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EVALUATION AND PHYTOCHEMICAL ANALYSIS OF *Aplotaxis auriculata* RHIZOME USING UV-VIS, FTIR AND HPLC

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ABSTRACT

In the present study an attempt has been made to establish the identification of functional components in *Aplotaxis auriculata* rhizome extract by using UV,FTIR and HPLC profiles. These techniques were strongly recommended for the aunthentation of traditional herbal medicines. UV-VIS profile showed different peaks ranging from 218nm to 945nm and confirmed the presence of phenol and its derivatives. In addition to this FTIR peak values revealed the presence of alcohols, alkanes, aromatic & carboxylic acids, sulphur compounds, amines & ketones in the plant extract. Hence, these quantitative analysis indicates the presence of important secondary metabolites in *Aplotaxis auriculata* rhizome extract and they used as a potent natural antioxidant in treatment of various oxidative stress and related diseases.

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INTRODUCTION

Nature provides everything for the well-being of human and its expensive gifts of medicinal plants are used traditionally over the thousands of years around the world [1]. Traditional medicine possess immense source of potentially useful compounds as therapeautic agents [2]. Now a days, natural formulations as medicines plays invaluable role in health problems in global level due to its continual production of valuable therapeutic agents with less adverse effects compared to synthetic drugs [3].

The therapeutic value of the medicinal plants based on some bioactive phytochemical constituents that produce a definite physiological action on the human body [4]. Even today, medicinal plants play a vital role in the development of drugs and its evaluation is

based on phytochemical and pharmacological approaches which leads to the drug discovery referred as natural product screening [5].

Plant based natural constituents can be derived from any part of the plant like bark, leaves, roots, flowers, seeds, fruits, rhizome etc [6]. The most important phytochemicals, generally used as markers of plant taxonomy and authenticity are secondary metabolites such as alkaloids ,carotenoids, chlorophylls / phenolic acids/ flavonoids / anthocyanins or terpenoids / unsaturated fatty acids / phytosterols, but also, in some specific cases, saponins, lignans and indoles, thiols and glycosinolates, , vitamins A, C, E [7]. Recent research suggested that diets rich in polyphenolic compounds such as quercetin, rutin, narigin, catechin, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents and flavonoids are associated with longer life expectancy [8] and found effective in many health - related properties, such as anticancer, antiviral, anti-inflammatory activities and an ability to inhibit human platelet aggregation [9] have correlated natural phenolic antioxidants with reduced coronary heart disease.

In order to promote the use of medicinal plants as potential sources, it is needed to thoroughly investigate their composition and activity and thus validate their use [10]. The appropriate identification and standardization of the herbs quality and authenticity is successfully determined either by chromatography and spectroscopic methods [11].

The evaluation of herbal product by metabolomic fingerprinting can be accomplished by complementary analytical techniques of liquid or gas chromatography coupled with MS or NMR detectors (GC / MS, LC / MS, LC / MS / MS, LC / NMR, LC / PDA / MS / FTIR) in specific platform is yet functional in specialized EU centers, providing spectral libraries and metabolomicdatabases (www.phenol - explorer. eu; www. liberherbarum.com; www. metabolomics - lab.com; www.kegg.jp). Beside these sophisticated methods applied to identify or discover new biomarkers of plant recognition [12], many laboratories use rapid and cheaper, available methods to fingerprint and authentify the composition of such products,e.g. Fourier transform infrared spectroscopy (FTIR), a convenient method to fingerprint herbal medicines, especially when is validated with chromatographic (HPLC-UV or - PDA) analysis. The present study was aimed to explore the bioactive compounds present in *Aplotaxis auriculata* rhizome extract by using UV-VIS,FTIR,HPLC may provide an insight in its use in traditional medicine.

MATERIAL AND METHODS

The rhizome of *Aplotaxis auriculata* (Family: Astreaceae) were collected in the month of Janauary 2016 from the Koli hills, Tamil Nadu, India. The rhizome of the plant was identified and authenticated by Dr. S. Alagumanian, Assistant Professor in Botany, H.H. the Rajah's College, Pudukkottai, Tamil Nadu, India.

Preparation of extract

Aplotaxis auriculata rhizome was washed several times with distilled water to remove the traces of impurities from the rhizome. The rhizomes were dried at room temperature and finely powdered. The powder was extracted with 70% methanol for 48 hours [14]. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used.

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 a high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-1100 nm 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.

Quantitative analysis of secondary metabolites by HPLC Analysis

Sample preparation

The extraction was carried out using 2 ml of fermented broth with 50 mL of 95% ethanol under 80 KHz, 5°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 100 ml mobile phases. After filtering through a filter paper and 0.45 mm membrane filter (Millipore), the extract was injected into HPLC.

HPLC conditions

Samples were analyzed using an RP-HPLC method [15] Shimadzu Corp., Kyoto, consisting of a LC- 0ATVp 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 by 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 5 µ 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 minutes and subsequently increased to 80% in 10 minutes 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 secretory 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 [9].

UV visible 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 minutes and filtered through Whatmann No. 1 filter paper by using a high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 400-4000 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected.

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 218.7, 322.0, 575.2 …. 945.5 nm with the absorption 4.0, 1.4, 0.24 …0.31 respectively Table 1 and Figure 1. The result confirms the occurrence of peaks at 218-945 nm reveals that the absorption bands are due to the presence of some alkaloids, phenol and its derivatives in the *Aplotaxis auriculata* rhizome extract [16].

Table: 1 UV –Vis Spectral / Peak values of *Aplotaxis auriculata* rhizome extract

S.No	Wave length (nm)	Absorption Peak
01	218.7	4.0009
02	322.0	1.4717
03	575.2	0.2408
04	671.9	0.2609
05	724.2	0.2704
06	833.9	0.2822
07	945.9	0.3136

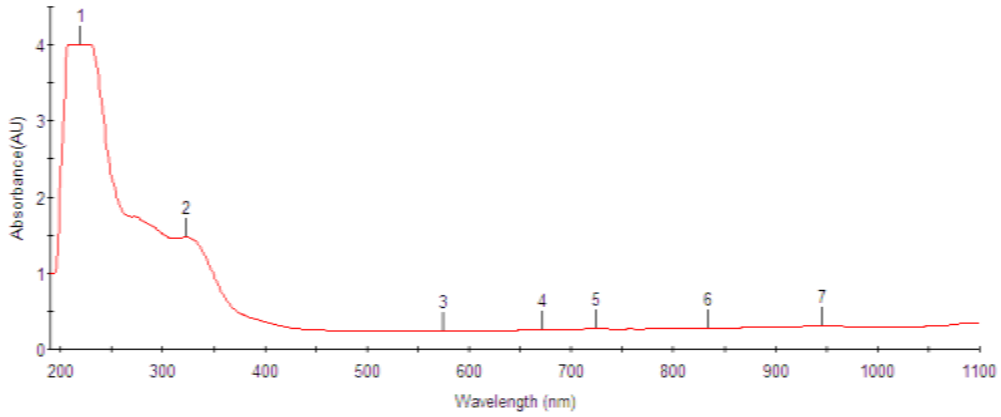

Figure: 1 UV –Vis Spectral Analysis of *Aplotaxis auriculata* rhizome extract

FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks 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 [17].

FTIR Spectrum identified the functional group of the active chemical components present in the fruit based on the peak value in the region of infra red radiation. When *Aplotaxis auriculata* rhizome extract was passed in to the FTIR, the functional group of the components was separated based on its ratio. The peak values and the functional groups were represented in Table: 2 and Figure 2. The results of the FTIR confirmed the presence of Alkenes, Alkenes, Ketones, Aromatics, Carboxylic acids, Esters, Ethers, Aliphatic amines, Halogen compounds, Sulphur compounds and Aromatic P substituted compounds.

Table: 2 FTIR Spectral / Peak values of *Aplotaxis auriculata* rhizome extract

S.No	Peak values (Cm^{-1})	Functional groups obtained
01	3340.85	-OH- (Alcohol, Phenol)
02	2930.83	C-H -(Alkane)
03	1765.92	C=O (Ketone)
04	1528.45	NH_2 (Amine)
05	1414.00	S=O (Sulphate)
06	1344.19	Sulphanic Acid
07	1289.03	COOH (Carboxylic acid)
08	1259.13	COO^- (Aromatic Ester)
09	1125	OR (Ether)
10	1033	C=N- (Cyano group)
11	938.5	COO^- (Aliphatic Ester)
12	817.25	C-C (Alkane)
13	779	Ester (with S group)
14	652.62	N-H (AromaticAmine)
15	620.94	N-H (AliphaticAmine)

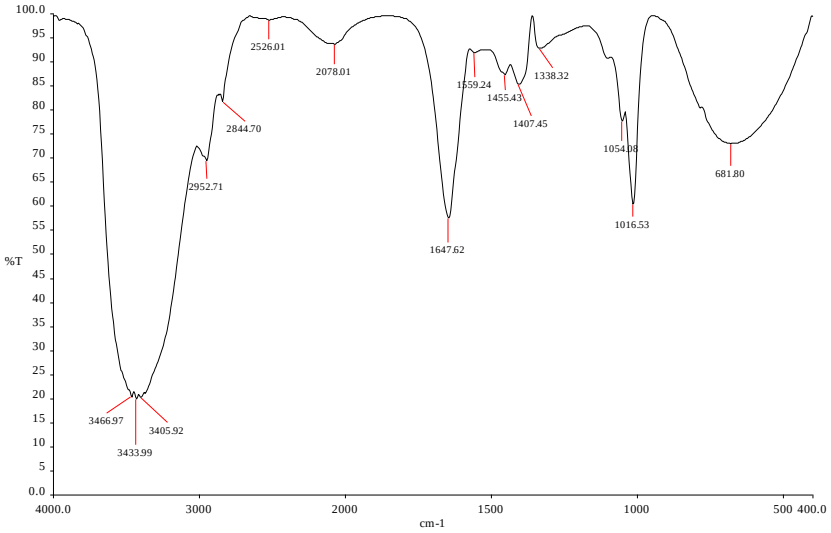

Figure: 2 FTIR Spectral analysis of *Aplotaxis auriculata* rhizome extract

Determination of HPLC retention times

HPLC profiles of *Aplotaxis auriculata* rhizome were analysed and five phenolic compounds namely Gallic Acid, Ellagic acid, Delphinidin -3-O-glucoside, Naringenin and Coumarin having different elution times could be obtained (Figure 2 and Table 2) 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 [18-22] supported the findings of these compounds.

Fig 2 HPLC analysis of *Aplotaxis auriculata* rhizome extract

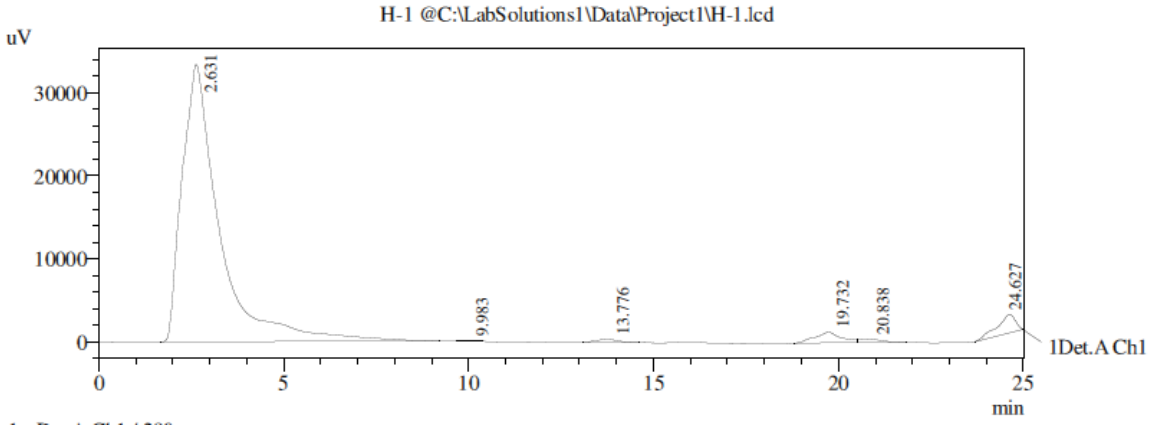

Table 2 HPLC analysis of *Aplotaxis auriculata* rhizome extract

Peak	Area %	Retention Time	Literature (RT)	Name of the compound
1.	93.110	2.631	2.679	Gallic Acid
2.	0.052	9.983	9.596	Ellagic acid
3.	0.557	13.776	13.3	Delphinidin -3-O-glucoside
4.	2.510	19.732	20.18	Naringenin
5.	0.701	20.838	Unknown	
6.	3.070	24.627	24.20	Coumarin
Total	100.000			

CONCLUSION

HPLC analysis provided a good platform for identification and quantification of five phenolic compounds as Gallic Acid, Ellagic acid, Delphinidin -3-O-glucoside, Naringenin and Coumarin present in *Aplotaxis auriculata* rhizome. The UV- VIS profile and FTIR analysis confirmed the presence of phenol, alkanes, aldehyde, secondary alcohol, amino acid, aromatic amines and halogen compound. The results of this study offer a platform of using *Aplotaxis auriculata* rhizome as herbal alternative for various diseases including diabetic, cardiovascular etc.

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