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PHYSICO-CHEMICAL EVALUATION OF WHOLE PLANT OF *COCCULUS PENDULUS* (J.R. & G. FORST.) DIELS

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ABSTRACT: The whole plant of *Cocculus pendulus* (J.R. & G. Forst.) Diels is reported to have quality medicinal values in traditional system of medicines. The various physico-chemical parameters included determination of extractive value, loss on drying, ash values, fluorescence analysis, HPTLC analysis and preliminary phytochemical analysis were performed. The preliminary identification of *Cocculus pendulus* was observed by studying macroscopical features and the final authentication was confirmed by the botanist. Preliminary phytochemical analysis of whole plant of *Cocculus pendulus* revealed the presence of various phytoconstituents like alkaloids, carbohydrates, phytosterols, saponins, proteins and mucilage. HPTLC fingerprint analysis of ethanolic extract of plant *Cocculus pendulus* reveals presence of 13 peaks and 10 peaks in 256 nm and 366 nm respectively. The physico-chemical evaluation of *Cocculus pendulus* was a useful tool to identify quality, purity and authenticity of *Cocculus pendulus*.

INTRODUCTION: The genus *Cocculus* specified to the family Menispermaceae. The plants of this genus are shrubs or woody climbers. It constitutes about 35 species, scattered throughout the tropical and subtropical countries of the world. The members of this genus are used in the classic system of medicine for various infirmities^{1, 2, 3}. Only two species of *Cocculus* are found in the Tirumala locality of Andhra Pradesh, i.e. *Cocculus pendulus* and *Cocculus hirsutus*. The plant *Cocculus pendulus* (synonym: *Cocculus leaeba*) is a scandent shrub which is originating particularly in accomplished areas, along rocks and in the dry mountainous areas of Seshadri and Venkatadri hills of Chittoor district.

The fruits are consumable and used to make an intoxicating drink from the fruits and flowers are mixed with food. Diluted leaf-juice with sugar is a good tonic, the juice contains mucilage when it is mixed with water forms a jelly substance which is applied outwardly in skin diseases. This is taken as a cooling medicine for gonorrhoea, stem bark and root bark decoctions are used antonym to the intestinal parasites and gonorrhoea^{4, 5}.

Roots are part of medicines resistance to constipation as laxative, helminthic, malaria and used as cholagogue. The root has a great regard against biliousness and menstrual causes and as a diuretic. Root decoction is used together with *Tinospora bakis* (A. Rich.) Miers, to formulate a stimulating tonic. Roots and leaves are used in jaundice, yellow fever, leprosy, syphilis, inflammation in rheumatic pains and aphrodisiac. An infusion of the plant is used to facilitate in removing thorns from the feet. Wood infusion is taken as an emetic. In the dried parts of the plant are used for livestock^{4, 5}.

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The present investigation was prospective to study the detailed physico-chemical aspects of *Cocculus pendulus* (J.R. & G. Forst.) Diels. The evaluation of herbal drugs has experience of various changes over the years. During ancient days the plant materials were identified and analysed with standard descriptions available in standard reference books by comparison. The present work highlighted on physico-chemical evaluation of whole plant of *Cocculus pendulus* (J.R. & G. Forst.) Diels to conform quality and authenticity⁶.

Taxonomical Classification:

Kingdom : Plantae
 Phylum : Tracheophyta
 Class : Magnoliopsida
 Superorder : Ranunculanae
 Family : Menispermaceae Juss
 Genus : *Cocculus*
 Species : *Cocculus pendulus*

Common Names:^{6,7}

Rajasthani : Pilwan
 Punj : Vehri, Parwatti
 Arab : Ssag-el-ghorab
 Sind : Ullarbillar
 Telugu : Dusaratige
 Gujarati : Parwatti
 Tamil : Villumbi

Morphology of *Cocculus pendulus*:^{8,9}

Flower: Flowers of the *Cocculus pendulus* are small and unisexual, which consist of 6 sepals, fleshy to membranous, ovate-elliptical, 3 inner ones larger finely hairy and 3 outer ones slightly hairy, 6 petals which are ovate-obovate and it contains notched apex; and peduncles are long; flowering period is nearly whole time of the year.

Tepal or petal colour is green. Free Female flowers: With pedicel (up to 1 cm long), Male flowers: Sessile or with short pedicel, 6-9 stamens, stigma (0.5 mm long), staminodes (61 mm long), ovary superior, consisting of 3(-6) free, ovoid and laterally compressed carpels (1 mm long).

Fruit: Fruits are one seeded and dark red in colour, stone ribbed on lateral faces. Each drupe are of 4 - 7 mm × 4 - 5 mm, drupes are ovoid to obovoid or reniform and flattened reddish, black when dry;

endocarp ribbed on lateral faces, not crested, not perforated in middle.

Root: Roots are dark brown in colour externally.

Leaf: Arranged Alternate (one leaf per node), simple, margin is smooth; stipules absent; petiole 2 - 10 mm long; blade oblong-lanceolate, in lower leaves sometimes ovate, 1.5 - 5 cm × 0.5 - 2 cm, base cuneate, rounded or sometimes spear-shaped, apex obtuse, with micro, sometimes notched, leathery, glabrous, basal veins 3, conspicuous. Stems: Branches long, slender, terete, puberulous, up to 15 cm in diameter at base, striped, bark grayish brown to dark brown or dark grey.

MATERIALS AND METHODS:

Collection, Authentication and Processing of Plant Materials: The plant materials for the present research was gathered from the wild source of the region of Tirumala hills. While collecting the plant material, care was taken to identify the healthy plant. After immediate collection the plant material was authenticated by Dr. T. Madhava Shetty Botanist Sri Venkateswara University, Tirupati. Plant (*Cocculus pendulus*) voucher no. 1122. The collected whole plant materials were shade dried for 15 days and reduced the size by using grinding mill into coarse powder. It was stored in a well closed transparent container, it can be used for further process¹⁰.

Physical Evaluation: Physical constants of crude drugs like loss on drying, total ash, acid insoluble ash, water soluble ash, extractive values, fluorescence analysis and TLC analysis were determined by using official standard methods prescribed in various pharmacopoeias and reference books¹¹.

Method of Extraction: Dried whole plant of *Cocculus pendulus* 1.5 kg was extracted by using three different solvents (Petroleum ether, Ethyl acetate, Ethanol) with successive hot continuous percolation method in Soxhlet apparatus. The extracts were concentrated on a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Preliminary Phytochemical Investigation: The extracts obtained during the extraction process were subjected to preliminary phytochemical

screening to determine the presence of various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, fixed oils, fats, saponins, phenolic compounds, triterpenes, flavonoids, proteins, aminoacids, gums, mucilage, tannins and volatile oils by using reported methods^{12, 13}.

HPTLC Analysis:

Instrument Details:

Name of the Instrument: CAMAG (CAMAG - Automatic TLC sampler, Scanner and Visualiser).

Spray Gas: N₂

Lamp used: Deuterium and Tungsten Lamp.

The sample - *Cocculus pendulus* (ethanolic extract - 5µl) was applied in TLC aluminium sheet silica gel 60 F 254 (E. Merck) and plate was developed using the solvent system Toluene: Ethyl acetate: Formic acid (8.5: 1.5: 01). After development the plate was allowed to dry in air and examined under UV - 254 nm, 366 nm and visible light after derivatised using vanillin – sulphuric acid^{14, 15}.

RESULTS AND DISCUSSION:

TABLE 1: PHYSICAL PARAMETERS OF WHOLE PLANT OF COCCULUS PENDULUS

S. no.	Parameters	Determined value (%w/w)
1	Extractive values	
	Alcohol soluble	4.56±0.8
	Water soluble	9.25±1.3
	Ethyl acetate	3.81±0.9
2	Petroleum Ether	3.93±0.7
	Ash values	
	Total ash value	5.31±0.9
	Acid insoluble ash	1.85±0.6
3	Water soluble ash	1.27±0.4
	Loss on drying	3.12±0.8

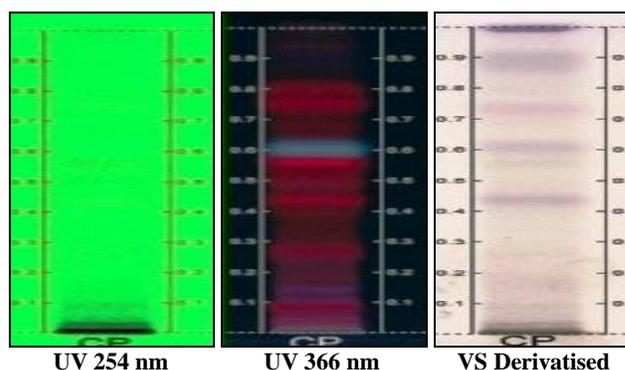


FIG. 1: TLC PLATES OF PLANT COCCULUS PENDULUS

TABLE 2: FLUORESCENCE ANALYSIS OF WHOLE PLANT OF COCCULUS PENDULUS

Treatment	<i>Cocculus pendulus</i>		
	Day light	UV Light	
		Short UV (264 nm)	Long UV (366 nm)
Powder as such	Pale Brown	Green	Pale Green
Powder + 1N NaOH (alc.)	Dark Yellow	Dark Green	Pale Green
Powder + 1N NaOH (aq.)	Dark Green	Brownish Green	Dark Green
Powder + 1N HCl	Pale Brown	Dark Green	Green
Powder + 1N H ₂ SO ₄	Dark Brown	Black	Pale Black
Powder + 1N HNO ₃	Dark Yellow	Dark Green	Black
Powder + NH ₃	Brownish Green	Blackish Green	Black
Powder + 5% FeCl ₃	Dark Green	Brownish Green	Blackish Green
Powder + acetic acid	Pale Green	Dark Green	Brownish Green
Powder + 5% iodine	Dark Green	Blackish Green	Purplish Green

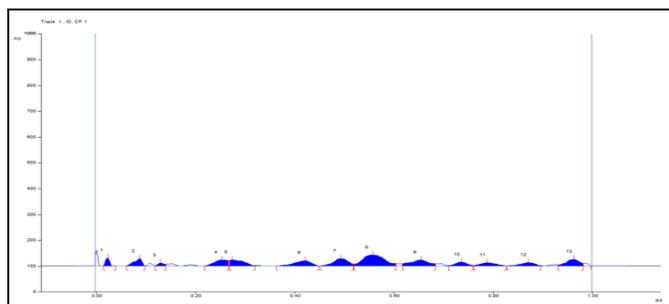


FIG. 2: HPTLC CHROMATOGRAM OF COCCULUS PENDULUS AT 254 nm

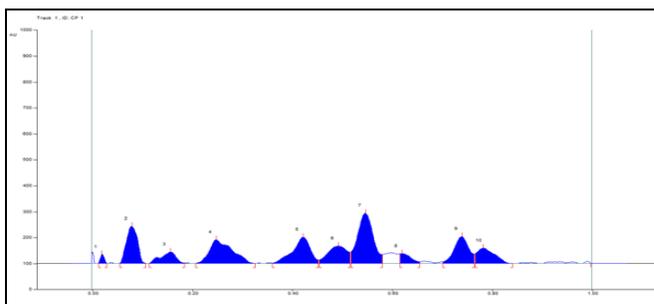


FIG. 3: HPTLC CHROMATOGRAM OF COCCULUS PENDULUS AT 366 nm

The present analysis reported in a detailed physico-chemical evaluation of whole plant of *Cocculus pendulus*, its physico-chemical constants included

ash values, loss on drying and different extractives values, TLC analysis was studied to determine purity, quality and identity. The moisture quantity

was determined through loss on drying and it was 3.12% w/w. The ash values were identified to estimate amount of inorganic salts present in the whole plant of *Cocculus pendulus* to identify adulteration. The total ash, acid insoluble ash, water soluble ash values were 5.31% w/w, 1.85% w/w and 1.27% w/w respectively. The preliminary phytochemical analysis was studied to Petroleum ether, ethyl acetate and ethanol extracts resulted the presence of alkaloids, flavonoids, glycosides, saponins, sterols. Results from fluorescence

analysis supported the purity, identity, quality and authenticity. HPTLC finger print analysis shows the range of 0.02 to 0.93 at 256 nm and 0.01 to 0.73 at 366nm and derivatisation by vanillin - sulphuric acid shows presence of different colours bands and percentage of the peak area is more in R_f value 0.52, 0.62, 0.45 that is 23.04%, 10.53%, 10.44 at 256 nm and 366 nm the percentage of peak area is more in R_f value 0.52, 0.21, 0.36 that is 22.35%, 15.24%, 13.13%.

TABLE 3: PRELIMINARY PHYTOCHEMICAL SCREENING OF WHOLE PLANT EXTRACTS OF COCCULUS PENDULUS

	Chemical tests	Extracts		
		Pet. ether	Ethyl acetate	Ethanol
1	Carbohydrates and glycosides			
	Molisch's test	+	+	+
	Fehling test	-	-	+
	Benedict's test	-	-	+
	Legal's test	-	-	+
2	Alkaloids			
	Dragendorff's	-	+	+
	Wagner's	-	+	+
	Hager's	-	+	+
	Mayer's	-	+	+
3	Phenolic compounds and tannins			
	Ferric chloride test	-	-	+
	Bromine water	-	-	+
	Lead acetate test	-	-	+
4	Fixed oils and fats			
	Spot test	+	-	-
	Saponification test	+	-	+
5	Phytosterols			
	Salkowski test	+	+	-
	Liebermann-Burchard's test	+	+	-
6	Tannins	-	-	-
7	Proteins and Amino acids		+	-
8	Flavonoids		-	+
9	Triterpenes	+	-	-
10	Gums and mucilages	-	+	-
11	Volatile oils	-	-	-

TABLE 4: HPTLC FINGER PRINT ANALYSIS OF ETHANOLIC EXTRACT OF PLANT COCCULUS PENDULUS

Extract	Solvent system	Numbers of peaks	R _f	% Area
Ethanolic extract of plant <i>Cocculus pendulus</i> (254nm)	Toluene: Ethyl acetate: Formic acid (8.5: 1.5: 01).	13	0.02, 0.06, 0.12, 0.22, 0.27, 0.36, 0.45, 0.52, 0.62, 0.71, 0.76, 0.83, 0.93	3.08, 5.33, 1.74, 7.82, 8.13, 8.16, 10.44, 23.04, 10.53, 4.23, 4.62, 4.47, 8.42
Ethanolic extract of plant <i>Cocculus pendulus</i> (366nm)	Toluene: Ethyl acetate: Formic acid (8.5: 1.5: 01).	10	0.01, 0.06, 0.11, 0.21, 0.36, 0.45, 0.52, 0.62, 0.70, 0.77	1.00, 11.78, 5.04, 15.24, 13.13, 9.62, 22.35, 3.01, 11.26, 7.58

CONCLUSION: Preliminary screening tests may be useful in the identifying of the bioactive principles and subsequently may lead to the drug discovery and its improvement. Further, these tests provide their quantitative estimation and qualitative isolation of pharmacologically active compounds.

Phytochemical study was also useful to isolate the pharmacologically active principles present in the drug.

HPTLC finger printing helps in differentiating the adulterant and species. It can act as a biochemical

marker for *Cocculus pendulus* in the plant studies and pharmaceutical companies. Thus, as folk medicine *Cocculus pendulus* has many uses as a multipurpose medicinal agent so further clinical trials should be performed to prove its efficacy. Because of their wide utilization, the plant deserves special research attention of these uses and compounds.

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CONFLICT OF INTEREST: Nil

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