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ANALYSIS OF BIOACTIVE COMPOUNDS IN *Spermacoce hispida* LEAVES USING HPLC, UV-VIS AND FTIR TECHNIQUES

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ABSTRACT

Identification of the chemical nature of phytochemical compounds present in the medicinal plants will provide some information on the different functional groups responsible for their medicinal properties. The bioactive components of *Spermacoce hispida* leaves have been evaluated using HPLC, UV VIS and FTIR. HPLC profiles of *Spermacoce hispida* reported to contained five phenolic compounds namely Kaempferol, Tannic acid, Epigallocatechin, Quercetin and Caffeic acid. The UV- VIS profile showed the peaks at 219nm, 232, 398 and 664nm reveals the presence of phenolic and alkaloids derivatives. The results of FTIR analysis confirmed the presence of phenol, alkanes, aldehyde, alcohol, aliphatic amines, aromatic and nitro compounds. The results of this study offer a platform of using *Spermacoce hispida* leaves as herbal alternative for various diseases.

Keywords: HPLC, UV-VIS, FTIR, Spermacoce hispida , Phytochemical, Medicinal plants,

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INTRODUCTION

Phytochemical simply means plant chemicals. “Phyto” is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. In addition, these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures (Dahanukar *et al*., 2000). Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments (Ashis, 2003). Chemical principles from natural sources have become much simpler and

have contributed significantly to the development of new drugs from medicinal plants (Cox, 1990). The valuable medicinal properties of different plants are due to presence of several constituents i.e. saponins, tannins, alkaloids, alkenyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters (Cox and Balic, 1994). Among them some are act as synergistic and enhance the bioactivity of other compounds.

Within a decade, there were a number of dramatic advances in analytical techniques including HPLC, UV, FTIR, NMR and GC-MS that were powerful tools for separation identification and structure determination of phytochemicals (Roberts and Xia, 1995). The aim of this study is to determine the bioactive compounds present in the *Spermacoce hispida* leaves extract with the aid of HPLC, UV-VIS and FTIR Techniques which may provide an insight in its use in tradition medicine.

MATERIALS AND METHODS

Plant materials

The *Spermacoce hispida* leaves were collected in January 2014 from Karur, Karur District, Tamil Nadu from a single herb. The leaves were identified and authenticated by Dr. M. Kandhasamy, Department of Botany, Government Arts College, Karur- 639 005,Tamil Nadu, India.

UV and FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-600nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm^{-1} and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

HPLC analysis

Sample preparation: The sample was prepared according to the procedure. The extraction was carried out using 2 ml of fermented broth with 50 mL of 95% ethanol under 80 KHz, 45°C in ultrasonic extraction device for 30 min, repeated twice. The extract was collected and filtered; the filtrate was dried at 50°C under reduced pressure in a rotary evaporator. The dried crude extract was dissolved in the 100 ml mobile phase. After filtering through a filter paper and a 0.45 mm membrane filter (Millipore), the extract was injected into HPLC.

HPLC conditions: Flavonoids were analysed using a RP-HPLC method (Weerasak Samee, 2007), Shimadzu Corp., Kyoto, consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu SPD- 10ATVp UV VIS detector and a loop injector with a loop size of 20 µl. The peak area was calculated with a CLASSVP software. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250×4.6 mm i.d., particle size 5 µm, Luna 5µ C-18; phenomenex, Torrance, CA, USA) at 25°C. The gradient elution of solvent A [water-acetic acid (25:1 v/v)] and solvent B (methanol) had a significant effect on the resolution of compounds. As a result, solvent gradients were formed, using dual pumping system, by varying the proportion of solvent A [water-acetic acid (25:1, v/v)] to solvent B (methanol). Solvent B was increased to 50% in 4 min and subsequently increased to 80% in 10 min at a flow rate of 1.0 mL/min. Detection wavelength was 280 nm.

RESULTS AND DISCUSSION

The pharmacological activities of any plant sample are due to the presence of metabolites, secondary metabolites and secretary products in it. These usually consist of the phenolic compounds, alkaloids, tannins, saponins, carbohydrates, glycosides, flavonoids, steroids, etc. Most phenolic compounds such as flavonoids, glycosides, triperinoids, flavonons, carbohydrates and anthraquinones are found distributed throughout the plant kingdom (Harbone, 1973). Similarly, the polyphenolic compounds most commonly found in plant extracts are the phenolic acids, flavonoids and tannins (Naik *et al*., 2006). These compounds together with other phenolic structures of plant origin have been reported as scavengers of Reactive Oxygen Species (ROS) and are seen as promising therapeutic drugs for free radical mediated pathologies including diabetic, cardiovascular diseases (Velavan, 2011). Most flavonoidic compounds exhibit antipyretic, analgesic, anti-inflammatory, anti-arthritic, antioxidant and immuno-modulatory properties (Balasundram *et al*., 2006; Gill *et al*., 2011). These activities of flavonoidic compounds may be due to the presence of gallic acid, ellagic acid, quercitin, tannin acid, vanillin, resorcinol, catechin, etc.

HPLC profile of *Spermacoce hispida*

HPLC profiles of *Spermacoce hispida* were analysed and five phenolic compounds namely Kaempferol, Tannic acid, Epigallocatechin, Quercetin and Caffeic acid having different elution times could be obtained (Figure 1 and Table 1) when each compound was analyzed individually using the mobile gradient phase consisting of methanol and 1% acetic acid in water during 30 minutes run time. Earlier review of literature (Gupta Mradu *et al*., 2012; Nadia Alam *et al*., 2011; Michael

Vagiri *et al*., 2012; Paranthaman *et al*., 2012) supported the findings of these compounds
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Fig 1 HPLC analysis of *Spermacoce hispida* leaf extract

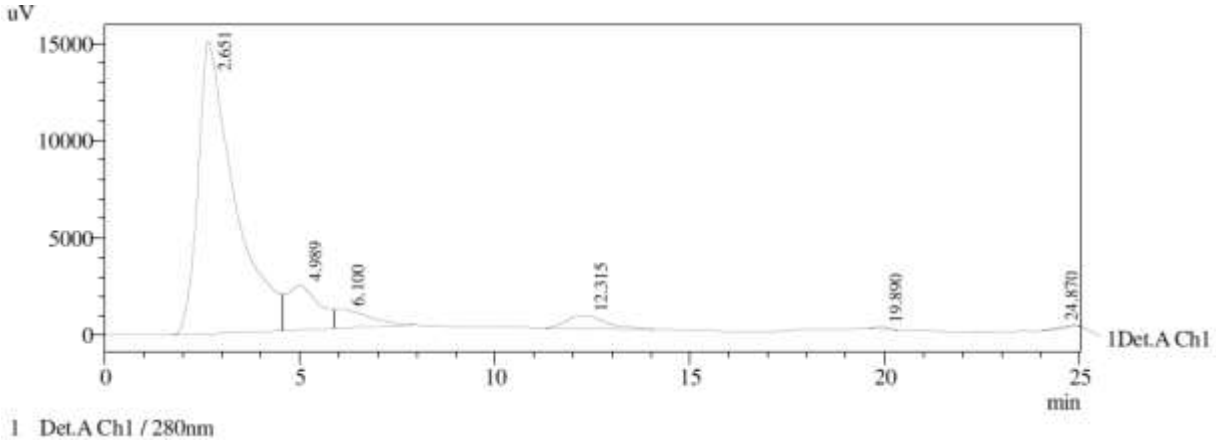

Table 1 HPLC analysis of *Spermacoce hispida* leaf extract

Peak	Area %	Retention Time (Plant)	Literature (RT)	Name of the compounds
1.	80.169	2.651	2.66	Kaempferol
2.	11.028	4.989	4.97	Tannic acid
3.	4.621	6.100	6.1	Epigallocatechin
4.	3.751	12.315	12.40	Quercetin
5.	0.215	19.890	19.10	Caffeic acid
6.	0.217	24.870	-	Unknown

Spectrophotometric analysis

The UV-VIS profile of plant extract was taken at the 200 to 800nm wavelength due to the sharpness of the peaks and proper baseline. The UV-visible spectra were performed to identify the compounds containing σ- bonds, π-bonds, and lone pair of electrons, chromophores and aromatic rings. The profile showed the peaks at 219nm, 232, 398 and 664nm with the absorption 3.599, 1.511, 0.453 and 0.142 respectively (Fig-2 and Table 2). Occurrence of peaks at 234-676 nm reveals the presence of phenolic and alkaloids in the *Spermacoce hispida*. On comparison of the spectra of seeds and flowers, shows that the extract has some similar alkaloid, flavonoids, and glycosides compounds reported (Jasper *et al*., 1958; Sofowora, 1993).

Fig 2 UV-Vis Spectral analysis of *Spermacoce hispida* leaf extract

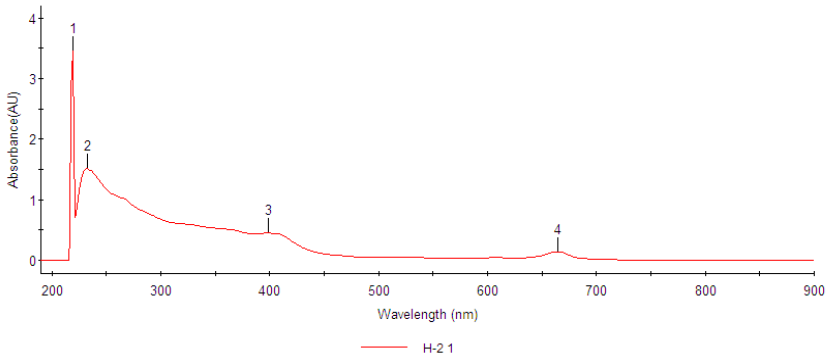

Table 2: UV-VIS Peak Values of Extract of *Spermacoce hispida* leaf

Peak	Wave length (nm)	Absorption Peak (O.D.)
1	219.3	3.599
2	232.9	1.511
3	398.8	0.453
4	664.5	0.142

Functional groups identification

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of phenol, alkanes, aldehyde, alcohol, aliphatic amines, aromatic and nitro compounds (Fig-3, and Table-3). FTIR analysis of *Aerva lanata* confirmed the presence of amide, alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, aromatics, esters, ethers, alkyl halides and aliphatic amines compounds which shows major peaks at 3675.36, 3618.49, 3587.12, 2918.08, 2849.76, 1771.81, 1733.59, 1652.96, 1636.03, 1457.06, 1318.57, 1243.66, 1053.77 and 510.63 respectively (Yamunadevi et al., 2012).

Fig 3 FTIR analysis of *Spermacoce hispida* leaf extract

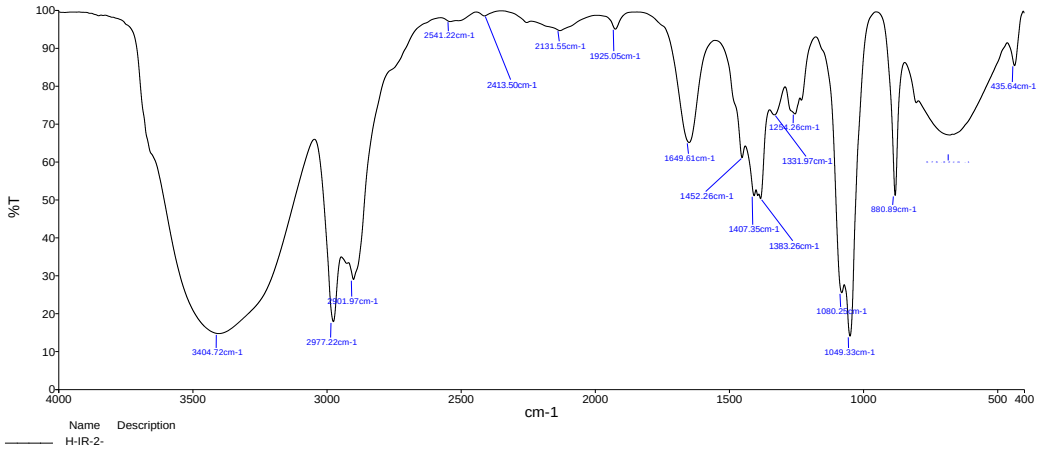

Table 3: FTIR Peak Values of Extract of *Spermacoce hispida* leaf extract

Peak Value	Bond	Functional group
3404.72	O-H stretch, free hydroxyl	Alcohols, phenols
2977.22	C-H strech	Alkenes
2901.97	C-H strech	Alkenes
2541.22	O-H strech	Carboxylic acids
2131.55	-C=C- strech	Alkynes
1649.61	-C=C- strech	Alkenes
1452.26	C-C stretch (in-ring), C-H bend	Aromatics , Alkanes
1407.35	C-C stretch (in-ring)	Aromatics
1254.26	C-N stretch	Aromatic amines
1331.97	N-O symmetric stretch	Nitro compounds
1080.25	C-O stretch C-N stretch	Alcohols, Carboxylic acids, esters, ethers Aliphatic amines
1049.33	C-N stretch	Aliphatic amines
880.89	N-H wag	1,° 2° amines

CONCLUSION

HPLC profiles of *Spermacoce hispida* reported to contained five phenolic compounds namely Kaempferol, Tannic acid, Epigallocatechin, Quercetin and Caffeic acid. The UV- VIS profile showed the peaks at 219nm, 232, 398 and 664nm reveals the presence of phenolic and alkaloids derivatives. The results of FTIR analysis confirmed the presence of phenol, alkanes, aldehyde, alcohol, aliphatic amines, aromatic and nitro compounds. The results of this study offer a platform of using *Spermacoce hispida* leaves as herbal alternative for various diseases including diabetic, cardiovascular etc.

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