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# Pharmacognostical and phytochemical analysis of leaf, stem and root of *Cocculus hirsutus*

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**Abstract**
Ethanomedicinal plant *Cocculus hirsutus* belonging to the family Menispermeace commonly known as Vevdi or vevati in Gujarat, India. Leaf, stem and root of *Cocculus hirsutus* were used for so many diseases as mentioned in any ancient literature. Pharmacognostical study of *Cocculus hirsutus* including the macroscopy and microscopy of leaf, stem and root of *Cocculus hirsutus.* Microscopical study of leaf, stem and root of *Cocculus hirsutus* were done by taking transverse section and examine powder microscopy. The study revealed the presence lamina and midrib regions in the transverse section of leaf. Surface of leaf consists of long and short unicellular trichomes with anomocytic stomata were found on lower epidermis only. Large amounts of sclerenchyma were present abaxial position, relative to protoxylem in the transverse section of stem. Transverse section of root of Cocculus *hirsutus* ceretenchymatous cells which is the main identifying character of this plant. As all the parts of plants contains different secondary metabolites having different activity, it will be further used for the treatment and management of different diseases.

**Keywords:** *Cocculus hirsutus*, leaf, stem, root, Pharmacognostical stuedy, vevdi

## 1. 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].
*Cocculus hirsutus* Linn. Belonging to family *Menispermaceae* consists of 20 different species which are mostly scandent or rarely sub erect herbs or climber, distributed in tropics and subtropics regions. *Cocculus hirsutus* Diels is a scandent shrub, known as Chilahinta in Ayurveda and Kattu kodi in Siddha system of medicine. The plant grows all over India, especially in dry regions. In India, it is known by various names in different regions viz, Vevati in Gujarat, Kattukkodi in Tamilnadu and Vasanvel at some places. Leaves are simple, alternate, ovate, sub deltoid or three lobed, obtuse and mucronate. The base of leaf is subcordate or truncate. Petioles are very short, dark green, usually subauriculate at the base. The fruit is a drupe which is size of small pea with dark purple endocarp. The flowers are very small, unisexual and green. The male flowers occur in axillary cymose panicles, stamens are six, free, embraced by the petals, anthers are subglobose in shape. Female flowers are in axillary clusters of 1-3 and staminooids are six and styles usually cylindrical. The plant is a climber with green flowers bloom in February-March and fruits in May-June. Seeds arecurved and fleshy with annular embryo. Roots are hairy and dark brown in colour [3, 5].
The roots and leaves are used to treat polyuria, eczema, dysuria, abdominal disorders, rheumatoid arthritis, fevers, piles, syphilis, disorders of blood and as an aphrodisiac. Roots and leaves possess antimicrobial, cardiotonic, hyperglycaemic, diuretic, laxative and epileptic activity. 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 gonorrhea [6, 7]. 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 root is also used as antipyretic, tonic; lessens thirst; good in fracture; good for tubercular glands when mixed with sesame oil (Yunani). A decoction of fresh roots, with a few heads of pepper, in a goat's milk is administered for rheumatic and old venereal pains; half a pint every morning is the dose. It is reckoned heating, laxative and sudorific. In the konkan, the roots rubbed with Bonduc nuts, are administered as a cure for belly-ache in children, in bilious dyspepsia, they are given in 6 massa doses, with ginger and sugar [8].
The present study was undertaken to evaluate the Pharmacognostical study of leaf, stem and root of *Cocculus hirsutus*.

## 2. Materials and Methods
### 2.1 Collection and authentication of leaf, stem and root of *Cocculus hirsutus*
Fresh plant material was collected from widely grown plant *Cocculus hirsutus* from Dhandhiya village of Rajkot district, Gujarat, India. Raw material was subjected to washing with distilled water and then allowed for drying for 5 days under shade and powdered to 60# separately and stored in well close container. The procured material of *Cocculus hirsutus* was authenticated by taxonomist

### 2.2 Pharmacognostical study of *Cocculus hirsutus*
#### 2.2.1 Macroscopy of leaf, stem and root of *Cocculus hirsutus*
Leaf, stem and root of *Cocculus hirsutus* was taken and morphological evaluation was done by evaluating way like size, shape, taste, odour, colour, and features like touch, texture, etc.

**2.2.2 Microscopical studies of leaf, stem and root of *Cocculus hirsutus* [9, 10]**

For microscopical studies free hand section of the leaf, stem and root of *Cocculus hirsutus* were taken, cleared with chloral hydrate solution and studied. The lignified elements were visualized by staining the section with a drop of hydrochloric acid and phloroglucinol in the cut sections. Macerates were prepared by the Schulz maceration method Photomicrographs were shot for histological observation (Labomed).

**2.2.3 Powder study of leaf, stem and root of *Cocculus hirsutus:***

For powder study very little amount of drug powder of leaf, stem and root 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 in the cut sections.

**2.2.4 Physicochemical parameters of *Cocculus hirsutus* [11, 12]:**

The freshly prepared leaf, stem and root extracts of *Cocculus hirsutus* were prepared by maceration method using different solvents. Physicochemical studies of leaf, stem and root of *Cocculus hirsutus* were carried out as per by using standard methods of pharmacopiea.

**2.2.4.1 Determination of ash values**

The ash remaining following ignition of medicinal plant materials was determined by three different methods which measures total ash, acid insoluble ash and water-soluble ash.

**2.2.4.1.1 Determination of total ash**

Four crucibles were taken, cleaned and make it air dry and weigh. Place two grams of powder of leaf, stem and root of *Cocculus hirsutus* was taken in all four crucibles. The material was spread in even layer in the crucible and places it in furnace and ignites it by gradually increasing the heat to 500ºC. Cool it by switching off plug and wait until temperature down up to 100 ºC. The crucibles were taken and weigh it when hot. The total ash was calculated in mg per gram of air-dried powder material.

**2.2.4.1.2 Determination of acid insoluble ash**

Two crucibles were taken, containing the total ash and 25 ml of hydrochloric acid was added in this. Beaker was covered with petridish and boiled gently for 5 min on water bath. Petridish was rinsed with 5 ml of hot water and that liquid was added in to the beaker. Two equal weight wattman filter paper were weighed. From that one filter paper was taken and filtered the solution of beaker. Wash the ash remains on the filter paper until filtrate become neutral with water. Same procedure was also done for the blank with another filter paper. Now, the filter paper containing ash was transferred in original crucible and blank filter paper was transferred in another crucible. Crucible was dried on the hot plate and placed it in to the furnace and ignites it by gradually increasing the heat to 500º C. Cool it by switching off plug and weigh until temperature down up to 100 ºC. Crucible was removed and weighed it when hot. The content of acid insoluble ash was calculated in mg per gram of air-dried powder material.

**2.2.4.1.3 Determination of water-soluble ash**

Two crucibles were taken, containing the total ash and 25ml of water was added in this. Beaker was covered with Petri dish and boiled gently for 5 minutes on water bath. Petri dish was rinsed with 5 ml of hot water and that liquid was added in to the beaker. Two equal weight wattman filter paper were weighed. From that one filter paper was taken and solution of beaker was filtered from them. The ash remaining on the filter paper was washed with water until filtrate become neutral. Same procedure was also done for the blank with another filter paper. Now, the filter paper containing ash was transferred in original crucible and blank filter paper was transferred in another crucible. Crucible was dried on the hot plate and placed it in to the furnace and ignites it by gradually increasing the heat to 500º C. Cool it by switching off plug and weigh until temperature down up to 100º C. Crucible was removed and weighed it when hot. The content of water-soluble ash was calculated in mg per gram of air-dried powder material.

**2.2.4.2 Determination of Extractive values**

This method determines amount of active constituents extracted with solvents from a given amount of medicinal plant materials. Extractive values indicate the nature of the constituents present in a crude drug.

**2.2.4.2.1 Alcohol- soluble extractive**

Accurately weighed 4 gm of air-dried powdered drug of leaf, stem and root of *Cocculus hirsutus* was macerated for 24 h with 100 ml of alcohol of the specified strength in a closed flask, shaken frequently during first 6 h and allowed to stand for 18 h. It was then filtered rapidly, taking precautions against loss of the solvent and 25 ml of the filtrate were evaporated to dryness in a tared flat-bottomed shallow dish and dried at 100º C to constant weight. The % w/w of alcohol soluble extractive value was calculated with reference to the air-dried drug.

**2.2.4.2.2 Water soluble extractive**

Accurately weighed 4 gm of air-dried powdered drug was macerated with 100 ml of water in a closed flask for 24 h, shaken frequently during first 6 h and allowed to stand for 18 h. It was then filtered rapidly and 25 ml of the filtrate were evaporated to dryness in a tared flat-bottomed shallow dish and dried at 100°C to constant weight. The % w/w of water-soluble extractive value was calculated with reference to the air-dried drug.

**2.2.4.3 Determination of moisture content**

**2.2.4.3.1 Loss on drying**

5 gm of the powered drug of leaf, stem and root were dried into a pre- weighed flat and thin porcelain dish then dried it in the oven at 100º C or 105º C and Cool it in desiccators. The loss in weight was usually recorded as moisture.

## 3. Results

**3.1 Collection and authentication of raw material of *Cocculus hirsutus***

Plant *Cocculus hirsutus* was collected in the month of June, when it fully grown and flowering. Total 1500 gm of herb was collected to get 1000gm of dry powder. Authentication was done by taxonomist and Herbarium sheet (PH/15/011)

was deposited at the Pharmacognosy and Phytochemistry department of K. B. Institute of Pharmaceutical Education and research.

### 3.2 Pharmacognostical study of *Cocculus hirsutus*

*Cocculus hirsutus* is climber or straggler throughout the greater part of India. Macroscopical observation of the plant was done according to size, shape, surface with a naked eye which provided a great deal of information about the drug material under consideration. Microscopical evaluations were done by taking transverse section of leaf, stem and root of *Cocculus hirsutus*.

#### 3.2.1 Morphology of leaf, stem and root of *Cocculus hirsutus*

##### 3.2.1.1 Morphology of leaf of *Cocculus hirsutus*

Leaves are greenish in colour, odourless, mucilaginous when fresh, brittle and powder on drying and with characteristic taste showed in figure 1 and figure 2. 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.

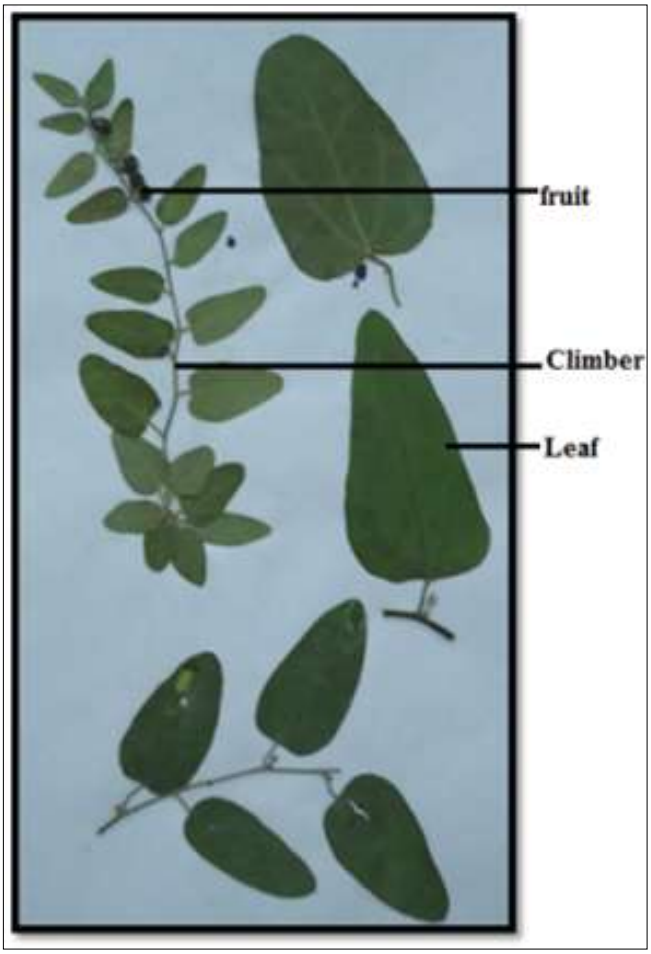


**Fig 1:** Climber and leaf of *Cocculus hirsutus*

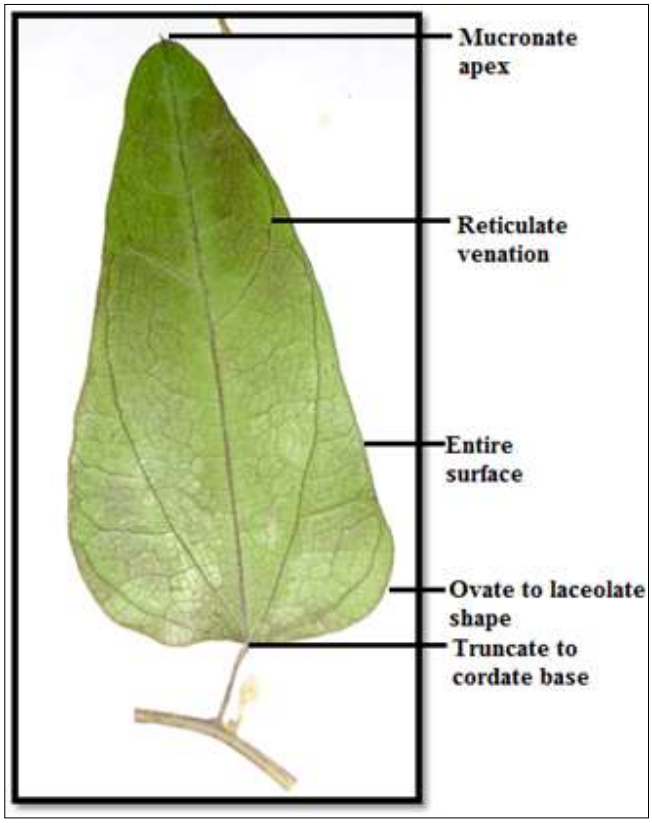


**Fig 2:** Morphalogy of leaf of *Cocculus hirsutus*

##### 3.2.1.2 Morphology of stem of *Cocculus hirsutus*:

Stem of *Cocculus hirsutus* was circular in shape, 1.0-1.5 cm diameter and long enough, smooth and somewhat hairy, color green when fresh and dark brown on drying with characteristic odour and taste. Therefore, the macroscopical and organoleptic characters of stem of *Cocculus hirsutus* were used to identify the plant materials as showed in Figure 3.

**Fig 3:** Morphalogy of stem of *Cocculus hirsutus*

##### 3.2.1.3 Morphology of root of *Cocculus hirsutus*

Root of *Cocculus hirsutus* was irregular in shape, 9-10 cm in diameter, very hard and somewhat hairy. Many rootlets were arising from the main root. Colour was light brown when fresh and pale brown on drying with characteristic odour and taste as showed in figure 4.

**Fig 3:** Morphalogy of root of *Cocculus hirsutus*

### 3.2.2 Microscopical studies of leaf, stem and root of *Cocculus hirsutus*

Authentification of leaf, stem and root of *Cocculus hirsutus* was further confirmed by the microscopical characters 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.

### 3.2.2.1 Microscopical characteristics of leaf of *Cocculus hirsutus*

The microscopy studies were carried out to authenticate leaf of *Cocculus hirsutus.* Transverse section of leaf of *Cocculus hirsutus* consist 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. 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 6) enclosed by sclerenchymatous bundle sheath. After sclerenchymatous bundle sheath, two bundle sheath lies in parenchymatous ground tissue as shown in Figure 5 and 6. Upper epidermis of leaf of *Cocculus hirsutus* consists of number of wavy epidermal cells and base of unicellular trichome as shown in Figure 7. Presence of anomocytic stomata were only found in lower epidermis, they were many in numbers, sunken and each surrounded by 4-6 epidermal cells as shown in Figure 8.

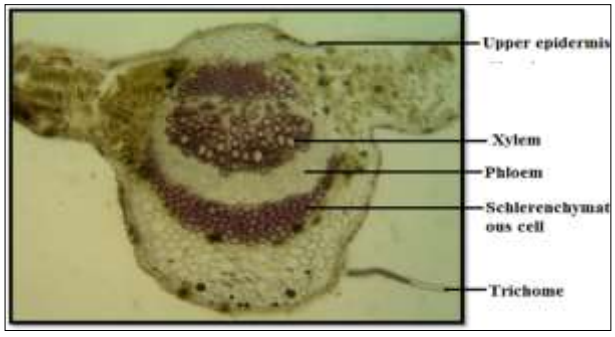


**Fig 5:** Tranasverse section of leaf of *Cocculus hirsutus*

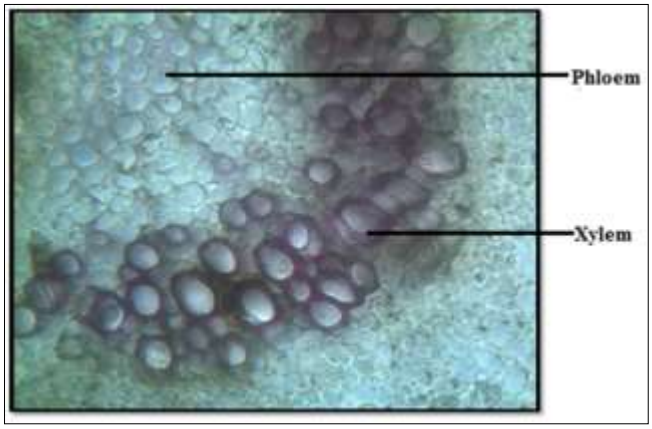


**Fig 5:** Tranasverse section of leaf of *Cocculus hirsutus*

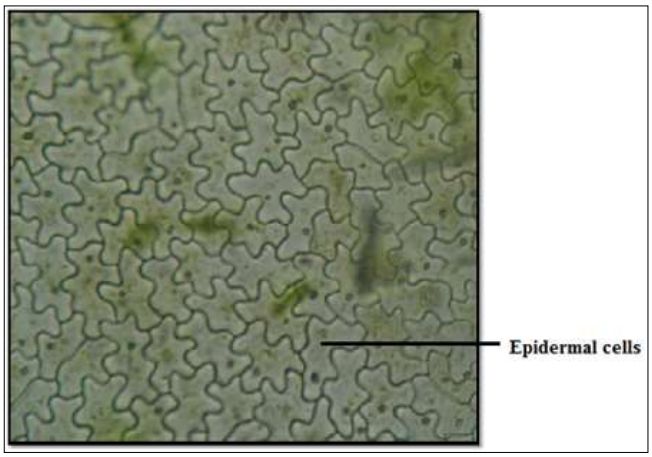


**Fig 7:** Upper epidermis of leaf of *Cocculus hirsutus*

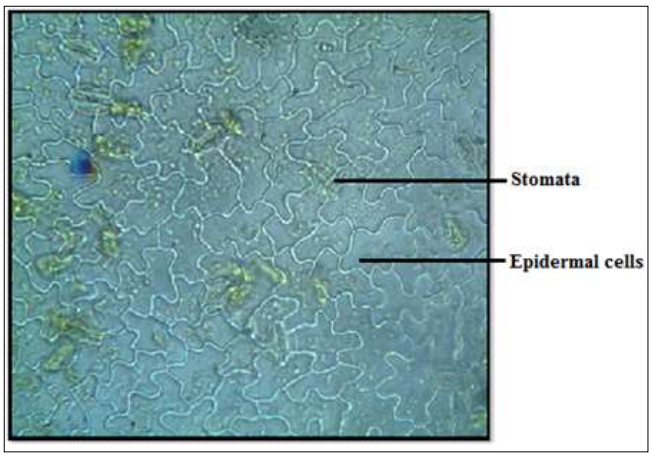


**Fig 8:** Lower epidermis of leaf of *Cocculus hirsutus*

### 3.2.2.2 Microscopy of stem of *Cocculus hirsutus*

Transverse section of stem of *Cocculus hirsutus* was consists of single layered epidermis with unicellular trichomes (figure 11a) and then two layers of collenchymatous cells (figure 11b) were present. Cortex was next to collenchymatous cells and endodermis were detected by presence of cells filled with starch grains only in places next to interfacicular zones and that zones called grooves. A sclerenchymatous cell (figure 11c) was the next layer, which was lignified. It was characterized externally by fiber cells and internally by parenchymatous cells surrounding phloem tissue (figure 11e). A large amount of sclerenchyma were present abaxial position relative to protoxylem (figure 11d)

was observed. At last pith was present as showed in figure 9, 10 and 11.

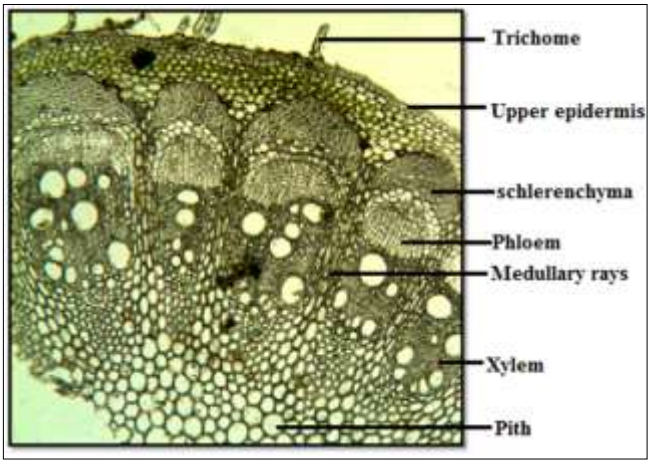


**Fig 9:** Transeverse se section of stem of *Cocculus hirsutus*

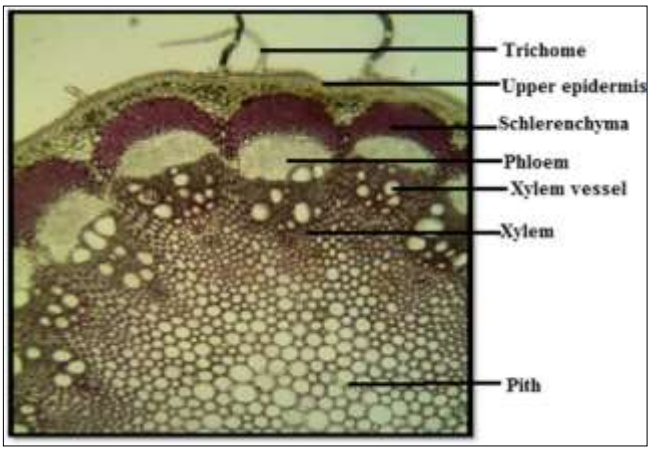


**Fig 10:** Transeverse se section of stem of *Cocculus hirsutus*

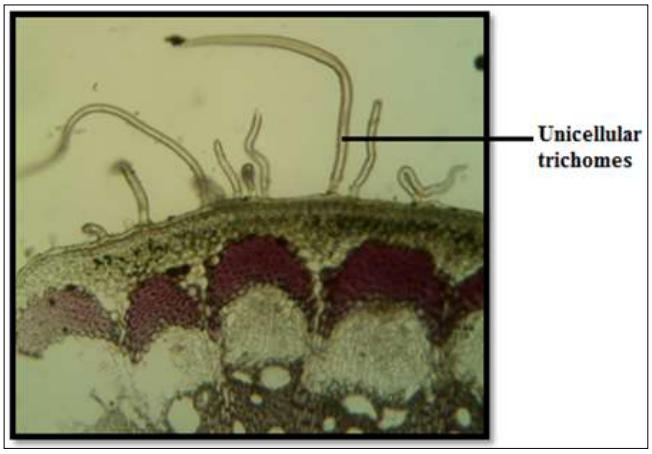


**Fig 11a:** Unicellular trichomes of transvere section of stem of *Cocculus hirsutus*

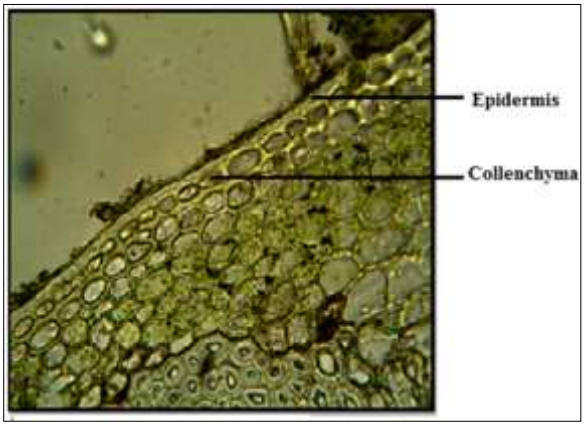


**Fig 11b:** Epidermis and Collenchyma of transeverse section of stem of *Cocculus hirsutus*

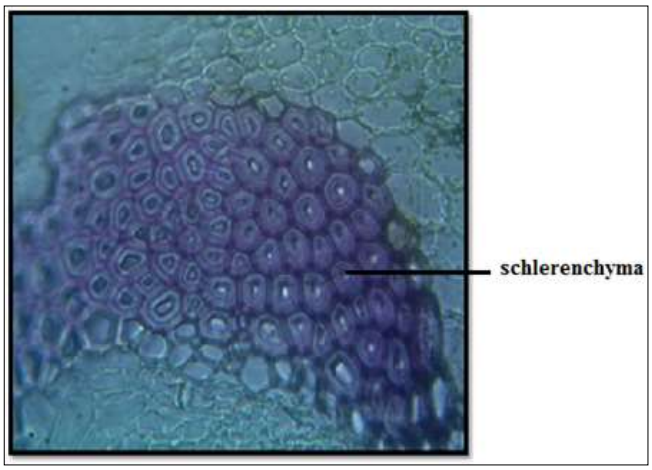


**Fig 11c:** Scherenchymatous cells of transverse section of stem of *Cocculus hirsutus*

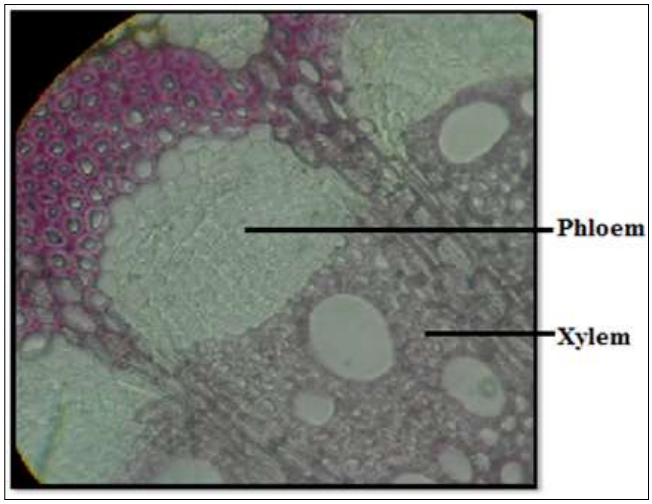


**Fig 11d:** Vascular bundle of transverse section of stem of *Cocculus hirsutus*

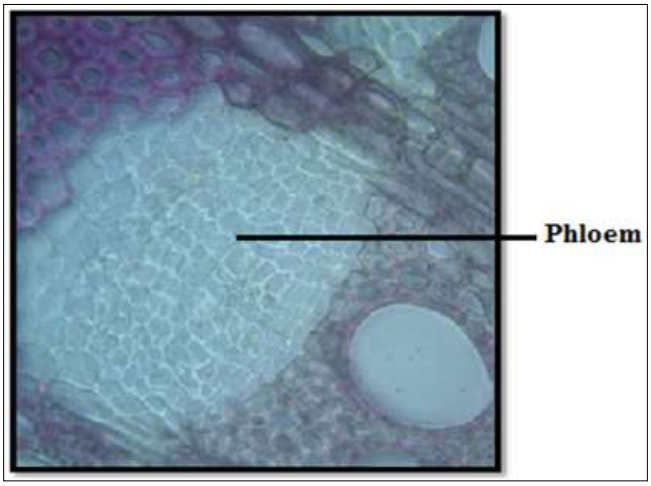


**Fig 11e:** Phloem of transverse section of srem of *Cocculus hirsutus*

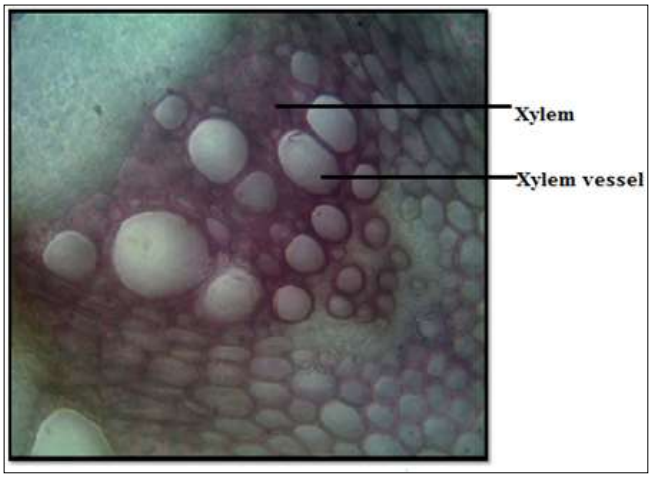


**Fig 11f:** Xylem of Transvers of stem of *Cocculus hirsutus*

**Fig 11:** Transverse section of stem of *Cocculus hirsutus* (40 X)

### 3.2.2.3 Microscopy of root of *Cocculus hirsutus*

Transverse section of root of *Cocculus hirsutus* was consists of cork cell layer (figure 13a) at the top. Two to three layer of cork cell was brown in colour and two to three layer of cork cell was transparent. Next to cork cell there was a three layers of stone cells (figure 13b). Stone cells were beaker in shape. Cortex consists of very small region. Xylem was the next to the cortex and schlerenchymatous tissue (figure 13c) followed by xylem. Schlerenchyma was semi-circular two layered cell, lignified. Ceretenchyma (figure 13d) was present, which was a type of phloem tissue, consisting of some brown matter and cells were compressed. Next layer was phloem, non-lignified. After that xylem was started. Medullary rays were tri to penta serreate (figure 13e). Pith was absent as showed in figure 12 and 13.

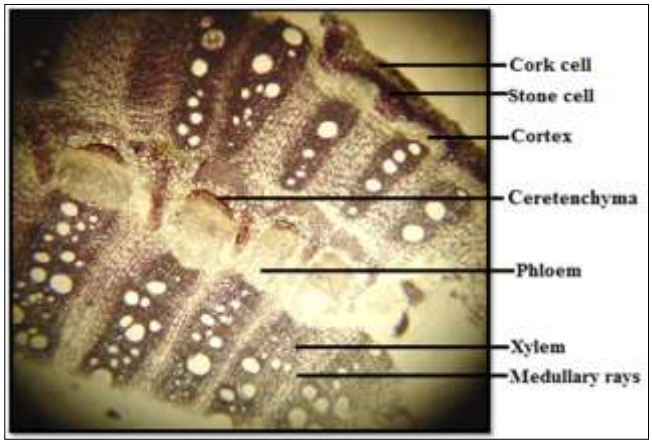


**Fig 12:** Transverse section of root of *Cocculus hirsutus*

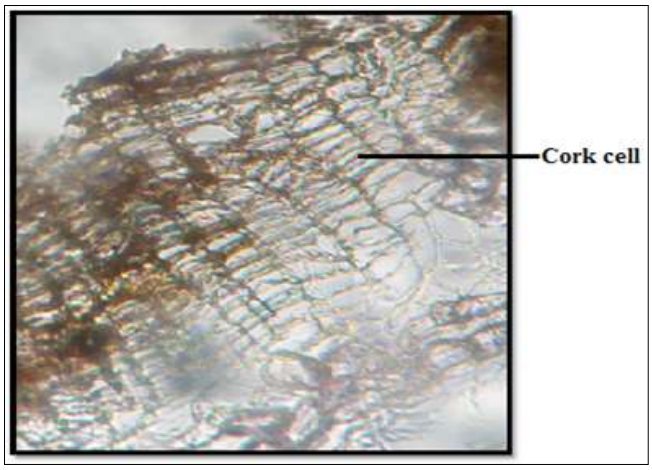


**Fig 13a:** Cork cells of transverse section of *Cocculus hirsutus*

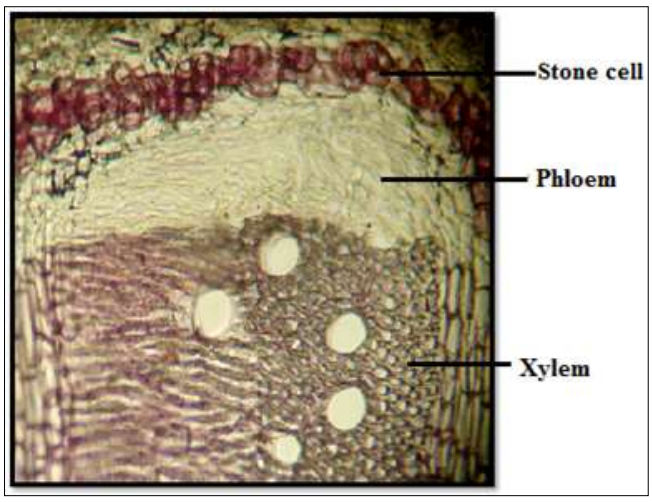


**Fig 13b:** Stone cell, Phloem and Xylem of transverse section of root of *Cocculus hirsutus*

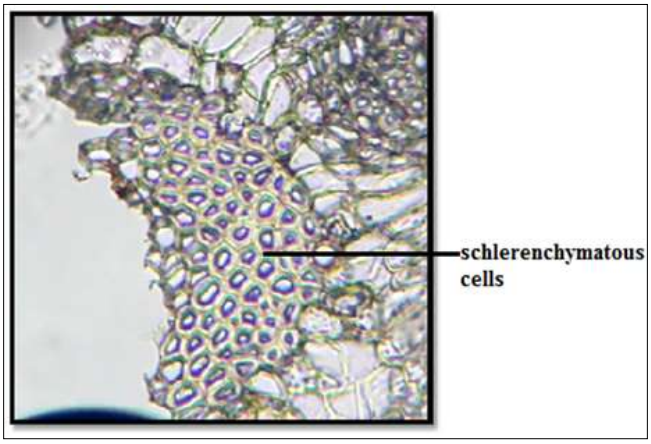


**Fig 13c:** Schlerenchymatous cells of transverse section of root of *Cocculus hirsutus*

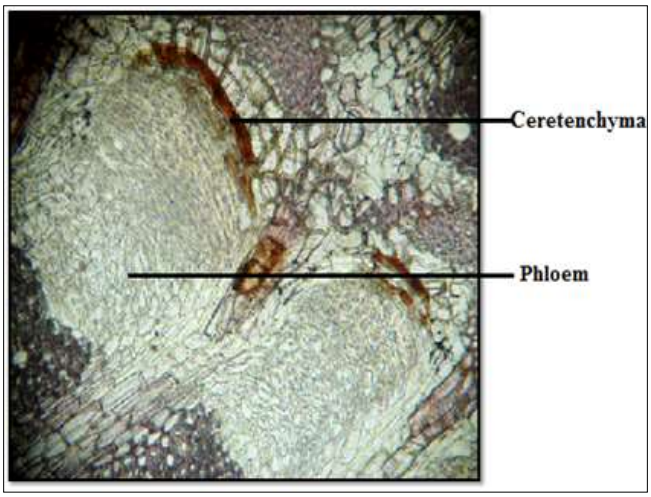


**Fig 13d:** Ceretenchyma and Phloem of transverse section of *Cocculus hirsutus*

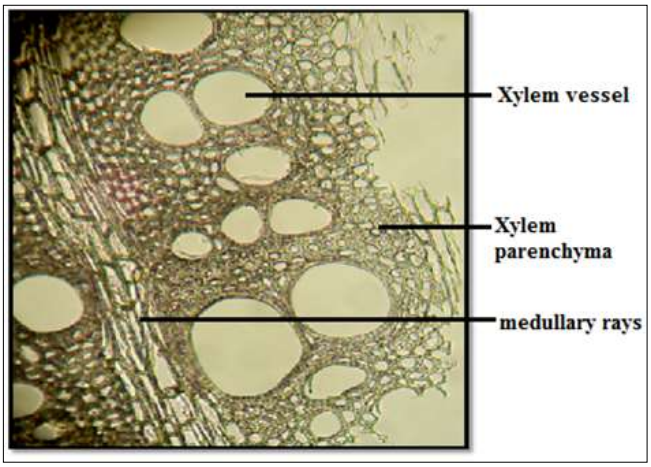


**Fig 13e:** Xylem of transverse section of root of *Cocculus hirsutus*

**Fig 13:** Transverse section of root of *Cocculus hirsutus* (40 X)

### 3.2.3 Powder study of leaf, stem and root of *Cocculus hirsutus*

#### 3.2.3.1 Powder study of leaf of *Cocculus hirsutus*

Powder study of leaf of *Cocculus hirsutus* showed the presence of anomocytic stomata (Figure 14a), pitted xylem vessel (Figure 14b) and long and short unicellular trichome (Figure 14c) as showed in Figure 14.

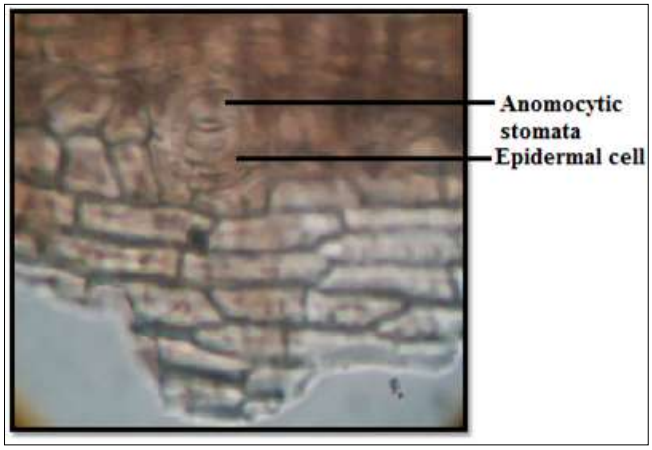


**Fig 14a:** stomata of leaf powder of *Cocculus hirsutus*

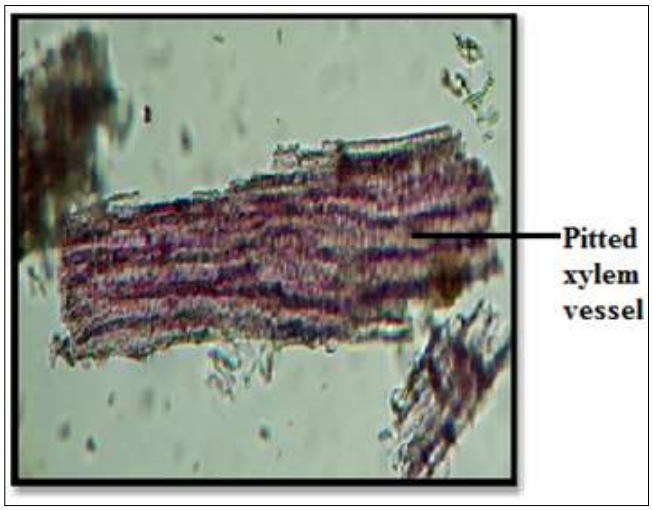


**Fig 14b:** Xylem vessel of leaf powder of *Cocculus hirsutus*

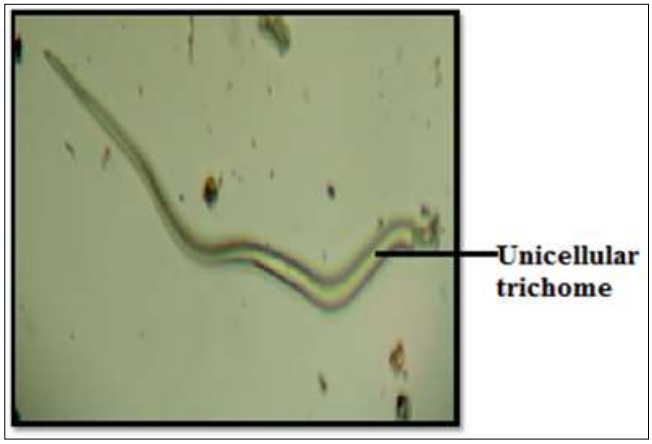


**Fig 14c:** Trichome of leaf powder of *Cocculus hirsutus*

**Fig 14:** powder study of leaf powder of *Cocculus hirsutus*

#### 3.2.3.2 Powder study of stem of *Cocculus hirsutus*

Powder study of stem was consisting of long and short unicellular trichome (Figure 15a) and pitted xylem vessel (Figure 15b).

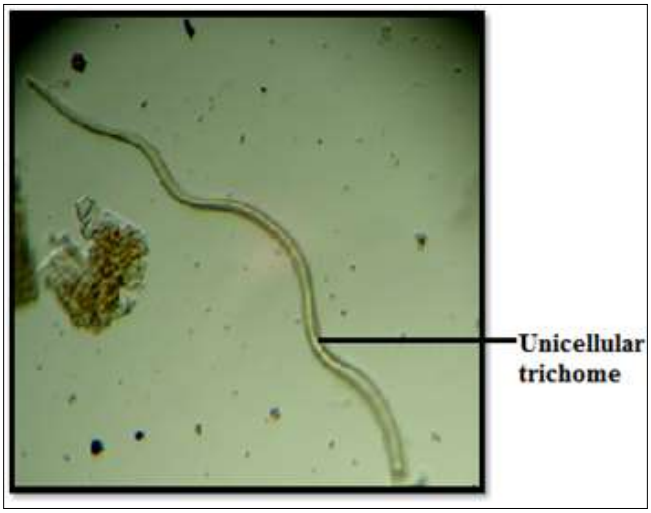


**Fig 15a:** Trichome of stem powder of *Cocculus hirsutus*

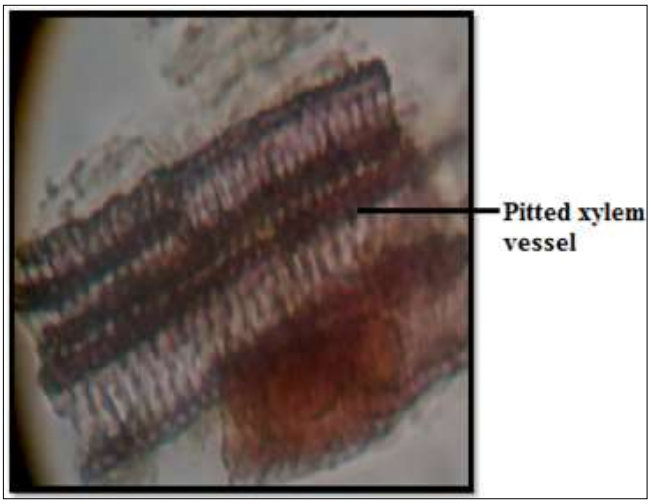


**Fig 15b:** Xylem vessel of stem powder of *Cocculus hirsutus*

**Fig 15:** powder study of stem of *Cocculus hirsutus*

### 3.2.3.3. Powder study of root of *Cocculus hirsutus*

Powder study of root was consisting of pitted xylem vessel (Figure 16a), cork cell (Figure 16b) and stone cell (Figure 16c) as showed in Figure 16.

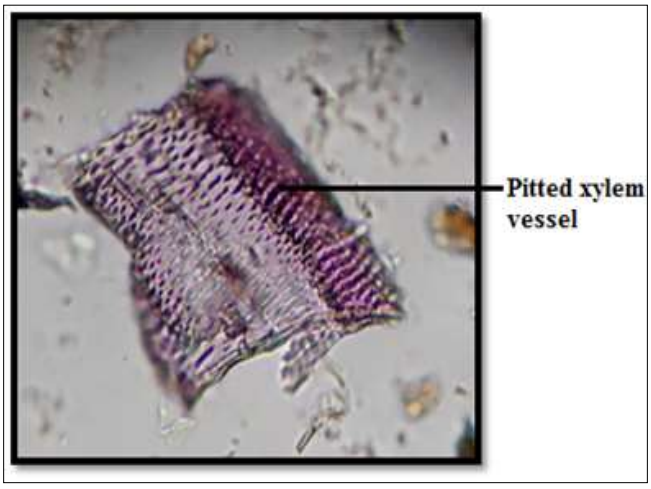


**Fig 16a:** Xylem vessel of root powder of *Cocculus hirsutus*

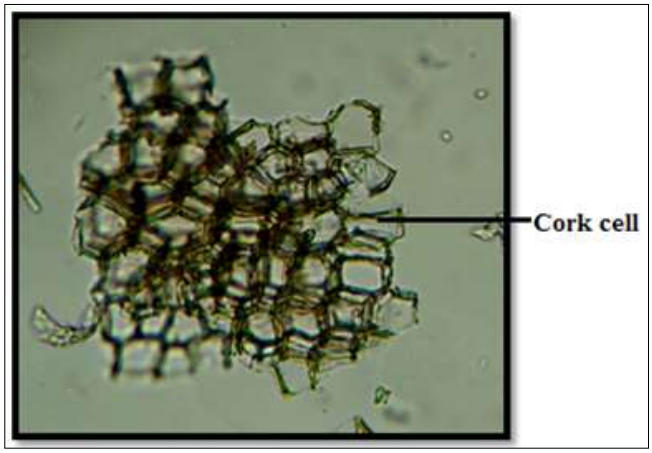


**Fig 16b:** Cork cell of root powder of *Cocculus hirsutus*


**Fig 16c:** Stone cell of root powder of *Cocculus hirsutus*

**Fig 16:** Powder study of root of *Cocculus hirsutus*

### 3.4 Physicochemical parameters of *Cocculus hirsutus*

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 and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica.

### 3.4.1 Determination of ash value

The results of ash values of crude powder of leaf, stem and root of *Cocculus hirsutus* were shown in table 1.

**Table 1:** Ash value of leaf, stem and root powder of *Cocculus hirsutus*

| Sr. No | Physicochemical evaluation | Leaf (% w/w) | Stem (% w/w) | Root (%w/w) |
|---|---|---|---|---|
| 1 | Total Ash | 12.40 | 03.20 | 04.30 |
| 2 | Acid Insoluble ash | 00.50 | 02.00 | 02.10 |
| 3 | Water soluble ash | 06.90 | 00.20 | 01.20 |

### 3.4.2 Determination of extractive value

The results of extractive values of leaf, stem and root of *Cocculus hirsutus* were showed in table 2. The water and ethanol extractive value of leaf of *Cocculus hirsutus* was highest as compared to stem and root.

**Table 2:** Extractive value of leaf, stem and root powder of *Cocculus hirsutus*

| Sr. no. | Name of different solvent | Colour of extract of leaf powder | Extractive value of leaf (% w/w) | Extractive value of stem (% w/w) | Extractive value of root (%w/w) |
|---|---|---|---|---|---|
| 1 | Petroleum ether | Light green | 02.50 | 00.56 | 01.50 |
| 2 | Toluene | Green | 04.00 | 01.00 | 02.30 |
| 3 | Chloroform | Brownish green | 02.00 | 01.11 | 01.00 |
| 4 | Ethyl acetate | Dark green | 04.37 | 01.25 | 02.40 |
| 5 | n-Butanol | Dark green | 04.00 | 03.33 | 03.00 |
| 6 | Ethanol | Dark green | 14.00 | 10.00 | 06.00 |
| 7 | Water | Green | 30.00 | 10.00 | 05.00 |

### 3.4.3 Determination of moisture content

The results of loss on drying of powder of leaf, stem and root of *Cocculus hirsutus* were showed in table 3.

**Table 3:** Loss on drying of *Cocculus hirsutus*

| Sr.no | Physicochemical evaluation | Leaf (%w/w) | Stem (%w/w) | Root (%w/w) |
|---|---|---|---|---|
| 1 | Loss on drying | 01.50 | 03.00 | 01.30 |

## 4. Discussion and Conclusion

The presence study was aim to evaluate pharmacognstical and phytochemical evaluation of leaf, stem and root of *Cocculus hirsutus.* Plants were collected and authenticate by taxonomist. Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs [13]. The organoleptic or macroscopic studies yielded important characteristics, such as the fractured surfaces of fresh and dried material, typical tongue sensitizing aromatic taste and aromatic and characteristic odour of the leaf, stem and root, which are useful diagnostic characters. Microscopical characters of leaf, stem and roots helps to authentic the material collected and useful for setting standards for crude drugs [14].

Physicochemical studies of leaf, stem and root of *Cocculus hirsutus* were determined by ash values, extractive values and loss on drying according to the WHO guidelines. The total ash method was designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which was derived from the plant tissue itself, and "non physiological ash", which was the residue of extraneous matter adhering to plant surface. Acid insoluble ash is the residue obtained after the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the difference in weight between the total ash and residue after treatment of total ash with water [15, 16]. From all three parts of *Cocculus hirsutus*, leaf powder showed maximum amount of ash value (12.40 %w/w) as it contains maximum amount of silica in its dried powder. Water extractive value of leaf of *Cocculus hirsutus* was maximum (30.00 %w/w) compared to all other selected parts of *Cocculus hirsutus.* It means higher water-soluble extractive value implies that water is a better solvent for extraction for plant material [17]. The percentage of Loss on drying of stem of *Cocculus hirsutus* was maximum (03.00%) and were compared with standard and it comply in the range of it.

## 5. Acknowledgement

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