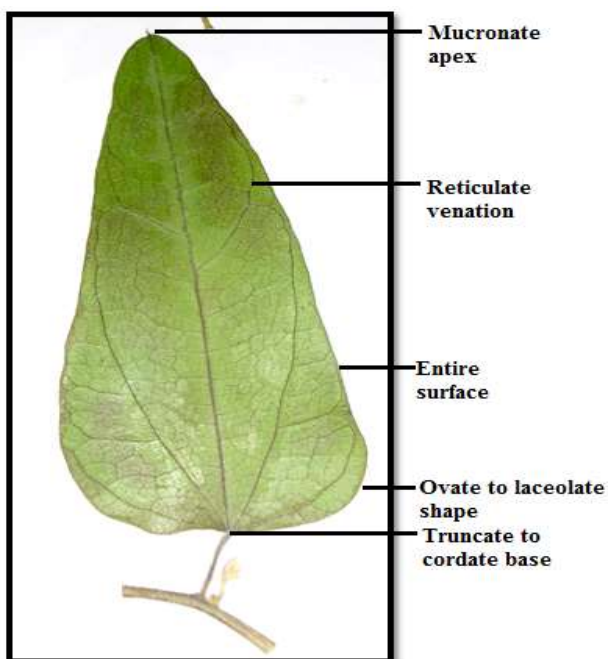


Figure 2: Morphology of leaf of *Cocculus hirsutus*

Microscopical study

Macroscopical and organoleptic characters of leaf of *Cocculus hirsutus* were usually not sufficient to enable the drug to be identified.

Therefore, authentication of leaf of *Cocculus hirsutus* was further confirmed by the microscopical studies of the plant material, which consists in an investigation of the natural distribution and relationship between various tissues and tissue components comprising the organ under study.

The microscopy studies were carried out to authenticate the leaf of *Cocculus hirsutus*.

Transverse section of leaf of *Cocculus hirsutus* (figure 3) consisting the lamina and midrib regions. Lamina showed the presence of upper and lower epidermis. Surface of leaf consist of unicellular trichomes which were long and short and upper epidermis where stomata are absent (figure 4).

Epidermal cells of upper epidermis were rectangular filled with chloroplast. Mesophyll comprises of palisade cells and spongy parenchyma. Parenchymatous cells contain sparingly distributed starch grains.

Palisade cells was one layered except near midrib where it is 2-3 layered, cells were elongated thin walled and enclose air spaces in between and excretory sacs.

Midrib exhibited crescent (semi-circular) shaped vascular bundle (figure 5) enclosed by sclerenchymatous bundle sheath.

After sclerenchymatous bundle sheath, two bundle sheath lies in parenchymatous ground tissue. Anomocytic stomata found only on lower epidermis (figure 6), they were many in numbers, sunken and each surrounded by 4-6 epidermal cells.

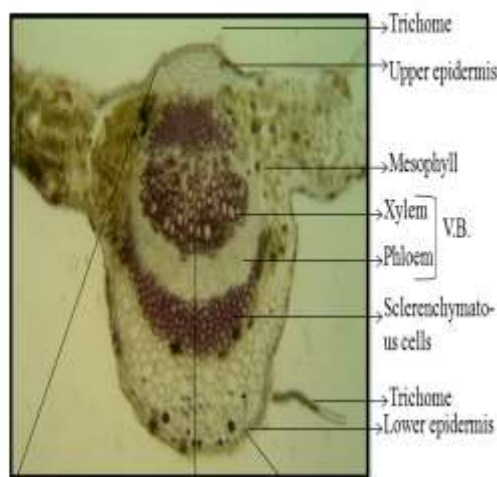


Figure 3: Transverse section of leaf of *Cocculus hirsutus*



Figure 4: Upper epidermis



Figure 5: Vascular bundle

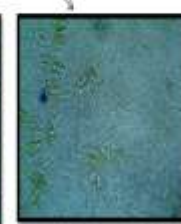


Figure 6: Lower epidermis

Powder microscopy of leaf of Cocculus hirsutus

Powder study of leaf of *Cocculus hirsutus* shows the presence of anomocytic stomata (Figure 7a), pitted xylem vessel (Figure 7b) and long and short unicellular trichome (Figure 7c).



Figure 7a: Anomocytic stomata of leaf powder of *Cocculus hirsutus*

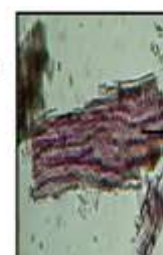


Figure 7b: Pitted xylem vessel of leaf powder of *Cocculus hirsutus*



Figure 7c: Trichome of leaf powder of *Cocculus hirsutus*

Figure 7: Powder study of leaf powder of *Cocculus hirsutus*

Physicochemical parameters of Cocculus hirsutus

The leaf of *Cocculus hirsutus* is evaluating for its physicochemical parameters such as ash value and moisture content are reported in table-1.

Table 1: Physicochemical parameters of *Cocculus hirsutus*

Parameters	% w/w Leaf
Total ash	12.40
Acid insoluble ash	00.50
Water soluble ash	06.90
Moisture content	01.50

Extractive value of leaf powder of *Cocculus hirsutus*

The results of extractive values of leaf of *Cocculus hirsutus* in different solvents are reported in table 2.

Table 2: Extractive values of leaf of *Cocculus hirsutus* in different solvents

S. No.	Name of different solvent	Colour of extract of leaf powder	Extractive value of leaf (% w/w)
1	Petroleum ether	Light green	02.50
2	Toluene	Green	04.00
3	Chloroform	Brownish green	02.00
4	Ethyl acetate	Dark green	04.37
5	n-Butanol	Dark green	04.00
6	Ethanol	Dark green	14.00
7	Water	Green	30.00

Phytochemical screening of *Cocculus hirsutus*

Preliminary Phytochemical screening of leaf of *Cocculus hirsutus* showed the presence of phytoconstituents like flavonoids, phenolics, saponins and steroids. They were showed in table 3.

Table 3: Phytochemical screening of different extracts of leaf powder of *Cocculus hirsutus*

Phyto-constituents	Different extracts						
	Petroleum ether (dark green)	Toluene (dark green)	Chloroform (greenish)	Ethyl acetate (greenish yellow)	n-butanol (light green)	Ethanol (dark green)	Water (light green)
Terpenoids	+	+	-	-	-	-	-
Steroids	+	+	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-
Caumarins	-	-	-	-	-	-	-
Flavonoids	-	-	-	+	-	+	+
Anthraquinones	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	+	+
Carbohydrates	-	-	-	-	+	+	+
Proteins	-	-	-	-	-	-	-
Phenolics	-	-	-	+	+	+	+

+ = Present, - = Absent

Total Flavonoid content from the different extracts of leaf of *Cocculus hirsutus*

Total Flavonoid content from the different extracts of leaf of *Cocculus hirsutus* are shown in Table no. 4

Table 4: Flavonoid content in leaf extracts of *Cocculus hirsutus*

Extract	% Flavonoids (w/w)
Ethanolic Leaf extract	0.0172
Hydro-alcoholic Leaf extract	0.618

Total Phenolic content from the different extracts of leaf of *Cocculus hirsutus*

Total Phenolic content from the different extracts of leaf of *Cocculus hirsutus* are shown in Table no. 5

Table 5: Phenolics content in leaf extracts of *Cocculus hirsutus*

Extract	% phenolics (w/w)
Ethanollic Leaf extract	0.605
Hydro-alcoholic Leaf extract	0.678

DISCUSSION

Any medicinal plant requires detailed study prior to its use because the therapeutic efficacy is absolutely dependent on the quality of the plant material used. The original and basic approach towards pharmacognosy includes study of morphological system, study of the cell structures and organization and study of tissue system, which still holds a key in the identification of the correct species of the plant and also to help us to differentiate between closely related species of the same genus. It is also first step to standardize a drug, which is the need of the day.

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus in recent years there has been an emphasis standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identifications evaluation of plant drugs by Pharmacognostic studies is still more reliable, accurate and in expensive means. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its Identity and purity and should be carried out before any tests are undertaken.

Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. The organoleptic or macroscopic studies yielded important characteristics, such as the fractured surfaces of fresh and dried leaf, typical tongue sensitizing aromatic taste and aromatic and characteristic odour of the leaf which are useful diagnostic characters.

This is of great interest for quality control in basic research and drug production, especially for imported items and for raw materials sold by traditional herbalists.

The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacteria, fungi or yeast growth. Equally important in the evaluation of crude drugs, is the ash value. The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude

drug or adhering to it or deliberately added to it, as a form of adulteration. The ash value was determined by three different methods, which measured total ash, acid-insoluble ash, and water-soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition. This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non physiological ash', which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially ash and siliceous earth. Water-soluble ash is the water soluble portion of the total ash. These ash values are important quantitative standards.

Extractive values help us in determining the amount of active constituents and is done on plant materials for which as yet no suitable chemical or biological assay exists. The result of physicochemical study; ash value, extractive value and moisture content were complies in its limit.

The plant material was subjected to preliminary photochemical screening involving successive solvent extraction by different solvents in order of increasing polarity to obtain diverse polar and non polar phytoconstituents possessing different solubility pattern, followed by various chemical constituents. The percent extractives in different solvents indicate the quantity and nature of constituents in the extract. Preliminary Phytochemical screening of leaf of *Cocculus hirsutus* showed the presence of phytoconstituents like flavonoids, phenolics, saponins and steroids and terpenoids. The phytoconstituents quantified in the present study exhibit great deal of medicinal importance like terpenoids acts as anti-bacterial and antineoplastic, anti-inflammatory and anti-oxidants activity. In the plant *Cocculus hirsutus* was consisting mainly steroids, terpenoids, phenolics, flavonoids and saponin. Presence of phenolic components in the leaf of *Cocculus hirsutus* may act as an antioxidant, anti microbial activity. Many flavonoids are shown to have antioxidant, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, and anti-inflammatory, anti cancer, anti arthritis while some flavonoids exhibit potential antiviral activities. The quantified values of the above phytoconstituents can be used as a major tool for obtaining a quality control profile for a drug.

Total Flavonoid content of ethanolic extract of leaf and hydro alcoholic extracts of leaf of *Cocculus hirsutus* were 0.0172 and 0.618 respectively and Total Phenolic content of ethanolic extract of leaf and hydro-alcoholic extract of leaf were 0.605 and 0.678 respectively. Hydro-alcoholic extract of leaf of *Cocculus hirsutus* containing higher amount of flavonoid and phenolic content, so might be the hydro-alcoholic extract of leaf of *Cocculus hirsutus* having more medicinal importance.

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