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Physico-chemical, phytochemical and FT-IR analysis of ethanolic leaves extract of *Cardiospermum halicacabum* L.

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Abstract
In the present study was carried out to investigate the Physico chemical, phytochemical and FTIR analysis of *Cardiospermum halicacabum* L. leaves. Physico chemical parameters such as Extractive value, Ash value, Loss on drying and fluorescence analysis was determined quantitatively. It will be helpful in assessing the quality of the plant powder. Preliminary phytochemical screening of ethanolic leaves extract of *C. halicacabum* showed the presence of alkaloids, carbohydrates, glycosides, flavonoids, phenol, proteins, amino acids, saponins and tannins. The quantitative analysis of phytochemicals such as alkaloids, flavonoids, phenols, proteins and tannins were also estimated from ethanol extract by various methods. The alkaloid content was found to be 3.23mg/g and the protein content was found to be 2.82mg/g. Total flavonoid content 11.08 mg/g, total phenolic content 14.07mg/g and the tannin content was found to be 1.73 mg/g. The results showed that Ethanolic extract of *C. halicacabum* leaves contain more phenolic compounds which can be beneficial in the therapeutic approach. The FTIR analysis results showed that the ethanol leaf extract of *C. halicacabum* having the presence of Hydroxyl group, Alcohols, Amides, Alkanes Aldehydes, Alkenes, Aromatic compounds, Nitro compounds, Carboxylic acids, Alkyl and Aryl halides, which shows major peaks at 3888.85, 3784.86, 3709.89, 3450.j76, 1036.61, 821.27, 772.24, 658.02 and 560.83 respectively. The results of the FTIR analysis are significant with the preliminary qualitative analysis.

Keywords: *Cardiospermum halicacabum*, ethanolic extract, FT-IR analysis, functional groups, phytochemical screening

Introduction
Herbal medicine is based on traditional medicine and exists all over the world. Each of these traditional medicines has its origin and a private basic philosophy (Vogel, 2003) [43]. India is one of the countries with the greatest medical and cultural diversity in the world (Archna Sharma *et al.*, 2013) [5]. Here Ayurveda, Siddha and Unani are among the most important traditional medical systems. The systems are heavily dependent on medicinal plants. India has a rich source of medicinal plants, around 8000, distributed in 16 agro-climatic zones (Rajalaxmi Nayak *et al.*, 2015) [33]. Herbal medicines make up the majority of complementary medicines. Since the cost of most Western medicines is beyond human affordability, millions of the world's population in developing countries depend entirely on herbal medicines. Herbal medicines are on the advance in developing countries, and herbal medicines are now firmly established in medical care (Mukherjee *et al.*, 2003) [26]. Phytochemicals are naturally found in medicinal plants in all the parts namely, leaves, stems, flowers, fruits, seeds, bark, and roots which play an important role in curing diseases. The phytochemical components are extremely important to facilitate the search for the particular effectiveness of the plant in medicine. Bioactive compounds normally accumulate as secondary metabolites (flavonoids, alkaloids, terpenoids, steroids, saponins, etc.) are present in all plant cells, but their concentration varies depending on the parts of the plant, the season, the climate, and the phase of growth (Raza *et al.*, 2013) [34]. Many of these were the basis for the development of new lead chemicals for pharmaceuticals. *Cardiospermum halicacabum* L. is one of the most important medicinal plants of the Sapindaceae family. The common names for this plant are Balloon vine, Puff-ball, Heartseed Vine, Heart pea, Love in a puff (Krishna Murti *et al.*, 2010) [20]. "Mudakattan" is a green backyard healer traditionally used for various diseases such as rheumatism, lumbago, cough, hyperthermia, nervous disease, limb stiffness, and snakebite, etc. (Jayabalan., 2006, Jeyadevi *et al.*, 2013, Patil *et al.*, 2011, Ponmari *et al.*, 2011, Raza *et al.*, 2013) [16, 17, 30, 31, 34]. *Cardiospermum halicacabum* L. contains certain phytochemicals that support its role in the medicinal field. It has anti-inflammatory (Sheeba & Asha, 2009, Selloum *et al.*, 2003) [38, 37], antidiarrheal (Kurian., 1995, Venkat Rao *et al.*, 2006) [21, 41], antiparasitic, antipyretic (Asha & Ushpangadan, 1999) [6], antifungal (Khunkitt *et al.*, 2000, Veeramani *et al.*, 2015) [19, 40], anxiolytic activity (Raza *et al.*, 2013) [34], adulticidal activities, urinary tract infections suppression and antihyperglycemic properties (Krishna Murti *et al.*, 2010, Patil *et al.*, 2010) [20, 29]. While there is traditional and experimental evidence to support various claims and benefits of these plants, proper evaluation and use are still required. A full investigation is inevitable, for this reason that we study the leaves of this plant using strict scientific protocols in order to capitalize on the enormous economic potential of this culture, which can be determined by its consumption. The employment opportunity for a farm labourer is sufficient.

Materials and Methods

Collection and Processing of Plant Material

Cardiospermum halicacabum L. was collected in February 2021 in Rettaimalai, Tiruchirappalli District**,** Tamil Nadu, and South India. The collected plants were washed with water to remove soil and dust. The plant material was dried for four to five days in the shade and cut into small pieces**.** Then it was pulverized into a coarse powder. The powder was used to extract the active principles.

Preparation of the Plant Extract

The coarse powder was placed in the Soxhlet apparatus and extracted successively with ethanol solvent. The extract was then filtered and evaporated on a rotary evaporator. The dried extracts were stored in the refrigerator for further experiments.

Physicochemical Properties

Physicochemical properties such as the percentage of extraction values, total ash, acid-insoluble ash, water-soluble ash; values were calculated according to the guidelines of the World Health Organization (WHO, 1998). In fluorescence analysis the plant powders have been treated with various acids and alkalis such as HCl, H_2SO_4, HNO_3, HCl in methanol, and NaOH, observed in both daylight and ultraviolet light to evaluate the purity of the plant powder.

Qualitative Phytochemical Screening

The plant extract was qualitatively determined for the presence of alkaloids, carbohydrates, glycosides, proteins and free amino acids, fixed oils, phenolic compounds, tannins, flavonoids, saponins, and sterols according to the standard method provided by Harborne, (1984) [13].

Quantitative phytochemical studies

Assessment of the Alkaloids

The alkaloid content of raw drugs was estimated by the Indian Pharmacopoeia, (1996) [15]. Five grams of the extract was weighed and transferred to a 250 ml beaker. An aliquot of 8 ml of 20% Acetic acid and 32 ml of ethanol was placed in the same beaker, covered, and left to stand for 4 hours. After 4 h, the extract was filtered and it was concentrated to a quarter of the original volume using a water bath. A few ml of concentrated ammonium hydroxide was added to the concentrate until the precipitation was complete. The entire solution was able to settle. The precipitate was collected by filtration and weighed.

Assessment of the Total Flavonoids

The total flavonoid content of leaf extract was estimated using the aluminum chloride method described by (Hossain & Nagooru, 2011) [14]. 1 ml of extract was mixed with 4 ml of deionized water and 0.3 ml of 10% sodium nitrate. The solution was allowed to stand for 5 min. 0.3 ml of 10% strength aluminum chloride solution was added to this solution. The solution is left to stand for one minute. A 2 ml aliquot of 1M sodium hydroxide solution was added to this. The test tube was shaken well to mix the reagents and allowed to stand for a while. The OD was measured at 510 nm. Rutin as the standard for creating the standard curve. Using the standard curve, the total flavonoid content of the extract was obtained.

Assessment of the Phenols

The total polyphenols present in the extracts were estimated using the Folin's ciocalteau method (Bray & Thorpe, 1954) [8]. One ml of extract was placed in a test tube, into which 1 ml of Folin phenol reagent and 2 ml of 20% sodium carbonate solution were added. 1 ml of 80% methanol was used as a blank and all of the above reagents were added. One ml of Catechol solution was used as the standard for creating the standard curve. The test tubes were shaken well and heated in a boiling water bath for 1 minute. After cooling, the solution turns blue and dilutes to 25 ml with distilled water. The absorbance of the solution was read at 650 nm against the 80% methanol-solvent blank value.

Assessment of the Tannins

Tannins were determined using the Folin-Ciocalteu method (Govindappa *et al.,* 2011) [11]. Approximately 0.1 ml of the sample extract was placed in a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution, and dilutes to 10 ml with distilled water. The mixture was stirred well and kept at room temperature for 30 minutes. A range of tannin reference standard solutions (20, 40, 60, 80, 100 µg/ ml) was prepared in a manner equivalent to that described above. The absorbance for the test and standard solutions was measured against the blank value at 700 nm with a UV/ visible spectrophotometer.

Assessment of the Proteins

The protein content of the extract was determined using the method of Lowry *et al.,* (1951) [22]. 5.5 ml of alkaline copper tartrate reagent was added to each test tube and allowed to stand for 15min. Add a 0.5 ml aliquot of Folin phenol was mixed thoroughly and allowed to stand at room temperature for 30 minutes. The absorbance at 650 nm was measured. The value from the standard graph was used to calculate the protein concentration in the plant material.

FTIR Analysis

The Fourier Transform Infrared (FTIR) spectrophotometer is possibly the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of the absorbed light is characteristic of the attraction, as will be seen within the annotated spectrum. The chemical bonds in a molecule can be determined from the infrared absorption spectrum. Dry powder made from ethanolic plant material extracts was used for the FTIR analysis. 10 mg of the dry extract powder was encapsulated in 100 mg of KBr pellet, to prepare translucent sample disks. The powder sample was placed in an FTIR spectroscope (PERKIN ELMER, IR) with a scanning range of 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The probable functional groups have been identified based on the maximum values of the IR radiation range (Ashokkumar & Ramaswamy 2014; Mohrig *et al.,* 2006; Nandiyanto *et al.,* 2019) [7, 25, 27].

Results

The Physicochemical parameters such as extractive value, ash value, loss on drying, and fluorescence analysis were quantified and the results were tabulated in Table 1. Fluorescence analysis of plant powders treated with various acids and alkalis such as HCl, H_2SO_4, HNO_3, HCl in methanol and NaOH, observed the powders in daylight as

well as in ultraviolet light presented in Table 2. The phytochemical analysis of the *Cardiospermum halicacabum* leaf extract was analyzed for compounds such as carbohydrates, glycosides, alkaloids, phytosterols, fixed oils, saponins, proteins, and amino acids, phenolic compounds, flavonoids, and tannins. A preliminary phytochemical examination of the ethanol leaf extract revealed the presence of eight compounds i.e. Carbohydrates, glycosides, alkaloids, saponins, phenolic compounds, proteins, flavonoids, and tannins, as well as the absence of phytosterols, and fixed oils which are listed in Table 3. The quantitative phytochemical analysis of the ethanolic leaf extract of *C. halicacabum* L. is represented in Table 4. The results showed that the phenol content was more in ethanolic extract of C. halicacabum L. leaf and was followed by flavonoids, alkaloids, proteins, and tannins, which are shown in Fig. 1. The FTIR spectrum of the extract of *C. halicacabum* leaves is shown in Fig. 2. The results of the FTIR analysis showed that the *C. halicacabum* ethanol leaf extract contains hydroxyl group, alcohols, amides, alkanes aldehydes, alkenes, aromatic compounds, nitro compounds, carboxylic acids, alkyl, and aryl halides.Table 5 shows the major peaks at 3888.85, 3784.86, 3709.89, 3450.76, 1036.61, 821.27, 772.24, 658.02 and 560.83 respectively.

Discussion

The results of the analysis of physicochemical parameters showed that the water-soluble extractive value was higher than the alcohol-soluble extractive value. This result agrees with the result of (Patil *et al.,* 2010) [29]. Apart from total ash, water-soluble ash value was higher in plant powder, which was followed by acid-insoluble ash value. The ash or residue produced by an organic chemical compound. A ruler is used to measure the amount of inorganic matter that is present as an impurity. The water-soluble ash is used to determine the presence of anhydrous material. Many flavonoids showed characteristic colors under ultraviolet light. In the case of non-fluorescent compounds, the fluorescence can be imparted by impurities present in the sample. Therefore, some raw materials are often qualitatively assessed in this way and it is an important parameter of pharmacognostic evaluation (Ansari, 2006, Gupta *et al.,* 2006) [4, 12]. Phytochemical screening of the plant was reported as a positive test for the presence of various phyto-components such as alkaloids, flavonoids, and tannins (Annadurai *et al.,* 2013, Mohaddesi *et al.,* 2016, Sagadevan *et al.,* 2013) [2, 23, 36]. The whole plant extracts (i.e. petroleum ether, alcohol, and water) from *C. halicacabum* (Linn) contain tannins, flavonoids, saponins, sterols, and triterpenes (Kurian., 1995, Venkat Rao *et al.,* 2006) [21, 41]. The alcoholic extract of the root of this plant contains fixed oils, fats, proteins, flavonoids, phenols, tannins, carbohydrates, saponins, phytosterols, and triterpenoids (Dhayabaran *et al.,* 2012) [10]. Chloroform extract from this plant has reported the presence of carbohydrates, steroids, terpenoids, glycosides, alkaloids, phenols, and tannins. The ethyl acetate extract from this plant contains carbohydrates, steroids, terpenoids, phenols, tannins, proteins, amino acids, glycosides, flavonoids, and alkaloids. The methanolic extract of this plant contains carbohydrates, amino acids, phenols, tannins, alkaloids, glycosides and the aqueous extract of this plant contains carbohydrates, flavonoids, amino acids, proteins, steroids, terpenoids, phenols, tannins, glycosides, alkaloids (Aishwarya *et al.,* 2014, Deepan *et al.,* 2012, Parameshappa *et al.,* 2012) [1, 9, 28]. Vadivazhagi, (2020) [39] examined the extract of the total phytochemicals from the *C. halicacabum* leaf using Petroleum ether and qualitatively identified secondary metabolites. The extracts had a high phenol content and showed strong radical scavenging activity and adequate iron reduction (Annamalai *et al.,* 2011) [3]. A similar result was found in Jeyadevi *et al.,* (2013) [18], the ethanolic extract from the leaf of *C. halicacabum* L. contains more phenol with 17.94 mg/g and 14.97 mg/g of flavonoid. Phytochemical screening of the plant was reported as a positive test for the presence of various phyto-components such as alkaloids, flavonoids, and tannins. The amount of total phenol in various extracts was found to be between 29.697 ± 0.232 to 187.372 ± 0.615 mg pyrogallol equivalent/g (Mohaddesi *et al.,* 2016) [23]. Previous research shows the results from FTIR analysis of *C. halicacabum* leaves confirmed the presence of amines, lipids, amino acids, proteins, halogen derivatives, lactams, sulfur compounds, alkanes, and iodine compounds. (Prabakaran *et al.,* 2014, Viji *et al.,* 2010) [32, 42]. FT-IR analysis of the methanolic extract of *C. halicacabum* leaves shows the presence of alcohol, isocyanides, alkyl Compound, alkane, primary alcohol, chloric components (Mohammed Junaid *et al.,* 2020, Sabeerali *et al.,* 2018) [24, 35]. The results of the FTIR analysis are significant with the preliminary qualitative analysis**.**

Conclusion

Extractions of any crude drug with a certain solvent result in a solution that contains various phyto-components. The composition of these phyto-components in the respective solvent provides initial information about the quality of a drug sample. The presence or absence of a certain component plays an important role in the decision about the medicinal properties of the plant. The ethanolic extract from the leaves of *C. halicacabum* L. contains phyto-compounds such as phenols, flavonoids, tannins, saponins, etc. It contains more phenolic compounds than other compounds, which can be beneficial in a therapeutic approach.

Table 1: Physicochemical parameters of *C. halicacabum* L. Leaves

Parameters	Observation
Extractive value	
a. Water Soluble (%)	16.03
b. Alcohol Soluble (%)	13.5
Ash value	
a. Total Ash (%)	9.8
b. Acid Insoluble Ash (%)	1.7
c. Water Soluble Ash (%)	4.10
Loss on drying (%)	12.04

Table 2: Fluorescence analysis of *C. halicacabum* L. leaves

Treatment	Observation under	
	Daylight	UV light
Powder as such	Green	Green
Powder+1N HCl	Brownish green	Black
Powder+H_2SO_4	Dark green	Dark brown
Powder+HNO_3	Brownish green	Brown
Powder+1N NaOH in	Methanol Green	Green

Table 3: Preliminary phytochemical analysis of an ethanolic extract of *C. halicacabum* L. leaves

Phytoconstituents	Ethanol extract
Carbohydrates	+
Glycosides	+
Alkaloids	+
Phytosterol	-
Fixed oils	-
Saponins	+
Proteins and amino acids	+
Phenolic compounds	+
Flavonoids	+
Tannins	+

Note: (+) = Present and (-) = Absent

Table 4: Quantitative phytochemical analysis of an ethanolic extract of *C. halicacabum* L. leaves

Phytoconstituents	Quantity (mg/g) dry. wt.
Alkaloids	3.23
Proteins	2.82
Flavonoids	11.08
Phenols	14.07
Tannins	1.73

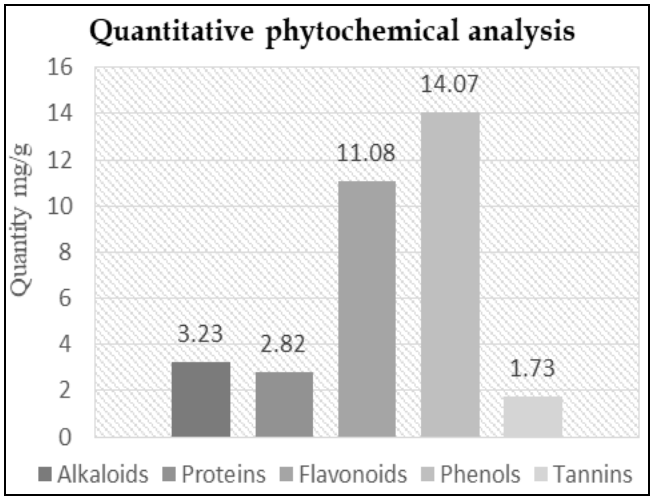

Fig 1: Quantitative phytochemical analysis of an ethanolic extract of *C. halicacabum* L. leaves

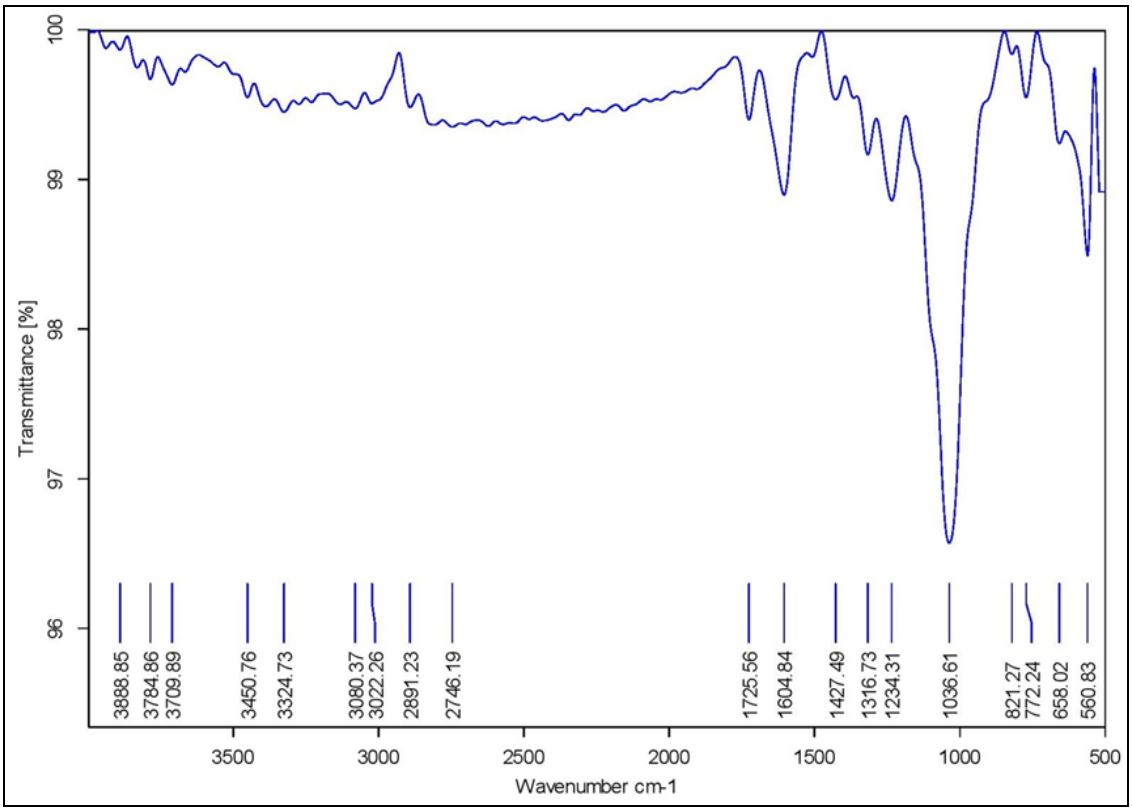

Fig 2: FTIR spectrum of ethanol extract of *C. halicacabum* L leaves.

Table 5: FTIR Interpretation of compounds of ethanol extract of *C. halicacabum* L.

Frequency range	Molecular motion	Functional group
3888.85 - 3709.89	O-H (Non-bonded)	hydroxyl group
3450.76	O-H stretch	Alcohol
3324.73	N-H stretch	Amides
3080.37 - 3022.26	C-H stretch	Aromatic compounds
2891.23	C-H stretch	Aldehydes, Alkanes
2746.19 - 1725.56	O-H stretch, C=O stretch	Carboxylic acids
1604.84	C=C stretch	Aromatic compounds, Alkenes
1427.49	C-H stretch	Alkenes
1316.73	NO_2 stretch	Nitro Compounds
1234.31 - 1036.61	C-F stretch	Alkyl & Aryl Halides
821.27 - 772.24	=C-H bend	Alkenes
658.02 - 560.83	C-Br stretch, C-Cl stretch	Alkyl & Aryl Halides

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