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Physicochemical, phytochemical and spectroscopic profile of ethanolic leaf extract of *Cocculus hirsutus* L. prepared by ultrasound-assisted extraction

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KEYWORDS

Cocculus hirsutus (L.). ultrasound-assisted extraction, physicochemical, phytochemical screening; total phenolic content, total flavonoid content; FT-IR, UV–Visible spectroscopy

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Abstract

Cocculus hirsutus (L.) (Menispermaceae) is a medicinal plant with a well-established history of traditional use and a chemically diverse profile. This study is aimed at characterizing selected physicochemical properties of powdered leaves of *C. hirsutus*, as well as the qualitative phytochemical and spectroscopic profile of a 70% ethanolic leaf extract obtained through ultrasound-assisted extraction (UAE). The loss on drying, ash values, and solvent-extractive values will be assessed using standard pharmacognostic techniques. Preliminary phytochemical screening will focus on identifying glycosides, alkaloids, flavonoids, terpenoids, saponins, tannins, phenolics, carbohydrates, and proteins/amino acids. Total phenolic and flavonoid contents will be measured through colorimetric assays, and FT-IR and UV-Visible spectroscopy will be utilized to create preliminary spectral fingerprints. Collectively, the physicochemical, phytochemical and spectroscopic findings demonstrate that the 70% ethanolic leaf extract of *C. hirsutus* is a rich source of phenolic and flavonoid constituents and provides a characteristic preliminary phytochemical fingerprint. These findings support the potential of *C. hirsutus* leaves as a valuable source of bioactive plant metabolites and provide a basis for further compound-specific chromatographic and spectrometric investigations.

Introduction

Historically, medicinal plant species have been extensively utilized in traditional medicine, and they continue to be regarded as potential sources for the development of therapeutic agents for a variety of diseases. Therefore, it is crucial to critically assess and comprehend the existing scientific literature regarding traditional medicinal plant species and their significance. *Cocculus hirsutus* (L.) Diels is a perennial climbing plant that is commonly found in tropical and subtropical regions [1]. Historically, the plant *Cocculus hirsutus* has been valued for its distinctive ability to heal various wounds, cuts, and boils rapidly and with minimal discomfort. Additionally, it is employed in the treatment of conditions such as spermatorrhoea, gonorrhoea, diarrhea, urinary issues, and hyperglycemia[2]. The entire aerial portion of the plant is utilized for the treatment of ocular ailments. A decoction made from the leaves is administered for dysentery, eczema, and urinary issues. Both

the roots and leaves are employed for Sarsaparilla, serving as a diuretic and in the management of gout[3].

Different parts of the plant, particularly the leaves, roots, and aerial components, have been traditionally used for healing wounds, addressing urinary disorders, treating dysentery, managing eczema, alleviating gastrointestinal issues, and combating inflammatory conditions [5,8]. Experimental investigations have validated some of these traditional uses, including diuretic [8], nephroprotective [9], antibacterial [11], antidiabetic, and spermatogenic effects [12].

The significance of C. hirsutus in biological contexts is attributed to its rich phytochemical diversity, which includes a variety of compounds such as alkaloids, flavonoids, glycosides, terpenoids, tannins, saponins, and phenolic substances [5,7]. Research has also focused on specific constituents, such as the alkaloid jamtine [6]. Advanced methodologies like LC-MS/MS and UPLC-MS have elucidated a complex array of alkaloids, flavonoids, triterpenoids, and steroids [14]. Additionally, further alkaloids have been discovered in the roots, emphasizing the chemical richness of this species [15]. Investigations employing quantitative and instrumental techniques have examined marker compounds, including rutin, quercetin, and liquiritin, while GC-MS has been utilized to profile bioactive components in the leaves [10,13]. The reported antioxidant [16], hepatoprotective [17,18], immunomodulatory, and other biological properties [19] underscore the critical need for thorough phytochemical characterization of C. hirsutus. However, it is important to note that preliminary phytochemical screenings cannot definitively ascertain the identity, concentration, or individual roles of specific metabolites in relation to the observed biological activities.

The phytochemical characterization of medicinal plant extracts is an important preliminary approach for establishing their chemical richness and potential biological significance. Previous investigations by Mungole and co-workers have demonstrated that medicinal plant extracts can contain diverse classes of secondary metabolites, including phenolics, flavonoids, alkaloids, tannins, saponins, steroids and terpenoid compounds, with considerable variation depending on the plant species, plant part and extraction solvent used [20,21]. In particular, Mungole et al. reported the occurrence of several bioactive phytoconstituents in different solvent extracts of *Ipomoea obscura* and emphasized the importance of solvent selection in recovering phytochemically active constituents [20]. Similarly, Mungole and Chaturvedi demonstrated the presence and quantitative occurrence of phenolics, flavonoids, alkaloids, tannins and saponins in *Hibiscus sabdariffa*, highlighting the contribution of these secondary metabolites to the biological potential of medicinal plants [21]. Furthermore, the phytochemical and antibacterial potential reported by Mungole et al. in *Canscora decurrens* further supports the significance of systematic phytochemical profiling of medicinal plant materials [22]. These observations provide a useful basis for the present investigation of the ethanolic leaf extract of *Cocculus hirsutus* L., in which physicochemical parameters, phytochemical composition and spectroscopic characteristics

are collectively employed to establish a comprehensive chemical profile of the extract.

Extraction methods significantly influence the recovery of phytochemicals and the resulting extract composition. Ultrasound-assisted extraction (UAE) provides several benefits compared to conventional extraction techniques by employing acoustic cavitation to enhance the penetration of solvents, break down plant tissues, and aid in the release of intracellular metabolites, which may result in decreased extraction duration and solvent usage [23,24]. The efficiency of the extraction process is determined by several factors, including the solvent's composition, temperature, sonication duration, ultrasonic conditions, and the solid-to-solvent ratio [25]. In this study, aqueous ethanol, particularly at a concentration of 70%, was selected for its effectiveness in recovering a diverse range of polar and moderately polar phytochemicals.

The measurement of total phenolic content (TPC) and total flavonoid content (TFC) is instrumental in understanding the prevalence of key phytochemical groups. The Folin–Ciocalteu assay is a commonly employed method for TPC estimation; however, it reflects the overall reducing capacity and may be influenced by other reducing substances. Therefore, it is recommended that results be reported as phenolic equivalents rather than as absolute concentrations of individual phenolic compounds [26,28]. In a similar vein, the aluminium chloride colorimetric method is widely used for TFC assessment, although its response is dependent on the structure of flavonoids and the conditions under which the assay is conducted [27].

Spectroscopic methods, including FT-IR and UV-Visible spectroscopy, can enhance phytochemical studies by delivering swift chemical fingerprints of plant extracts. FT-IR facilitates the identification of specific molecular vibrations and functional group regions, while UV-Visible spectroscopy offers insights into chromophoric, aromatic, and conjugated components.

Taxonomical Classification:[4]

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Ranunculanae

Family: Menispermaceae

Genus: Cocculus

Species: Cocculus

Materials and Methods

Collection and preparation of plant material

The leaves of *Cocculus hirsutus L.* were gathered from the rural area of Ambad, Jalna District, Maharashtra, India, between January and March 2026. After collection, the material was washed with tap water to eliminate any dust and particulate matter that adhered to it, followed by drying in the shade at room temperature for a duration of 6 to 7 days. Subsequently, the dried leaves were ground into a relatively uniform powder and stored under suitable conditions until further analysis.[7]

Ultrasound-assisted extraction

The powdered dried leaves were combined with 70% ethanol in a 1:5 ratio of sample to solvent and underwent ultrasound treatment in an ultrasonic bath. The extraction process involved a cycle of 15 minutes of sonication, followed by a 15-minute resting period, and concluded with an additional 15 minutes of sonication, all while maintaining the bath temperature at approximately 40 °C. Subsequently, the mixture was processed to isolate the extract from the plant residue, and the solvent was eliminated under the same conditions as those employed in the initial experiment. The extract obtained was then utilized for qualitative phytochemical and spectroscopic analyses.

Physicochemical evaluation

Selected physicochemical parameters of the powdered leaves were determined using established pharmacopoeial or standard pharmacognostic procedures. The measurements included loss on drying, total ash, acid-insoluble ash, water-soluble ash, water-soluble extractive and alcohol-soluble extractive values.[7]

Loss on drying

About 4 g of powdered leaf material was precisely measured and subsequently dried in a hot-air oven at a temperature of 105 °C. The sample was allowed to cool and was weighed at regular intervals until a stable mass was achieved. The loss on drying was determined by calculating the difference between the initial and final weights, and this value was expressed as a percentage of the initial weight of the sample.

Total ash

About 2–3 g of air-dried powder was introduced into a silica crucible that had been weighed beforehand and was then incinerated gradually at a temperature not surpassing 450 °C until a residue devoid of carbon was achieved. The crucible was subsequently cooled in a desiccator and weighed. The total ash content was determined as a percentage of the mass of the air-dried sample.

Water-soluble ash

A total of 25 mL of water was added to the total ash and heated to boiling. The insoluble portion was isolated using an ashless filter medium, subsequently washed with hot water, and then ignited. The mass of the water-soluble ash fraction was determined by subtracting the mass of the water-insoluble residue from the total ash.

Acid-insoluble ash

The ash underwent treatment with 25 mL of dilute hydrochloric acid and was subjected to

gentle heating. The insoluble residue was isolated through filtration, subsequently washed with water, and then ignited until a constant mass was achieved. The percentage of acid-insoluble ash was calculated based on the original air-dried sample.

Water-soluble extractive

A quantity of one gram of powdered substance was subjected to maceration with 20 mL of the designated chloroform-water solvent mixture in a sealed flask for a duration of 24 hours, during which intermittent shaking was performed. Following this, the mixture was filtered, and a specific volume of the filtrate was evaporated to dryness in a pre-weighed dish. The resulting residue was then weighed, and the water-soluble extractive value was determined in accordance with the established protocol.

Alcohol-soluble extractive

A gram of the powdered sample was subjected to maceration with 20 mL of alcohol of the designated strength for a duration of 24 hours, with regular shaking during the initial phase, followed by a period of standing. The resulting mixture was then filtered, and a specific volume of the filtrate was evaporated to dryness in a pre-weighed shallow dish. The remaining residue was dried until a constant mass was achieved, allowing for the calculation of the alcohol-soluble extractive value.

Preliminary phytochemical screening

The 70% ethanolic extract was analyzed through qualitative chemical tests aimed at detecting glycosides, terpenoids, saponins, tannins, flavonoids, alkaloids, proteins/amino acids, phenolic compounds, and carbohydrates. The results of these tests were interpreted based on characteristic changes in color, the occurrence of precipitates, or frothing responses that are indicative of the respective reactions. The methodologies employed were consistent with established phytochemical screening protocols.[5,7,13,14]

Phytochemicals	Test/reagent	Positive indication
Alkaloids	Wagners reagent	Brown/reddish precipitate
Saponins	Froth test	Persistent foam
Glycosides	Keller Killiani test	Interfacial coloration
Tannins	Ferric chloride reaction	Green/brown coloration
Flavonoids	Alkaline reagent test	Yellow coloration
Carbohydrate	Benedict test	Orange /Red precipitate
Protein/Amino acids	Ninhydrin reaction	Blue coloration
Phenolics	Iodine reaction	Transient red color

Total phenolic content

The total phenolic content was determined using a Folin–Ciocalteu colorimetric method. An aliquot of the extract was combined with Folin–Ciocalteu reagent and sodium carbonate under the experimental conditions, leading to color development, which was then measured

at 650 nm against the appropriate solvent blank. Catechol was utilized as the calibration standard in this experimental procedure. Therefore, the final results should be expressed in terms of catechol equivalents, unless the calibration was performed with gallic acid. The numerical result is provided below, awaiting verification of the standard employed. [26,28,29]

Total flavonoid content

The total flavonoid content was assessed using an aluminium-chloride colorimetric technique, with rutin serving as the calibration standard. The extract was subjected to a sequential reaction involving sodium nitrite, aluminium chloride, and sodium hydroxide, after which the absorbance was measured at 510 nm. The concentration was calculated from the rutin calibration curve and expressed in accordance with the equivalence determined by that calibration.[27]

FT-IR spectroscopy

FT-IR spectroscopy was utilized to obtain a vibrational fingerprint of the dried leaf extract and to facilitate the initial identification of key functional groups. The dried extract was analyzed using a Shimadzu FT-IR spectrometer over the range of 400–4000 cm^{-1}. The observed bands were interpreted by comparing them with commonly reported vibrational regions linked to functional groups derived from plants. These assignments are considered provisional, as FT-IR spectra of complex extracts often display overlapping contributions from multiple constituents. [5,7]

UV–Visible spectroscopy

The UV–Visible absorption spectrum of the ethanolic leaf extract was recorded over 200–800 nm. The principal absorption maxima were noted and interpreted in relation to aromatic, conjugated and pigment-containing constituents. Spectral assignments were treated as indicative rather than compound-specific. [5,7]

Results and Discussion

Physicochemical characteristics

The powdered leaves showed loss on drying, total ash, acid-insoluble ash and water-soluble ash. The alcohol-soluble and water-soluble extractive values were respectively. These measurements provide preliminary information on moisture, inorganic residue and solvent-soluble matter in the powdered plant material. The relatively low acid-insoluble ash value is compatible with a limited proportion of acid-insoluble mineral material in the tested sample. Extractive values should be interpreted in relation to solvent composition and experimental conditions rather than as direct measures of pharmacological activity.

Parameter	Value (%)
Loss on drying	3.23
Total ash	5.10
Acid-insoluble ash	0.59
Water-soluble ash	0.68

Alcohol-soluble extractive	31.63
Water-soluble extractive	25.65

Table 1: Physicochemical parameters of ethanolic extract of Cocculus hirsutus L. leaves.

Preliminary phytochemical screening

The 70% ethanolic extract gave positive qualitative reactions for glycosides, terpenoids, saponins, tannins, flavonoids, alkaloids, proteins/amino acids, phenolic compounds and carbohydrates. The pattern is broadly consistent with previous phytochemical reports for C. hirsutus L. These reactions indicate the presence of broad classes of constituents but do not establish their individual molecular identities, concentrations, or biological activities.

Constituent class	**Result**
Glycosides	+
Terpenoids	+
Saponins	+
Tannins	+
Flavonoids	+
Alkaloids	+
Proteins and amino acids	+
Phenolic compounds	+
Carbohydrates	+

Table 2: Preliminary phytochemical screening of ethanolic extract of Cocculus hirsutus L. leaves.

Total phenolic and flavonoid contents

he ethanolic leaf extract of *Cocculus hirsutus* L. exhibited appreciable levels of phenolic and flavonoid constituents. The total phenolic content, determined using the Folin–Ciocalteu colorimetric method with catechol as the calibration standard, was found to be 48.82 ± 1.84 mg catechol equivalents per gram of dry extract (mg CE/g). The total flavonoid content, estimated by the aluminium chloride colorimetric method using rutin as the calibration standard, was 32.67 ± 1.26 mg rutin equivalents per gram of dry extract (mg RE/g). The results are expressed as mean ± standard deviation of replicate determinations. The observed phenolic and flavonoid contents indicate that the ethanolic leaf extract of *C. hirsutus* is a potential source of bioactive secondary metabolites. Phenolic and flavonoid compounds are widely associated with antioxidant properties and may contribute to the biological activities of medicinal plant extracts.

Parameter	Result
Total phenolic content	48.82±1.84 mg catechol equivalents/g dry extract (mg CE/g)
Total flavonoid content	32.67±1.26 mg rutin equivalents/g dry extract (mg RE/g)

Table 3: Quantitative phytochemical measurements of ethanolic extract of Cocculus hirsutus L. leaves.

FT-IR spectral profile

FTIR analysis confirmed the existence of various functional groups in the ethanolic leaf extract of Cocculus hirsutus. The broad band observed at 3317 cm^{-1} signified hydroxyl groups typical of phenolic and flavonoid components, while the bands at 1649 cm^{-1} and 1519 cm^{-1} indicated the presence of unsaturated and aromatic structures. Absorptions at 1444 cm^{-1} and 1334 cm^{-1} were primarily linked to C–H deformation and vibrations of phenolic or other functional groups, whereas the bands at 1207, 1166, and 1062.78 cm^{-1} pointed to C–O/C–O–C functionalities characteristic of alcohols, ethers, phenolics, carbohydrates, and glycosides. Additionally, the bands at 862 and 759 cm^{-1} further corroborated the presence of substituted aromatic structures. In summary, the FTIR profile suggests that the ethanolic leaf extract is abundant in hydroxylated, aromatic, and oxygen-containing phytoconstituents, particularly phenolic and flavonoid compounds, aligning with the previously documented phytochemical composition of C. hirsutus.

Frequency Range (cm^{-1})	Functional Group	Phytochemicals/Biomolecules
3983, 3825	O–H stretching of free/weakly H-bonded hydroxyl groups	Alcohols, phenols and other hydroxylated constituents
3317	Broad O–H stretching	Phenols, flavonoids, tannins, alcohols and carbohydrates hydrogen-bonded –OH
1649	C=O stretching and/or C=C stretching; contribution from adsorbed water is also possible	Carbonyl-containing compounds, conjugated phenolics/flavonoids; moisture contribution may occur
1519	Aromatic C=C skeletal vibration	Aromatic compounds, particularly phenolic and flavonoid constituents
1444	C–H bending / CH_2 or CH_3 deformation; may overlap with	Terpenoids, lipids and other aliphatic constituents

	aromatic skeletal vibrations	
1334	O–H bending and/or C–O vibration	Phenolic compounds and flavonoids
1207	C–O stretching	Alcohols, phenols, ethers and flavonoid structures
1166	C–O–C / C–O stretching	Flavonoids, glycosides and carbohydrates

Table 4: FT-IR interpretation of compounds of ethanolic extract of Cocculus hirsutus L. leaves.

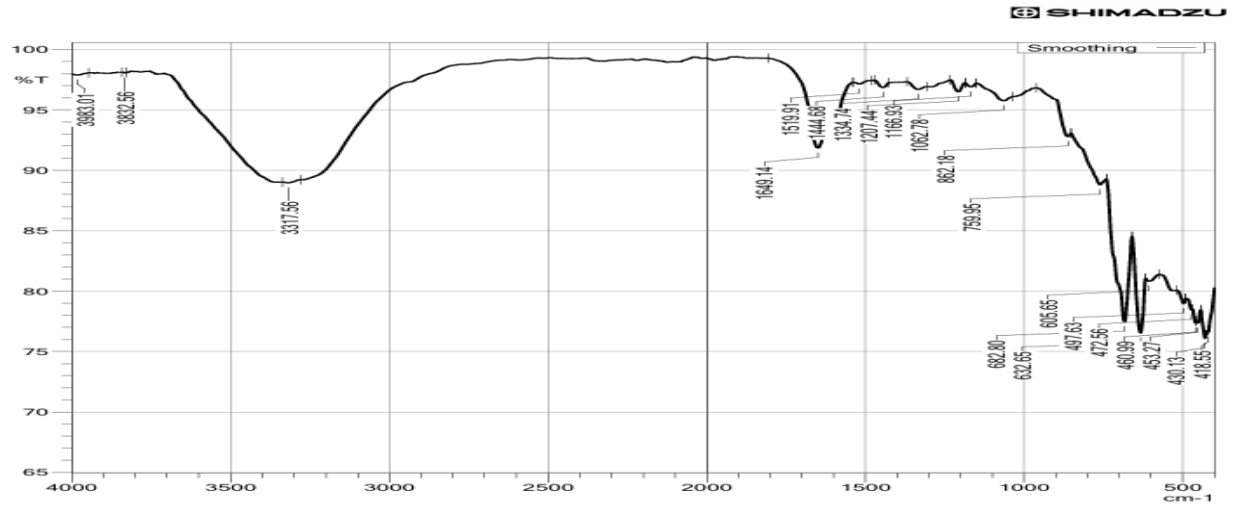

Fig.1: FT-IR spectrum of ethanolic extract of Cocculus hirsutus L. leaves

UV–Visible spectral profile

The UV–Visible spectrum of the ethanolic leaf extract of *Cocculus hirsutus* L. showed characteristic absorption maxima at 218 nm (0.60), 260 nm (0.21), 350 nm (0.17), and 650 nm (0.05). The absorption bands at 260 and 350 nm are consistent with the characteristic UV spectral regions of phenolic and flavonoid-type chromophores, particularly the Band II and Band I regions of flavonoids, respectively. The weak absorption near 650 nm may be associated with residual chlorophyllous pigments. The spectral profile thus provides a characteristic fingerprint supporting the presence of conjugated phenolic/flavonoid constituents in the ethanolic leaf extract.

λmax (nm)	**Possible phytochemicals/bioactive compounds**
218	Phenolics, flavonoids and other conjugated secondary metabolites
260	Phenolic compounds and flavonoids

350	Flavonoids, particularly flavones/flavonols
650	Chlorophyll-related/photosynthetic pigments

Table 5: Characteristics UV-Vis absorption band of ethanolic extract of Cocculus hirsutus L. leaves.

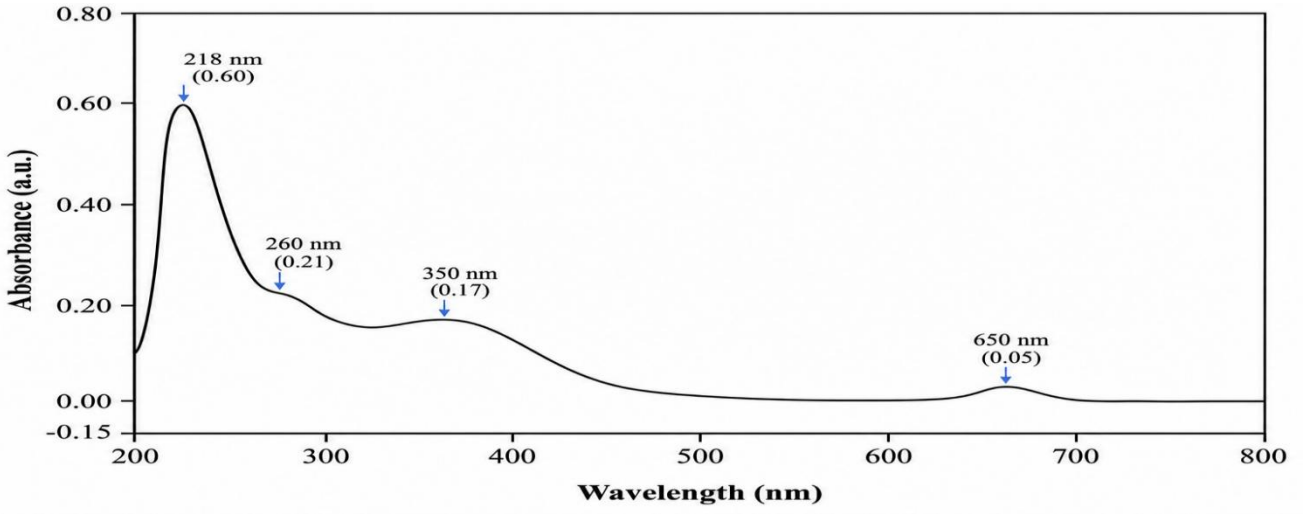

Fig.2: UV-Visible spectrum of ethanolic extract of Cocculus hirsutus L. leaves

Conclusion

The investigation provided a foundational physicochemical, phytochemical, and spectroscopic profile of the 70% ethanolic leaf extract of Cocculus hirsutus L., which was derived using ultrasound-assisted extraction techniques. The extract was found to contain a range of phytoconstituents, including glycosides, terpenoids, saponins, tannins, flavonoids, alkaloids, phenolics, carbohydrates, and proteins/amino acids. It also demonstrated considerable total phenolic (48.82 ± 1.84 mg catechol equivalents/g) and flavonoid (32.67 ± 1.26 mg rutin equivalents/g) concentrations. FT-IR analysis revealed the presence of hydroxyl, aromatic, carbonyl, and C–O/C–O–C functionalities, while UV–Visible spectroscopy indicated characteristic absorption maxima at 218, 260, 350, and 650 nm. Overall, these findings contribute a significant preliminary chemical fingerprint and quality profile of C. hirsutus leaves, supporting subsequent chromatographic, spectrometric, and biological explorations of its bioactive constituents.

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