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Original article

Phytochemical Screening, FTIR Spectroscopic Analysis and Antioxidant Activity of *Spermacoce hispida* Linn. Leaves

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ABSTRACT:
Spermacoce hispida Linn. is one of the important plant belonging to the family of Rubiaceae and is commonly used herb in Siddha medicine. The present study was aimed to screen the phytochemicals, FTIR analysis and to evaluate the antioxidant activity of *Spermacoce hispida* leaf extracts. The results revealed that the Alkaloids, Steroids, Flavonoids, Phenolic compounds, Tannins, Coumarins, Cardiac glycosides and Saponins were present in the leaf extracts. The FTIR Spectroscopic studies revealed different characteristics peak value with various functional compounds in the extracts. The FTIR analysis confirmed the presence of amide, alkenes, alkyne, alkane, ether, alcohol, ketone, alkyl halides and aromatic group in the leaf extracts. The antioxidant activity was determined by DPPH free radical scavenging method. The leaf extracts of *Spermacoce hispida* showed strong radical scavenging activity. The study concluded that the leaf extracts of *Spermacoce hispida* has potential bioactive compounds and strong antioxidant activity, the plant could be utilized to discover various bio active natural products for the development of new pharmaceuticals.

Keywords: *Spermacoce hispida*. Ethanolic extracts, Phytochemicals, FTIR and Antioxidant activity.

1. INTRODUCTION

In recent years, there has been a rising attention in drugs from medicinal plant origin in compare to the synthetics which are considered as unsafe to humans [1]. Natural products and their derivatives exhibit minimal side effects and improved efficacy than other synthetic counterparts. The medicinal plants derived drug is a key resource in developing countries to fight with serious disease [2]. The medicinal value of the plant species is due to presence of bioactive compound like Alkaloids, Tannins, Flavonoids, Saponins, Phenols etc., which can conduct certain biological functions that enhance therapeutic activities such as anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant properties [3].

Flavonoids and Phenolic compounds are the secondary metabolites which are responsible for the antioxidant activity of plants. These compounds are suggested to contribute to the health promoting properties. Antioxidant agents of natural origin have attracted special interest because of their free radical scavenging abilities [4]. The use of medicinal plants with high level of antioxidant constituents has been proposed as an effective therapeutic approach for hepatic damages [5].

Awareness of the herbal plants chemical constituents is helpful in the discovery of effective therapeutic agents. Extraction and characterization of several active phytocompounds from the medicinal plants is the foundation for the formation of some high activity profile drugs [6]. Fourier Transform Infrared Spectroscopy (FTIR) is a high resolution analytical technique used to identify the chemical constituents and elucidate the structural compound [7]. FTIR offers a rapid and non destructive investigation to fingerprint plant extracts (or) powder. The present study was carried out to screen the phyto chemical constituents of the leaf extract of *Spermacoce hispida*, to evaluate its antioxidant activity and to find out the functional groups present in the plant using FTIR spectroscopic technique.

Description of the plant

Spermacoce hispida Linn popularly known as 'Nattaiccuri' in Tamil and shaggy button weed in English and belongs to the family of Rubiaceae. The plant is widely distributed in the Western Ghats of Kerala and Maruthamalai forest of Tamil Nadu. The whole plant is used for various medicinal properties. Seeds of Spermacoce hispida are crushed into paste and taken orally to treat stomach problems. The seed extract has been used as a remedy for curing internal injuries of nerves and kidney. The plant is rich in flavonoids. The

plant exhibits various pharmacological activities like anti-inflammatory, analgesic, hypolipidemic, antidiabetic, antihypertensive, antifungal, anticancer and hepato protective activity.

2. MATERIALS AND METHODS

Collection of plant material

The plant *Spermacoce hispida* was collected from the herbal garden of Queen Mary's College, Chennai. The plant was identified and authenticated by the taxonomist at the Department of Botany, Queen Mary's College, Chennai and the herbarium specimen has been deposited at the college for further reference.

Preparation of plant extract

The fresh leaves were collected, shade dried and ground into a coarse powder. The powder was stored in an air tight container and used for further analysis. About 5gm of dried leaf powder was extracted with aqueous and ethanol (75%) for 1min using an ultra Turax mixture (13000 rpm) and soaked overnight at room temperature. The samples were then filtered through Whatman No: 1 paper in a funnel. The filtered solution was evaporated under vacuum in a rota-evator at 40˚C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in refrigerator below 10˚C.

Phytochemical analysis

Phytochemical screening was performed using the standard procedure of Trease and Evans (2005) [8], Alkaloids, Carbohydrates, Tannins, Phenols, Flavonoids and Saponins etc., were qualitatively analyzed.

Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristics of the chemical bonds as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a compound can be determined. The powdered sample of plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a scan range from 400 to 4000 cm-^{1} with a resolution of 4 cm^{-1}.

Free Radical Scavenging Activity

The antioxidant activity of the extracts was evaluated through the DPPH (1.1 diphenyl-2 picrylhydrazyl) radical scavenging assay as described by Lee et.,al.,(2003) [9]. Leaf extracts of 100 µl were mixed with 2.7 ml of methanol and then 200 µl of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank containing the same amount of methanol and DPPH solution was prepared and measured as a control. Subsequently, at every 5 minutes interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517 nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of butylated hydroxyl toluene (BHT). The experiment was carried out in triplicates. The capacity of scavenging free radical was calculated as scavenging activity (%) =

$$\frac{\text{(Absorbance of control) – (Absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

3. RESULTS AND DISCUSSION

Phytochemical Screening

The results of the various phytochemical screening tests obtained during the experiment are shown in **Table 1.** Ethanol and Aqueous solvents were used to detect the various compounds. Saponins, Tannins, Alkaloids, Flavonoids, Terpenoids, Coumarins, Steroid and Phenols were the phyto constituents found in the leaf extracts. The presence of different phytocompounds in the plant are responsible for the various pharmacological properties [10]. The curative properties of medicinal plants are due to the presence of various secondary metabolites such as Alkaloids, Flavonoids, Glycosides, Phenols, Saponins, Steroids etc. These compounds served as natural antibiotics, which help the body to fight infections and microbial invasion [11]. Phenolic acids are the most commonly occurring natural products noted for allelopathic activities. The flavonoids are also widely distributed in plants which have been reported to exert multiple biological benefits, including antioxidant, free radical scavenging abilities, anti inflammatory and anti-carcinogenic [12, 13] studied the phytochemical screening of the extract of *Rosmarinus officinalis*, *Ocimum basilicum* and *Origanum syriacum* and observed the presence of alkaloids. Alkaloids has important biological property like cytotoxicity and are used in allophatic systems [8]. Steroids and Sterols are great importance in pharmacy as they possess compounds like sex hormones and can be used for drug production [14]. Plant derived natural products such as flavonoids, terpenoids and steroids are known to be important for their cardiotonic activities, possess insecticidal and antimicrobial activity have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity. Thus the preliminary screening study revealed the presence of various secondary metabolites in the leaf extracts of *S.hispida* and it showed that it has high medicinal values.

Table 1: Phytochemical screening of leaf extracts of *Spermacoce hispida*

Sl. No	Phytochemicals	Different Solvent used	
		Aqueous	Ethanol
1	Tannin	++	+
2	Saponins	+	+
3	Quinines	+	-
4	Flavonoid	++	+
5	Alkaloid	+	++
6	Glycoside	-	-
7	Cardiac. Glycoside	+	-
8	Terpenoid	++	-
9	Phenols	++	+

10	Steroid	++	+
11	Coumarin	++	+
12	Anthocyanin	-	-
13	Betacyanin	+	+

'++' Highly present, '+' Moderately present, '-' Absent

Fourier Transform Infrared Spectrophotometer (FTIR)

The FT-IR spectrum was used to identify the functional group of the active compounds based on the peak value in the region of infrared radiation. **Fig. 1** shows the FTIR spectra of *S. hispida* leaf extracts. The peak at 3276.0 cm^{-1} revealed the presence the alcohols (O-H stretch). The peak at 2919.7 cm^{-1} refers to the presence of alkanes (C-H stretch). The peak at 1733.2 cm^{-1} corresponds to ketone (C=O stretch). A peak of 1606.9 cm^{-1} showed the presence of bezene ring (C=O stretch). The peaks of 1419.6, 1318.7, 1236.6, 1145.7 and 1007.4 cm^{-1} indicate the organo phosphorus compounds, nitric compound, ethers, phosphorus oxide and alcohols respectively.

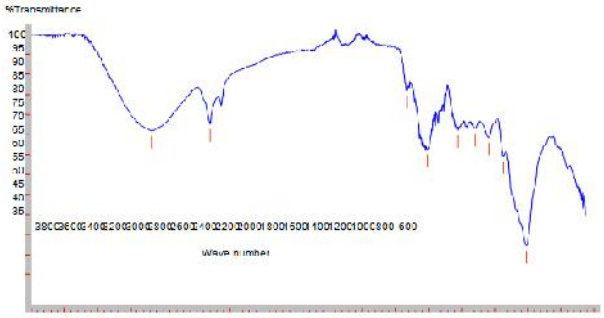

Fig 1: FT-IR Spectra of *Spermacoce hispida*

Table 2: FT-IR analysis of *Spermacoce hispida*

S. No	Absorption peak	Bond	Type of Bond
1	1007.4	O-H	Alcohols
2	1145.7	P-O	Phosphorus oxide
3	1236.6	C-O	Ethers
4	1318.7	N-O	Nitric compound
5	1419.6	P-C	Organophos Phorus compound
6	1606.9	C=C	C=O with benzene ring
7	1733.2	C=O	Ketone
8	2919.7	$C=CH_2$	Vinyl
9	3276.0	O-H	Alcohols, Phenols

The functional groups present in *S. hispida* are aldehydes, alkenes, alcohols, phenols, aromatics, ethers, ketone, vinyl and nitric compounds **Table 2**. All these compounds belong to secondary plant metabolites. These were confirmed by FT-IR Spectrophotometer study that predicted the presence of the groups: O-H, C-H, C=O, C=C, P-C, N-O, C-O, P-O and C-O stretching. The presence of characteristic functional groups of phytochemicals could be responsible for the various medicinal properties of *S. hispida*. Similar research was carried out in FTIR spectral analysis of *Ampelocissus latifolia* extract and reported that the presence of functional groups such as metal carbonyl compounds, alkanes, amides and aliphatic fluoro compounds were responsible for potential medicinal properties [15, 16] analyzed the FTIR spectral analysis of medicinal plants such as *Eclipta alba* and *Eclipta prostrate* and reported that the very strong absorption band appearing in the region 2933–2922 cm^{-1} for whole plant parts is due to N–H stretching and also reported the presence of functional groups like carboxylic acids, amines, polysaccharides, nitrates and carbohydrate. FTIR spectrum analysis of *Carallumafim briyata* was reported and found the presence of phenols, alkanes and aromatic amines [17].

Table: 3 DPPH free radical scavenging activity of *Spermacoce hispida*

Concentration (mg/ml)	Percent inhibition
1	74.44
2	82.22
3	86.67
4	92.22
5	97.78
IC_{50} (mg/ml)	<1

Scavenging activity for free radicals of DPPH has widely used to evaluate the antioxidant activity of natural products from plants. In this study, the leaf extracts of *S. hispida* showed strong radical scavenging activity with 97.8% **Table. 3**. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties [18]. The high phenolic content in the leaf extract can explain its high free radical scavenging activity. An IC_{50} value is the concentration of the sample required to scavenge 50% of the free radicals present in the system. IC_{50} value is inversely related to the antioxidant activity of crude extracts. The lower the IC_{50} value the higher the antioxidant activity of samples [19]. In the present study, the lowest IC_{50} value with highest antioxidant activity was found in ethanolic extracts of *S.hispida*.

The antioxidant activities of *S.hispida* extracts may also be related to their total flavonoid content. Several studies have reported the biological activity of flavonoids [20]. As antioxidants, flavonoids have been reported to be able to interfere with the biochemical pathways involved in the generation of reactive oxygen species (ROS), quenching free radicals, chelating transition metals and rendering them redox inactive in the Fenton reaction [21]. Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases [22]. Natural antioxidants from plants are potent and safe due to their harmless nature; many wild herbs have been investigated for their antioxidant properties [23]. The leaf extracts of the plant *S.hispida* have shown the antioxidant and free radical scavenging activity, which revealed that the plant can be used as a potent source of natural antioxidant**.**

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