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PHARMACOGNOSTIC AND PHYTOCHEMICAL SCREENING OF *COCCULUS PENDULUS* Diels. STEM AND ROOT

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ABSTRACT

Cocculus pendulus Diels. belongs to family Menispermaceae locally known as "Parwatti" is a part of medicines against jaundice, yellow fever, leprosy, syphilis and as an aphrodisiac. To authenticate the *C. pendulus* from adulterations, in the present study the stem and root has been evaluated for different pharmacognostic studies. Morphologically the plant is dioecious, woody climber (Liana) and about 4-8m tall. The macroscopic study showed that stem is cylindrical in shape, characteristic odor, acceptable taste and brittle fracture while the root is irregular in shape with characteristic odor and bitter taste. Histologically both plant parts showed typical dicot anatomy. Preliminary phytochemical screening showed the presence of proteins, carbohydrates, alkaloids, tannins, saponins, steroids, flavonoids, glycosides and phenolic compounds in both plant parts. Quantitatively highest amount of sterols 85.32±2.42mg/g followed by alkaloids 30.45±0.32mg/g in root while stem with 71.43±1.24mg/g amount of sterols and 21.47±0.54mg/g alkaloids has been recorded. Fluorescence analysis showed diverse colors by treating with different reagents at ordinary and UV light. Powdered drug study and percent extractive values determination were also carried out. Pharmacognostic studies will be helpful in the standardization and authentication of the species from adulteration as the above studies has been carried out for the first time for the said plant.

Key words: *Cocculus pendulus*, fluorescence analysis, pharmacognosy, phytochemical screening.

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INTRODUCTION

Plants have always been the principal source of medicine in the world. Several drugs has been derived from plant sources that are being used today (Rao *et al.* 2010). It is thought that in developing countries 80% of the people are dependent on traditional medicines for their primary healthcare (Kim *et al.* 2001). About 80% compounds has been derived from 94 species of plants, which are being used for ethnomedical purposes (Kokate *et al.* 2008). So, the present study has been carried out to explore *Cocculus pendulus* which may be helpful for the treatments of many ailments.

Cocculus pendulus Diels. Synonym: *Cocculus leaeba* DC. belongs to family Menispermaceae locally known as "Parwatti (Fig.1). It is a dioecious, shrubby plant with 15cm diameter of the stem at the base. Leaves are broad, oblong lanceolate, with obtuse apex, generally glabrous or puberulous. Fruit composed of 1–3 flattened drupes. Seed horse-shoe shaped, laterally flattened (Rabari *et al.* 2010). People in Pakistan and Afghanistan use the plant parts, especially the roots to cure fevers, including intermittent fever. In Nigeria the root and leaves are used for this purpose while in Senegal the Toucouleur and Paul people use both root and stem bark for the same purpose. In Senegal the Toucouleur and Paul people use root and stem bark decoctions against intestinal parasites and gonorrhea (Rabari *et al.* 2011). The root has a great reputation in Senegal against biliousness and menstrual problems and as a diuretic. It is also a part of medicines against jaundice, yellow fever, leprosy, syphilis and as an aphrodisiac in the area (Rabari *et al.* 2010). The literature studies also showed that *C. pendulus* stem and root has not been analyzed for pharmacognostic work. So, in the present studies stem and root has been investigated for pharmacognostic profile.

MATERIALS AND METHODS

Specimen collection and preservation: The fresh stem and root of the experimental plant were collected in January 2015 from the Tribal area, Sama Bada Bera, Labi Khel, F.R Peshawar located at 33.8078° North latitude and 71.7356° East longitude and elevation of the area above the sea level is 1,000m. This area comes under subtropical zone. The relative humidity varies from 46% in June to 76% in August. Specimens were identified by Dr. Barkatullah, Lecturer, Department of Botany, University of Peshawar mounted on Herbarium sheet and provided with a Voucher number Bot-UOP-2015, and deposited in the herbarium. Both stem and root were completely dried under the sun and grinded by electric grinder. After grinding the powdered drugs were kept in

impermeable bottles and were used for different pharmacognostic studies.

Pharmacognostic evaluation

Morphology and Macroscopy: Morphological and macroscopic features of the stem and root were conducted by organoleptic method following the methodology of Evans (2002); Wallis (2009). Both plant parts were studied for color, shape, inner surface, outer surface, fracture, taste and odor.

Microscopic evaluation: The microscopic and histological features of the leaf were carried out by hand sectioning using the standard procedure of Chaffey (2001); Evans (2002); Wallis (2009). Sharp razor were used for making thin fine transverse sections of the stem and root. Fine sections were selected, stained properly and observed under Digital Labomed microscope (Model iVu 3100 and no. Lx 400).

Preparation of extract: 250g dried powder of stem and root separately were treated with 1 liter ethanol and shake at regular intervals for 48 hours. After 48 hours the extract was filtered, the filtrate were collected and processed in rotary to make the final volume 1/5 of the original volume and stored in impermeable glass bottles at 4ºC for further evaluation Evans (2002).

Qualitative Phytochemical evaluation: For the determination of different phytoconstituents like alkaloids, fats, phytosterols, fixed oils, glycosides, proteins, flavonoids, saponins and tannins, various phytochemical tests of the ethanolic extract were carried out.

Carbohydrate detection test

- **Molisch's test:** Few drops of Molish reagent were added to 1 ml extract solution. Through the side of the test tube concentrated sulphuric acid were added. Purple to violet color ring appearance at the junction will be the detection of carbohydrates Evans (2002).

- **Benedict test:** The addition of Benedict reagent to 1 ml extract solution followed by placing on water bath and the appearance of reddish brown precipitate appearance will be the indication of reducing sugar otherwise absent Evans (2002).

Proteins and amino acids detection test

- **Biuret test:** The addition of equal volumes of NaOH (5%) and CuSo4 (1%) to 1 ml extract solution and the appearance of violet color will be the indication for the presence of free amino acids and proteins otherwise absent Kumar and Kiladi (2009).

- **Ninhydrin test:** Boiling of crude extract at 100°C with 1 ml Ninhydrin solution and appearance of violet color will be the indication for the presence of amino acids and proteins otherwise absent Kumar and Kiladi (2009).

- **Xanthoproteic test:** The addition of few drops of concentrated of HNO3 to 2ml extract solution made in distilled water and the appearance of cloud upon heating changing to yellow which turns to an orange color by adding ammonia will indicate of the presence of amino acids otherwise absent Kokate *et al.* (2008).

Alkaloid detection tests

- **Dragondorff's test:** Add 2-3ml Dragondorff's reagent to 1 ml extract solution. If orange brown precipitate appears it will indicate the presence of alkaloids Khandelwal (2004).

- **Wagner's test:** The addition of Wagner's reagent to 1 ml extract solution and the formation of reddish brown precipitate will be the indication of the presence of alkaloids otherwise absent Khandelwal (2004).

- **Hager's test:** The addition of Hager's reagent to 1 ml extract solution followed by the formation of yellow precipitate will be the indication of the presence of alkaloids otherwise absent Khandelwal (2004).

Triterpenoids and Phytosterols detection

- **Salkowski's test:** The addition of 0.5 ml concentrated H_2SO_4 to 1 ml extract solution made in chloroform through the side of test tube and the appearance of red color at lower layer will indicate the presence of sterol, while yellow color appearance will be the indication of triterpenoids presence Harnborne (1998).

Phenol detection test

- **Ferric chloride test:** The addition of 1 ml $FeCl_3$ solution to 1 ml extract solution and the appearance of bluish green color appearance will be the indication for the presence of total phenols otherwise absent Dahiru *et al.* (2006).

Test for Flavonoids

- **Alkali reagent test:** The addition of NaOH to 1 ml extract solution and the appearance of yellow to red color precipitation will indicate for the presence of flavonoids otherwise absent Kokate *et al.* (2008).

Test for the Tannins

- **Ferric chloride test:** The addition of 1 ml 5% $FeCl_3$ to 1 ml extract solution followed by appearance of greenish black color will indicate the presence of tannins otherwise absent Wallis (2009).

- **Alkali reagent test:** The addition of Sodium hydroxide to 1 ml extract solution and the formation of

yellow red precipitate will indicate the presence of tannins otherwise absent Wallis (2009).

Saponins detection test

- **Frothing test:** The formation of persistent froth by vigorous shaking of extract in distilled water will be the indication of saponins prescence Evans (2002).

Steroidal glycosides detection test

- **Killaer Kilani test:** The addition of glacial acetic acid to 1 ml extract solution and addition of one drop of concentrated H_2SO_4 through the side of test tube and formation of reddish brown color precipitate at the junction will be the indication of glycosides presence Harnborne (1998).

Fixed oils presence test

- **Spot test:** The rubbing of extract between two filter papers and the existence of permanent spot will be the indication of fixed oil presence Kumar and Kiladi (2009).

- **Volatile oil:** The rubbing of a small amount extract between two filter papers and the absence of permanent stain will be the indication of volatile oil presence Kumar and Kiladi (2009).

Quantitative phytochemical screening

Alkaloids determination: The methodology of Wallis (2009) was adopted for the quantitative screening of total alkaloids. 100 ml acetic acid (10%) was taken in which 2g crude ethanolic extract of stem and root were dissolved. The solution was allowed to stand for 4 hours and then filtered. After filtration, the extracts were placed on a water bath for further concentration to reduce the volume to one-fourth. Precipitate formation occurred by the addition of Concentrated NH4OH drop wise. Dilute NH4OH was used for washing the collected precipitate. The obtained product was collected and weighted using the following formula.

Amount of alkaloid (mg/g) = $\frac{w2-w1}{\text{Weight of sample}}$

W_1= Weight of filter paperW_2 = Weight of filter paper + ppt

Saponins determination: The methodology of Obadoni and Ochuko (2001) was adopted to carry out the detection of total saponins contents quantitatively. 20ml distilled water were taken in which 2g crude ethanolic extract of stem and root were dissolved and transferred into a separating funnel. 20 ml diethyl ether were added and shake well. The ether layer was discarded and the aqueous layer was separated. 60 ml n-Butanol was added for the purification of the product followed by washing the extract twice by 10 ml 5% aqueous NaCl. The product obtained were boiled on water bath and dried. The saponins content were calculated using the following formula.

Amount of saponins (mg/g) = $\frac{w2-w1}{\text{Weight of sample}}$

W_2=Weight of filter paper + residue

W_1 =Weight of filter paper

Sterol determination: The methodology of Huang *et al.* (2010) was adopted to carry out the detection of total sterols contents quantitatively. 75 ml distilled water were taken to which 2g crude ethanolic extract of stem and root were added. 25 ml potassium hydroxide (10%) was added to the solution. This mixture was then poured into a separating funnel followed by the addition of 75 ml petroleum ether thrice for extraction. The ether fraction was separated from the funnel and transferred into a flask which was pre weighted (W_1). The extract were boiled on hot water bath followed by drying and cooling. The flask was again weighted (W_2) and total sterol contents were obtained using the following formula.

Amount of sterol (mg/g) = $\frac{w2-w1}{\text{Weight of sample}}$

W_2= Weight of flask+ residue W_1 = Weight of flask

Tannins determination: The methodology of Van-Buren and Robinson (1969) was carried out for the quantitative determination of tannins. 75 ml distilled water were taken in which 2g crude ethanolic extract were dissolved. The suspension was then filtered. Lead acetate saturated solution were added to the filtrate in order to get lead-tinnate precipitate. 20 ml distilled water was added followed by treating with dilute H2SO4, which acidified the solution. Then the mixture was filtered through the pre-weighted Whatman filter paper (W1) to get the tannins. The filter paper was then dried. By weighting the filter paper the amount of tannins were calculated.

Amount of tannins (mg/g) = $\frac{w2-w1}{\text{Weight of sample}}$

W_2=Weight of filter paper+ residue W_1 =Weight of filter paper

Powdered drug study: Both the plant parts were sun dried at for 10 days and grinded with the help of electric grinder. A little amount of fine powder drug were treated with chloral hydrate solution, iodine solution and glycerin and observed under Labomed microscope for different histological structures following Wallis (2009).

Fluorescence analysis: The fluorescence screening of the powdered drugs of the both plant parts were carried out by treating a little amount of powder with different reagents like methanol, NaOH in water, diethyl ether, iodine solution, HNO_3, H_2SO_4, $FeCl_3$ solution and NH_3 solution. All these treated powdered were examined under UV light and ordinary day light (UV 254 and UV 336) Nikam *et al.* (2009); Wallis (2009).

Extractive values analysis: The methodology of Ansari *et al.* (2006) has been followed for the determination of extractive values of stem and root powdered drug. 15 gram powdered drug was soaked in 250 ml each solvent (ethanol, n-Hexane, methanol, chloroform, acetone and phenyl ether) separately and kept for 8 days in air tight bottles with regular shaking every day. Each fraction was filtered after 8 days and the filtrate was dried. Following formula was used for the extractive values determination:

Percent (%) extractive value (w/w) = $\frac{\text{weight of the extract}}{\text{weight of the sample}} \times 100$

RESULTS

Morphological and macroscopic features: *Cocculus pendulus* is a woody climber (Liana) about 4-8m tall. In the present study the stem and root of *C. pendulus* were evaluated for its morphological features. The macroscopic study revealed that the stem is cylindrical in shape, outer surface light brown, inner surface light yellow, odor characteristic, acceptable taste and has brittle fracture while the root is irregular in shape, outer surface light brown, inner surface light yellow, odor characteristic with slightly bitter taste and brittle fracture (Table. 1). Upadhyay *et al.* (2010); Akbar *et al.* (2014) worked out on *Euphorbia hirta* and *Malva parviflora* respectively and said that organoleptic evaluation is the primary technique for the correct identification of a plant by sense organs and provide a base for the crude drug authentication from adulteration.

Microscopic evaluation: Histological evaluation is the most important method use for the authentication of a crude drug. Transverse section of *C. pendulus* stem showed that Epidermis comprised of 2-3 layers of cells protected by thick cuticle layer followed by irregular 2-3 layers of hypodermal cells. 4-6 layers of cortical cells followed by endodermis, which are arranged in biconvex manner. Below the endodermis a dome shaped pericyclic region is present. Xylem vessels are smaller in size while phloem tissues are circular shaped and larger in size. Pith is in the form of compact round shaped cells (Fig. 2).

Transverse section of *C. pendulus* root showed the external cork layer comprised of 4-5 layered irregular wavy shaped cells followed by stellar region made up of cortical cells arranged in 3-4 layers. Medullary rays, thread like structures extended towards the xylem tissues. Xylem tissues are irregularly arranged. Phloem tissues are adjacent with the cortex (Fig. 3).

Modi *et al.* (2010); Khyade and Vaikos (2014) analyzed the histological studies of *Syzygium cumini* and *Wrightia tinctoria* respectively and stated that anatomical study is very much important for identification and diagnostic feature of the drugs. Anatomy can provide additional evidences which correspond to the anatomy for the recognition of the taxon. According to Pachkore *et al.* (2012) anatomical perspective of medicinal plants is an integral component of pharmacognosy.

Phytochemical screening

Qualitative Phytochemical screening: Qualitative phytochemical analysis of the stem and root extracts of *C. pendulus* showed that both the plant parts consists of many important metabolites such as fats, proteins, carbohydrates, alkaloids, tannins, saponins, steroids, flavonoids, glycosides and phenolic compounds given in (Table. 2). These phytochemical constituents have a great curative properties against different diseases.

Quantitative phytochemical screening: Quantitative phytochemical screening of the stem and root were conducted for some important phytoconstituents such as saponins, sterol, alkaloids, flavonoids and tannins. The results has been listed in (Table. 3).

Prabhu *et al.* (2011); Uthayakumari and Sumathy (2011); Desai *et al.* (2012) worked out on three *Viburnum* species, *Jatropha maheswarii* and *Tinospora cordifolia* respectively and revealed that active constituents such as alkaloids, flavonoids, tannins and glycosides are very important secondary metabolites which are helpful in the treatment of different disorders. Alkaloids can be use as cardiac stimulant, analgesic and as respiratory stimulant. They also possess the vasoconstriction, muscle relaxant, antispasmodic, antineoplastic, anticancer, allelopathic and insecticidal potential (Goncalves *et al.* 2019). The literature revealed that flavonoids possess the vasoprotective, anti-inflammatory, antithrombotic, antiallergic potential, gastric mucosa protection and tumor promotion inhibition. Flavonoids also have the ability to alter immunological response, antioxidant, anticancer and antimicrobial potential (Rice *et al.* 1996). Tannins have antidiarrheal potential and can also be use as antidotes against heavy metals and alkaloids having poisonous effect. They also possess wound healing, antibiotic, soothing effect and various inflammatory effects. Immuno-regulation and most of the cardiac diseases can be cured by saponins and glycosides respectively (Ashok and Upadhyaya 2012). Terpenoids and saponins have the astringent properties while steroids have the potential to regulate the function of sex hormones and possessing strong pain killing potential (Al-Snafi, 2015). Phenolic compounds are valued to due to possessing anti-inflammatory potential such as quercetin and antihapatotoxic potential such as silybin. Other phenolic compounds such as genistein and daidzen possessing phytoestrogenic potential and naringenin have significant insecticidal effect. They also possess antioxidant and free radical scavenger potential which can fight against cancer (Lin *et al.* 2016). Several researchers did the same work such as Shah and Seth (2010) analyzed *Lagenaria*

siceraria and Kumar *et al.* (2012) carried out the phytochemical screening of *Holoptelea integrifolia* for phytochemicals are well in lineage with our findings and described the importance of phytochemical screening of crude drugs, which are helpful for the researchers in the field of pharmacology, phytotherapy and phytochemistry to conduct advance research on herbal plants. The present work on *C. pendulus* are helpful in exploration of the plant for further advanced research in above mentioned fields.

Powder drug study: Powder drug microscopy is an important method for the correct recognition of different cellular structures present in the powdered drug. This technique is helpful for the purity of a drug from adulterants. Powder drug analysis of stem of *C. pendulus* showed different fragments such as fibers, vessels, cortical cells, epidermal cells and pitted vessels while root showed different fragments such as cortical cells, fibers, parenchyma cells, endodermal cells, and xylem vessels (Fig. 4; Fig. 5).

Fluorescence analysis: Fluorescence analysis of stem and root powders were conducted. The powder were treated with different solvents and observed in ordinary day light and UV light (UV 254, UV 336). The results has been listed in (Table. 4). Similar studies were also conducted by other researchers like Pandya *et al.* (2012); Paul *et al.* (2012) worked out fluorescence analysis of *Cassia fistula* and *Mimosa pudica* respectively revealed that fluorescence analysis is one of the important method for the standardization and authentication of a crude drug as this parameter is helpful in detection of important secondary metabolites which show difference in fluorescence under ordinary and UV light.

Extractive values determination: Extractive values both plant parts powder drug were determined treated with various solvents. Significant extractive values were found in methanol which were 9.32% followed by ethanol 8.15% (Table. 5). The results showed that more polar solvent (methanol and ethanol) are significant for the extraction of *C. pendulus* stem and root. Similar studies were also performed by Rani and Lakshmi (2012); Bharti and Vasudeva (2013) on *Dioscorea oppositifolia* and *Oreganum vulgare* respectively and stated that extractive value determination is helpful in the identification of a crude drug being adulterated. Various type of adulterated or exhausted drugs can detect by solvent extraction. Extraction with petroleum ether is useful for the indication of lipid contents in crude drugs. Aqueous and alcohol extraction are helpful in the indication of defective processing and adulterants in the crude drug (Kokate *et al.* 2008).

Fig. 1. *Cocculus pendulus* plant morphology

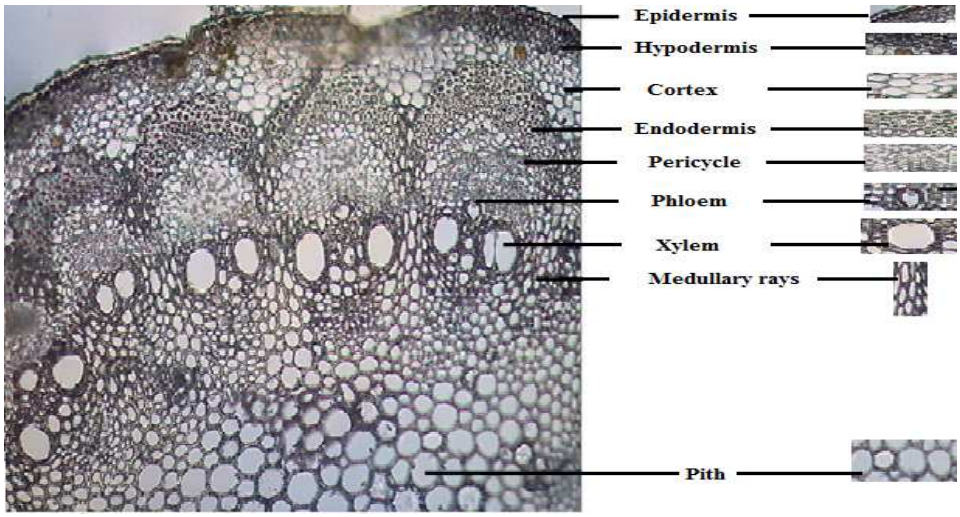

Fig. 2. Transverse section of *C. pendulus* stem.

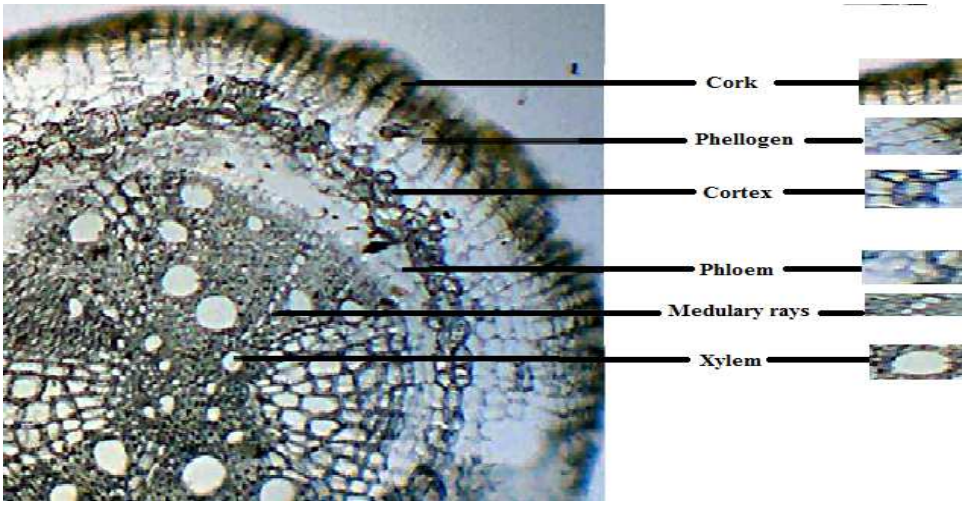

Fig. 3. Transverse section of *C. pendulus* root.

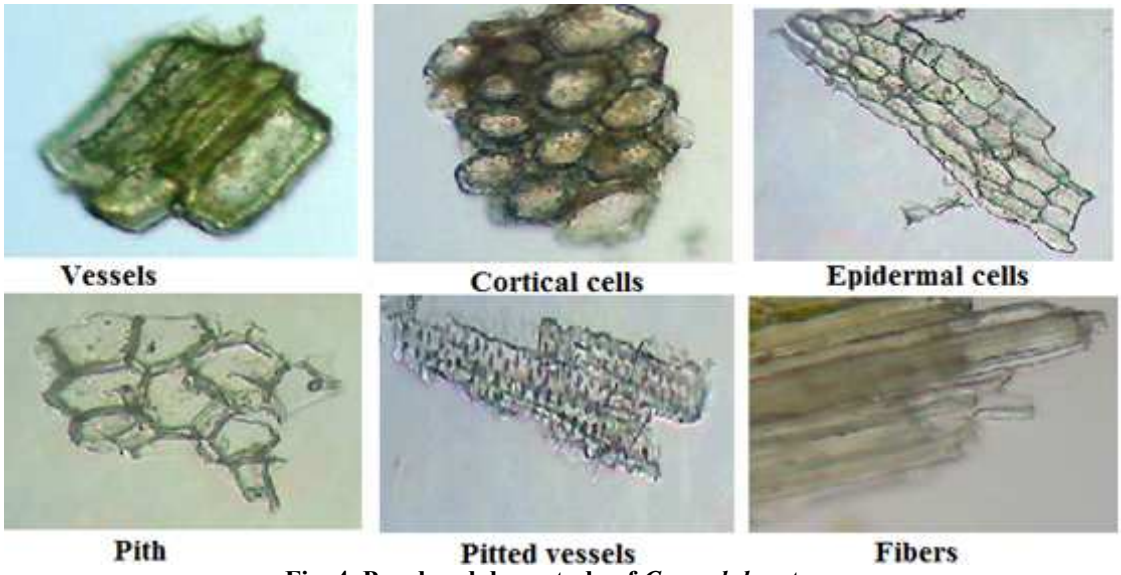

Fig. 4. Powdered drug study of *C. pendulus* stem.

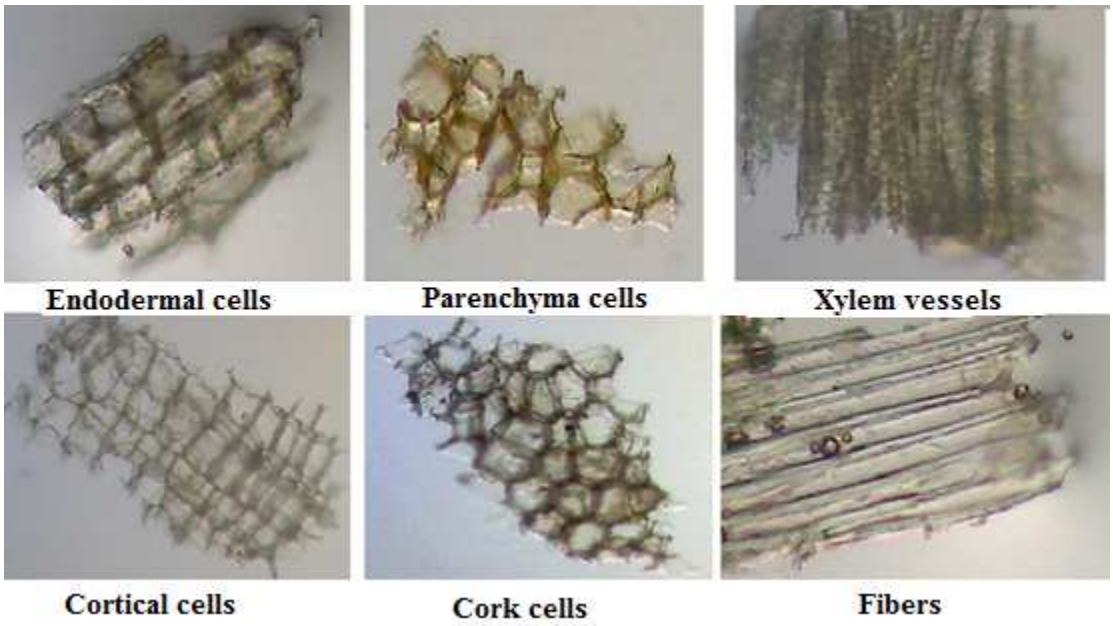

Fig. 5. Powdered drug study of *C. pendulus* root.

Table 1. Macroscopic features of *C. pendulus* stem and root.

S.No.	Characteristic	Stem	Root
1	Shape	Cylindrical	Irregular
2	Outer surface	Light brown	Light brown
3	Inner surface	Light yellow	Light yellow
4	Odor	Characteristic	Characteristic
5	Taste	Acceptable	Slightly bitter
6	Fracture	Brittle	Brittle

Table 2. Phytochemical screening of the stem and root extracts of *C. pendulus.*

S.No.	Constituents	Test name	Stem	Root
1	**Carbohydrates**	Molisch test	++	++
		Benedict's test	-	-
2	**Protein**	Ninhydrine test	++	++
		Xanthoproteic test	++	++
		Biuret test	+	+
3	**Alkaloids**	Wagner's test	+	++
		Hager's test	+	+
		Dragandorrf test	++	++
4	**Phytosterol and Triterpenoids**	Salkowskii's test	+	++
5	**Phenol**	Ferric chloride test	+	+
6	**Flavonoids**	Alkali reagent test	++	++
7	**Tannins**	Ferric chloride test	+	+
		Alkali reagent test	+	+
8	**Saponinss**	Frothing test	+	++
9	**Glycosides**	Killaer kilani test	++	++
10	**Fixed oil and fats**	Spot test	+	+
11	**Volatile oil**	Spot test	-	-

Key: ++ = Strongly detected, + = Detected, - = Not detected

Table 3. Quantitative phytochemical analysis of *C. pendulus* stem and root. All values are expressed in mg/g.

S.No.	Part	Alkaloids	Tannins	Sterol	Saponins
1	**Stem**	21.47±0.54	18.23±0.46	71.43±1.24	19.84±1.45
2	**Root**	30.45±0.32	21.43±0.96	85.32±2.42	28.42±0.86

Tab. 4. Fluorescence analysis of stem and root powders of *C. pendulus.*

S.No.	Reagents	Visible light	UV 254	UV 336
1	CS powder as such	Light yellow	Yellow	Yellow
2	CS powder+ 50%H2SO4	Light yellow	Brown	Green
3	CS powder+ 50%HNO3	Brown	Yellow	Light green
4	CS powder+ Diethyl ether	Yellow brown	Brown	Green
5	CS powder+ NaOH in H2O	Light yellow	Dark green	Bluish green
6	CS powder+ Methanol	Yellow brown	Light yellow	Yellow
7	CS powder+ Picric acid	Yellowish brown	Brown	Green
8	CS powder+ Iodine solution	Light yellow	Yellow	Yellow
9	CS powder+ NH3 solution	Light yellow	Light brown	Light yellow
10	CS powder+ 10%FeCl3	Yellow	Light yellow	Brown yellow
11	CR powder as such	Brown	Yellow	Greenish yellow
12	CR powder+ 50%H2SO4	Reddish brown	Brown	Dark brown
13	CR powder+ 50%HNO3	Greenish brown	Light brown	Purplish brown
14	CR powder+ Diethyl ether	Light brown	Yellow	Purple
15	CR powder+ NaOH in H2O	Brown	Green	Pinkish green
16	CR powder+ Methanol	Brown	Light brown	Yellow
17	CR powder+ Picric acid	Yellow	Brown	Yellow brown
18	CR powder+ Iodine solution	Yellowish brown	Brown	Green
19	CR powder+ NH3 solution	Dark brown	Brown	Yellow brown
20	CR powder+ 10%FeCl3	Brown	Light brown	Green

CS: *C. pendulus* stem CR: *C. pendulus* root

Table. 5. Extractive values of *C. pendulus* stem and root.

Part	Solvent	Percent extracts
Stem	Chloroform	1.22%
	Methanol	**8.32%**
	Ethanol	7.46%
	Petroleum ether	0.87%
	n-Hexane	0.66%
	Acetone	0.55%
Root	Chloroform	0.68%
	Methanol	**9.32%**
	Ethanol	8.15%
	Petroleum ether	0.66%
	n-Hexane	0.53%
	Acetone	0.67%

Conclusion and recommendations: *Cocculus pendulus* belongs to the family Menispermaceae locally known as Parwatti, is a dioecious shrubby plant. Morphological and histological features of both plant parts might be useful taxonomic information that can help the species and genera outlining. These characteristics will be more appreciated if other species of the said plant are also evaluated for the same studies. Standardization of the crude drug of both plant parts may be carried out by macroscopic examination. The phytochemical screening revealed the presence of various important phytoconstituents both qualitatively and quantitatively. Due to the presence of such important active constituents, further advance study is recommended to isolate the important secondary metabolites.

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