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MORPHOLOGICAL, ANATOMICAL AND PHYTOCHEMICAL CHARACTERIZATION OF COCCULUS SPECIES (MENISPERMACEAE) IN WESTERN GHATS

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ABSTRACT

The Menispermaceae family of flowering plants comprises of about 71 genera and 450 species. The genus *Cocculus* belongs to the family Menispermaceae comprises about 35 species, distributed throughout the tropical and subtropical countries of the world. Three species of *Cocculus* were found in Western Ghats - *Cocculus hirsutus* (L.) W.Theob., *Cocculus pendulus* (J.R. & G. Forst.) Diels and *Cocculus laurifolius* DC. Menispermaceae are mostly climbing plants and the majority of species contains scientifically recognized important pharmacological activities. The present work aimed at comparative morphology, anatomy and qualitative and quantitative phytochemical screening using two solvent systems (chloroform and methanol). The results revealed that the leaves of *Cocculus hirtusus* and *Cocculus pendulus* are heteromorphic whereas the leaves of *Cocculus laurifolius* are homomorphic. Results also showed that the chloroform leaf extracts show more positive result than that of methanolic extracts. The extractive value (yield percentage) in two solvent system showed that methanolic extract has the maximum yield percentage than that of chloroform extract. Quantitative phytochemical analysis of alkaloids, terpenoids and phenols showed that *Cocculus* species contained more phenolic

compounds followed by alkaloids and terpenoids. The study showed that chloroform and methanol leaf extracts contained improved bioactive compounds. Phytochemical study was also useful to isolate the pharmacologically active principles present in the drug. This justifies the use of these plants as drugs to treat various ailments.

Keywords: Morhological, Phytochemical, Qualitative, Quantitative, medicinal plants

INTRODUCTION

The Menispermaceae comprises of 71 genera with 450 species **[1]**. The genus *Cocculus* belongs to the family Menispermaceae comprises about 35 species, distributed throughout the tropical and subtropical countries of the world **[2]**. Three species of *Cocculus* are found in Western Ghats - *Cocculus hirsutus, Cocculus pendulus* and *cocculus laurifolius*. Menispermaceae are mostly lianas, sometimes small trees or shrubs and occasionally perennial herbs and are mostly dioecious plants. The leaves are alternate, petiolate, sometimes peltate and exstipulate. The lamina is simple, entire or lobed, palmately veined. The family contains a number of plants with scientifically recognized important pharmacological activities **[3, 4]**.

The genus *Cocculus* consist of three species in Western Ghats, *Cocculus hirsutus, Cocculus pendulus* and *Cocculus laurifolius.*

Cocculus hirsutus (Linn.) Diels is a straggling shrub, widely distributed in tropical climate especially in dry regions. In Ayurveda, C. hirsutus is known as Patalagarudi. The leaves and roots of *C. hirsutus* have proved to treat a variety of diseases such as jaundice, bronchitis, gonorrhoea, and leprosy **[5]**. It is used as a first aid medicine for small injuries. *C. hirsutus* have great potential in treating diabetes mellitus by controlling blood sugar **[6]**.

Cocculus pendulus (J.R. & G. Forst.) Diels is a scandent shrub which is found in the dry mountainous areas and also along rocks. The juice of the leaves contains mucilage when mixed with water forms a jelly like substance can be used as a cooling medicine for gonorrhea **[8]**. The alkaloid obtained from leaves and stem have hypotensive and anticancer activity **[9]**.

Cocculus laurifolius DC. is known as laurel leaved snail tree. The plant has also been used as a diuretic and as a vermifuge. *Cocculus laurifolius* have been reported for its muscle relaxant and hypotensive activity. The plant has significant importance as it has been used traditionally in the treatment of headache, hypertension, abdominal pain,

rheumatic pain, and in the healing of scalp wounds **[7]**. In US it is grown as a slow growing ornamental plant.

Chemotaxonomy is widely used as a taxonomic approach to identify, classify and position of closely related taxa. The taxonomic position of a particular plant can be determined by the presence or absence of a particular phytochemical in a plant along with the knowledge of its biochemical synthetic pathways **[10]**. Nature of chemicals is given more significance to the identification of species and in solving the taxonomic problems. Screening and quantification of bioactive compounds has lead to the invention of new medicinal drugs which have the efficient capacity to treat various ailments including cancer **[11]**.

MATERIALS AND METHODS

MORPHOLOGICAL STUDIES

Aerial parts (stem and leaves) were collected from Western Ghats. The healthy plants were selected for the morphological investigation. The specimens were identified with the help of floras, Revisions and Monographs and also referred to experts for accurate identification. Macroscopic analysis of the selected plant leaf was carried out according to the method of Evans **[12]**.

ANATOMICAL STUDIES

Stems were collected and a free hand section method was carried out for anatomical studies. For this 1% safranin were used and mounted in glycerine. The slides were analysed by stereomicroscope (Leica DM 500). Photographs were taken using Leica Las EZ software.

PREPARATION OF PLANT EXTRACT

The collected plant parts were cleaned, shade dried and powdered by a mechanical grinder. 10 g of the sample was extracted in a soxhlet apparatus using 150 ml of chloroform and ethanol as the solvent, and the soxhlet was run overnight until the sample loaded became colorless. After the soxhlet extraction is over, extracts were put inside rotor evaporator until it gave the solidified residues. The sample extracts were collected, weighed and stored in a freezer.

QUALITATIVE AND QUANTITATIVE ANALYSIS OF SECONDARY METABOLITES

The preliminary phytochemical analysis was performed using the standard procedures by Harborne to identify the active constituents **[13]**. Quantitative phytochemical study was done by standard procedure. The alkaloids estimation was performed by spectrophotometric method of Dragendroffs reagent as it was described by Sreevidya and Mehrotra **[14]**. The total terpenoid content

was determined by spectrophotometric method. The phenolic estimation was performed by spectrophotometric method of Folin Cio-calteau reagent **[15]**.

STATISTICAL ANALYSIS

All the experiments were performed in triplicates and the results were expressed as Mean±Standard Deviation.

RESULTS AND DISCUSSIONS

The three species of *Cocculus* were collected from Western Ghats. They were examined for their proper identification. Collection, locations and their vernacular names were given in **Table 1 and Figure 1** (A- *Cocculus hirsutus*, B- *Cocculus pendulus*, and C- *Cocculus laurifolius*).

MORPHOLOGICAL STUDIES

Cocculus hirsutus

The results showed that the lower branches of the plant *Cocculus hirsutus* have trilobed leaves and upper branches have ovate or lanceolate leaves with obtuse to rounded apex and cordate to truncate base. The macroscopic observations of the leaves highlighted that the leaf margins of *Cocculus hirsutus* had lobed margins and are densely pubescent on both sides when young and subglabrous later. Lamina is hairy, venation is reticulate with 5-6 pairs of alternating lateral veins **[16]**. Petiole is stout and glabrescent. Flowers are small, unisexual and found in axillary panicles. In male flower, six hairy sepals are present and six petals which are shorter than the sepal. Stamens are six, free, embraced by the petals. Female flowers are tricarpellary, free and six staminods are present. The fruit is drupe, small, globose and black when ripe.

Cocculus pendulus

Cocculus pendulus leaf is heteromorphic, lower branches of the plant have trilobed leaves and upper branches have ovate or lanceolate with mucronate apex and truncate base, lobed leaf margin and are glabrous or slightly pubescent on both sides. . Leaves of *C. pendulus* are glabrous or slightly pubescent on both sides with 3-5 basal nerves and the microscopical examinations revealed that the leaf was bilateral, mesomorphic and stomatiferous **[17]**. Petiole is stout and glabrescent. Flowers are small, unisexual and found in axillary panicles. In male flower, six sepals in two rows slightly hairy to glabrous and six petals auricled below. Stamens are six, free embraced by the petals. Female flowers are tricarpellary, free, ovoid and six staminoid are present. The fruit are reddish drupe, small globose and black when dry.

Cocculus laurifolius

Cocculus laurifolius leaf is ovate-lanceolate with acuminate apex and acute base. Petiole is short and glabrescent. Flowers are small,

unisexual and found in axillary panicles. Six sepal arranged in two rows outer elliptic and inner ovate in shape. Six petals are present and are glabrous. Stamens six, free and ovate. Female flowers are tricarpellary, free and contain six staminodes. The fruit is a drupe, glabrous, black when ripe.

ANATOMICAL STUDIES

Cocculus hirsutus

The cork comprises of an outer 2 layer of reddish highly compressed thick-walled cells and inner 3-4 layers of colourless thin-walled cells. The cortex consists of 6-8 layers of collenchymatous cells. The sclerenchyma forms a continous ring joined by stone cells and prism-shaped crystals around the stelar region. Stelar region composed of successive rings of vascular bundles **[18]**. 23-25 discrete wedge-shaped strips of xylem, surrounded by 6-8 layers of phloem externally. The adjacent vascular bundles are connected by parenchymatous narrow medullary rays which are lignified. Cambium composed of 3-4 layers of loosely arranged thin walled cells. Xylem consists of vessels, tracheid, parenchyma and fibers with more number of xylem vessels and large xylem vessels. Small round to oval shaped vessels are present mostly solitary sometimes in pairs. Pith comprises of large thin-walled compactly arranged parenchymatous cells. The length of fibres and the height and width of xylem rays increase gradually from the centre towards the periphery of the stem **[18]**.

Cocculus pendulus

The cork comprises of an outer 10-12 layer of dark brown tangentially elongated thick-walled cells and inner 3-4 layers of colourless thin-walled cells. The cortex consists of 4-6 layers of collenchymatous cells. The sclerenchyma forms a continous ring joined by stone cells around the stelar region. Stelar region composed of successive rings of vascular bundles with 20-22 discrete wedge-shaped strips of xylem, surrounded by 5-6 layers of phloem externally. The adjacent vascular bundles are connected by parenchymatous high and narrow medullary rays which are unlignified and contain numerous crystals. Cambium composed of 4-6 layers of loosely arranged thin walled cells. Xylem consists of vessels, tracheid, parenchyma and fibers with more number of xylem vessels and large xylem vessels. Small round to oval shaped vessels are present mostly solitary sometimes in pairs. Pith comprises of large thick walled compactly arranged parenchymatous cells. *C. pendulus* wood with wide xylem vessels enhances water flow to the upper canopy and the abundance of ray parenchyma cells between xylem provides great flexibility. These

features are crucial for the survival of the plant under the harsh conditions **[19]**.

Cocculus laurifolius

The cork comprises of an outer 4-5 layer of reddish brown highly compressed thick-walled cells and inner 2-3 layers of colourless thin-walled cells. The outer layer of cortex consists of 5-7 layers of collenchymatous cells and 3-5 parenchymatous layer of cells. The sclerenchyma forms a continous ring joined by stone cells. Stelar region composed of 2 successive rings of vascular bundles **[20]** with 30-32 discrete wedge-shaped strips of xylem, surrounded by 10-12 layers of phloem externally. The adjacent vascular bundles are connected by parenchymatous high medullary rays which are multiseriate and lignified and crystals are absent in the ray portion. Cambium composed of 3-4 layers of loosely arranged thin walled cells. Xylem consists of vessels, tracheid, parenchyma and fibers with more number of xylem vessels with narrow size **[20]**. Small round to oval shaped vessels are present mostly solitary sometimes in pairs. Tyloses are absent. Pith comprises of large thick walled loosely arranged parenchymatous cells and are irregular in shape.

PHYTOCHEMICAL SCREENING

The physical parameters of the extracts were examined and tabulated in **Table 3**. The parameters include colour and texture of the extract and yield percentage. The results showed that the extractive yields of leaves are more in methanolic extract than in chlorofomic extract. The extractive values provide a key role to evaluate the chemical constituents present in the crude drug and also helps in the estimation of specific constituents soluble in a particular solvent **[21, 22]**.

QUALITATIVE ANALYSIS OF SECONDARY METABOLITES

Phytochemical investigation of selected plants in two different solvent systems revealed that chloroform extract shows more secondary metabolites than methanolic extracts **(Table 2)**. The preliminary phytochemical investigation of *C. hirsutus* and *C pendulus* showed the presence of alkaloids, phenols, flavanoids, glycosides, saponins and steroids and similar observations were documented **[23]**. The phytochemical studies of *C. hirsutus* showed the presence of bis-benzyl isoquinoline alkaloids which are isolated from stem and root **[24]**. The qualitative examinations of *C. pendulus* revealed the presence of various phytoconstituents like alkaloids, carbohydrates, phytosterols, proteins and

mucilages in the leaf extracts **[23]**. Alkaloids, phenols, terpenoids, glycosides, saponins and resins were reported *C. laurifolius*.

QUANTITATIVE ANALYSIS OF SECONDARY METABOLITES

The present study showed that the extracts of cocculus species contain various bioactive molecules in different concentration. The cocculus species contained more phenolic compounds followed by alkaloids and terpenoids and similar observations were also documented **[25]**. Alkaloids are more in methanolic extract than in chlorofomic extract. The *C. hirtusus* extract showed more alkaloids than the other two species and were tabulated in **Table 4**. *Cocculus hirsutus* fruits were evaluated as major source of anthocyanin, flvanoids and polyphenols and the fruit extract showed potential antioxidant activity **[26]**.

Terpenoids are more in methanolic extract than in chlorofomic extract and the extracts showed significant terpenoid content in *C. laurifolius* (201.35 ± 0.63) than the other two species and were shown in **Table 4**. Phenols are also more in methanolic extract than in chlorofomic extract and the extracts showed significant phenolic content in *C. laurifolius* (347.97 ± 0.54) than the other two species and were shown in **Table 4** and in *C. laurifolius* bark contain high amount of alkaloids and flavanoids and phenolic content and saponins were present in leaf **[25]**.

Table 1: Location of Collected Species

Sl. No.	Taxon	Vernacular name	Location
1.	***Cocculus hirsutus***	**Broom creeper or Patalgarudi**	**Tirunelveli (8° 40' 4.836'' N 77° 49' 22.764'' E)**
2.	***Cocculus pendulus***	**Pilwan**	**Anaikatti (11° 5' 50.6688'' N 76° 47' 30.7968'' E)**
3.	***Cocculus laurifolius***	**Laurel-Leaved Snail Tree**	**Palode (8° 45' 16.794'' N 77° 1' 27.5592'' E)**

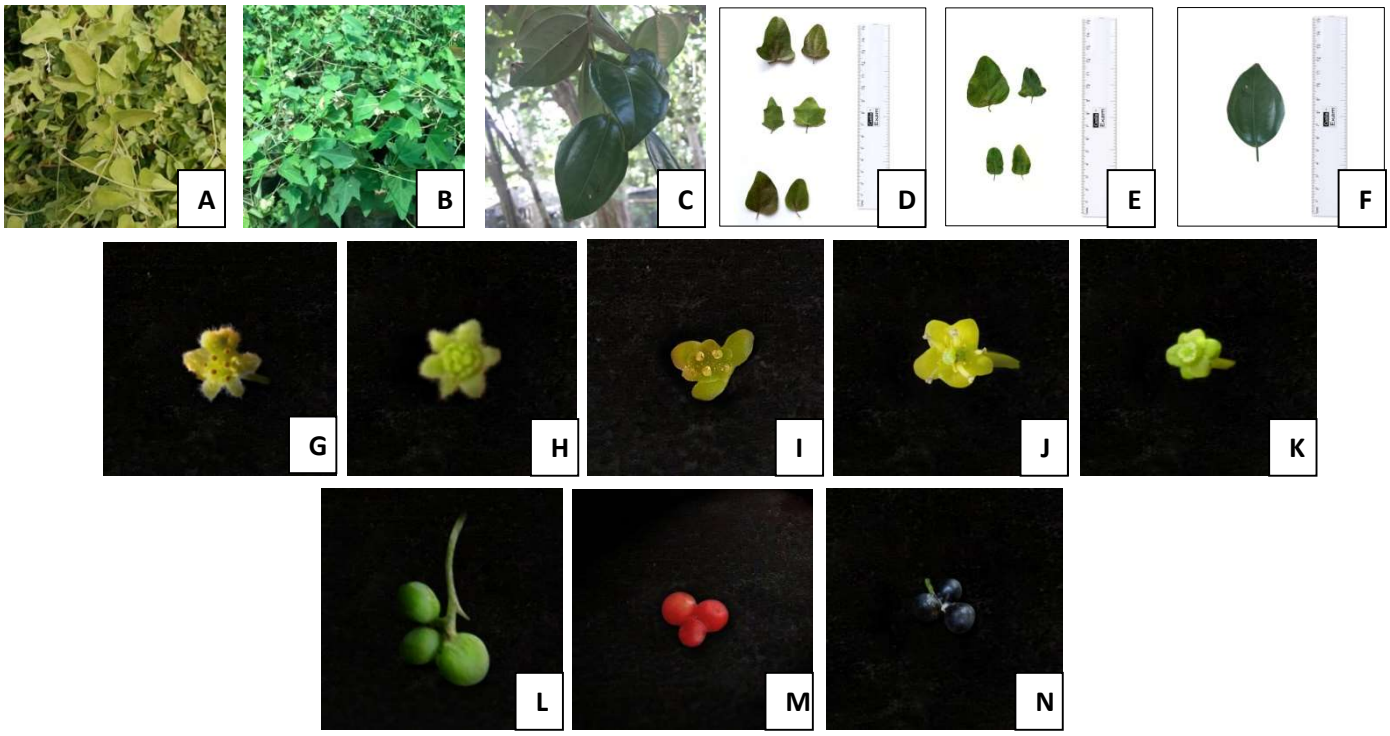
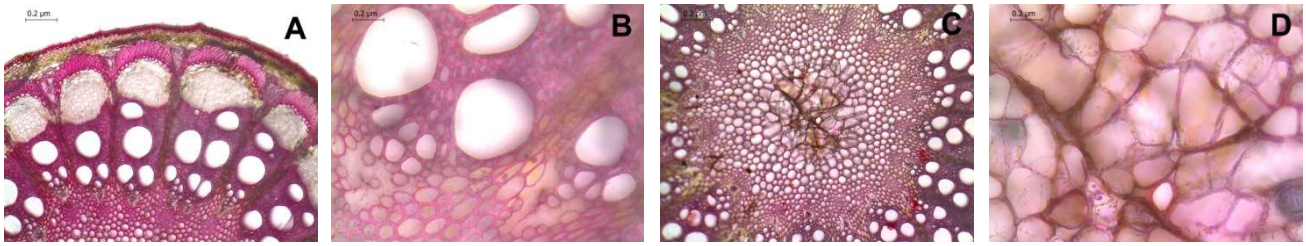
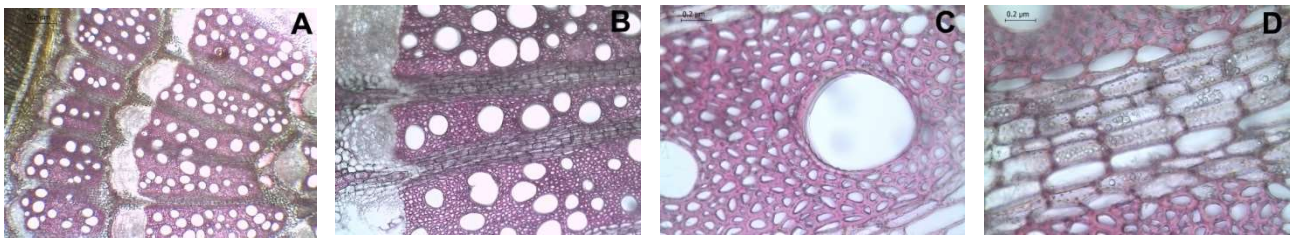


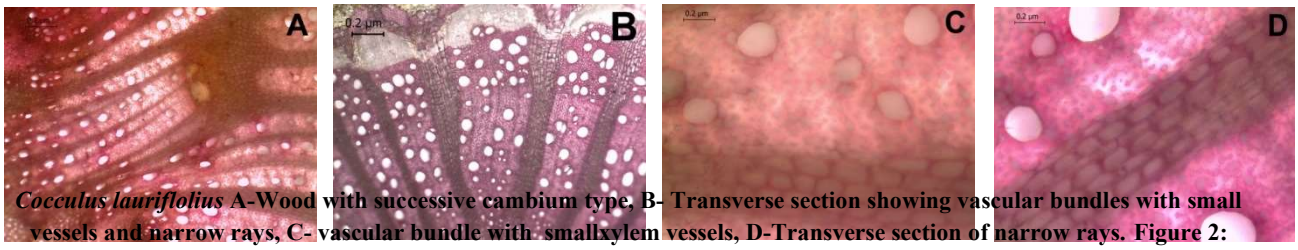
Figure 1: Morphology of selected plants
***Cocculus hirsutus* (L.) W.Theob. A-Habit, D-Leaf morphology, G-Male flower, H-Female flower, L- fruit *Cocculus pendulus* (J.R. & G. Forst.) Diels B-Habit, E-Leaf flower, I-Male flower, M-Fruit**
***Cocculus laurifolius* DC. A-Habit, F- Leaf morphology, J-Male flower, K-Female flower, N- Fruit**



***Cocculus hirsutus* A -Transverse section showing vascular bundles with less number of xylem vessels , B- vascular bundle with xylem vessels, C-Transverse section showing pith portion, D-section showing enlarged pith region**



***Cocculus pendulus* A-Wood with successive cambium type, B- Transverse section showing vascular bundles with more number of xylem vessels , C- vascular bundle with xylem vessels, D-Transverse section rays**



Cocculus lauriflolius **A-Wood with successive cambium type, B- Transverse section showing vascular bundles with small vessels and narrow rays, C- vascular bundle with smallxylem vessels, D-Transverse section of narrow rays. Figure 2: Stem anatomy of Cocculus species**

Table 2: Table Showing Colour, Texture of Extract And Extractive Value of Leaf

Sl. No.	Name of the plant	Name of different solvent	Colour of extract	Texture of extract	Extractive value of leaf (% w/w)
1.	Cocculus hirsutus	Methanol	Reddish Green	Semi Solid	5.67
2.		Chloroform	Dark Green	Waxy	3.46
3.	*Cocculus pendulus*	Methanol	Brown	Semi Solid	5.16
4.		Chloroform	Dark Green	Waxy	5.11
5.	*Cocculus laurifolius*	Methanol	Brown	Semi Solid	6.61
6.		Chloroform	Dark Brown	Semi Solid	5.93

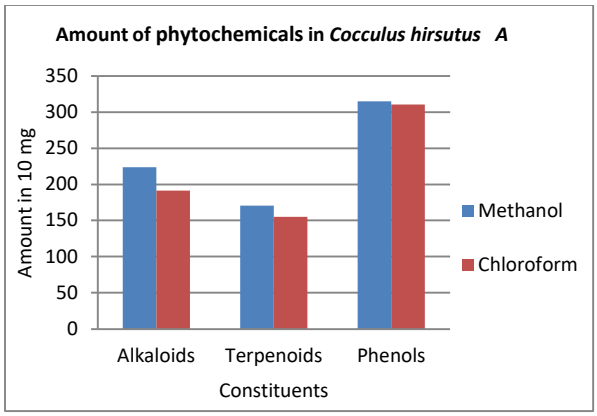
Table 3: Phytochemical Screening of Selected Plants

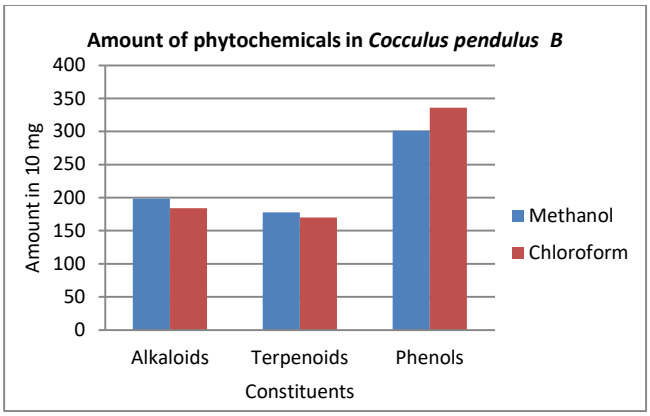
Sl. No.	Name of the test	Chloroform			Methanol		
		A	B	C	A	B	C
1.	Carbohydrate test	+	+	+	+	+	+
2.	Protein test	+	+	+	-	-	-
3.	Aminoacid	-	-	-	-	-	-
4.	Tannin and phenolic compound	+	+	+	+	+	+
5.	terpenoid	+	+	+	+	+	+
6.	Steroid	+	+	-	+	-	-
7.	Glycosides	+	+	+	-	-	-
8.	Quinone	-	-	-	-	-	-
9.	Anthraquinone	-	-	-	-	-	-
10.	Saponin	+	-	-	+	-	+
11.	Alkaloid	+	+	+	+	+	+
12.	Coumarin	-	-	+	-	-	+
13.	Flavanoid	+	+	+	+	-	+
14.	Resin	+	-	-	-	-	+

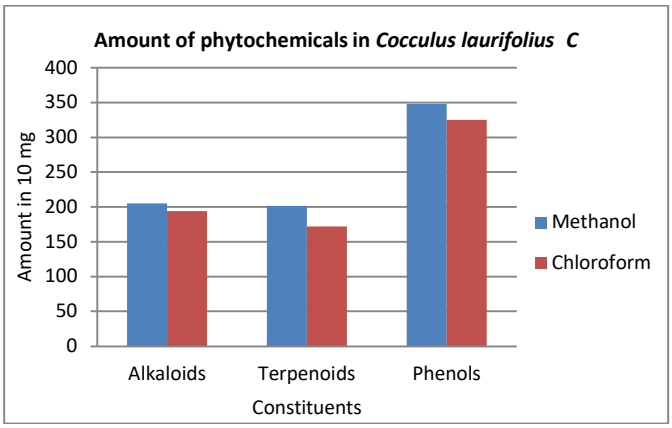
Table 4: Quantitative Analysis Of Phytochemical Compounds

Sl. No.	Name of the plant	Solvent used	Amount of Alkaloid	Amount of Terpenoids	Amount of Phenol
1.	Cocculus hirsutus	Methanol	223.79 ± 0.12	170.71 ± 0.48	314.77 ± 0.68
2.		Chloroform	191.26 ± 0.33	154.96 ± 0.44	310.54 ± 0.59
3.	Cocculus pendulus	Methanol	198.70 ± 0.30	177.80± 0.98	301.24 ± 1.14
4.		Chloroform	184.05 ± 0.70	169.94 ± 0.70	336.09 ± 0.48
5.	Cocculus laurifolius	Methanol	204.89 ± 0.27	201.35 ± 0.63	347.97 ± 0.54
6.		Chloroform	194.25 ± 0.36	172.02 ± 0.87	324.87 ± 0.73

A-*Cocculus hirsutus*, B-*Cocculus pendulus* and C-*Cocculus laurifolius*







Graphs showing the amount of phytochemicals (Alkaloids, Terpenoids and Phenols)
A-*Cocculus hirsutus*, B-*Cocculus pendulus*, C-*Cocculus laurifolius*

CONCLUSION

The morphological studies of the leaves highlighted that the leaf margins of *Cocculus hirsutus* and *Cocculus pendulus* had lobed margins and in case of *Cocculus laurifolius* the leaf margin is entire and leaves are shining. Size of the xylem vessels can be used as an identifying feature to distinguish the cocculus species. The study showed that the extracts contain major bioactive molecules such as alkaloids, phenols and terpenoids. Studies on phytochemical screening can provide a valuable source of information to determine the amount of secondary metabolite qualitatively and quantitatively. Phytochemical study was also useful to isolate the pharmacologically active principles present in the drug. This justifies the use of these plants as drugs to treat various ailments.

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