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LC-ESI-MS Determination of Bioactive Components from the aerial parts of *Stephania Wightii* (Arn.) Dunn (Menispermaceae) - an Endemic Medicinal plant from Western Ghats, Kerala, India.

Uthaman Danya^{1*}, Madathupatti Ramanathan Udhayasankar¹, David Punitha², Pandancode chandran Sajitha¹, Karupannan Arumugasamy¹.

1. Research Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India.

2. Department of Botany, Providence College for Women, Coonoor, The Nilgiris, Tamilnadu, India.

ABSTRACT

The present investigation was carried out for phytochemical profile from aerial parts of methanolic extracts of *Stephania wightii* by using Liquid Chromatography-Electronic Spray Ionization-Mass spectrometry (LC-ESI-MS). The results confirmed the presence of eighteen compounds in the crude extract and the pharmacological activities of the identified compounds were listed. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners.

Keywords: Liquid chromatography, mass spectrometry, *Stephania wightii*, aerial parts and bioactive compounds

*Corresponding Author Email: danya@gmail.com

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INTRODUCTION

LC coupled to mass spectrometry (LC/MS) is better suited for the analysis of nonvolatile polar compounds in their natural form¹. This method may be an alternative procedure for assessing the quality of end phytomedicines, in the selective analysis of low concentrations of toxic plant compounds².

The genus *Stephania* could be a potential source of biologically active compounds which might be used as lead molecules for development of new drugs³. *Stephania wightii* (Arn.) Dunn (Menispermaceae) is a slender climber with peltate and membranous leaves. The flowers are umbelliform cymes while inflorescence is axillary and arising from old leafless stem. It is commonly known as “Koloukone” in Tamil. The Kanikkar tribes, inhabitants of Agasthiarmalai Biosphere Reserve, Tamilnadu use the paste prepared from tubers with water to treat cancer⁴. These plants have traditionally have been used for the treatment of asthma, tuberculosis, dysentery, hyperglycemia, cancer, fever, intestinal complaints, sleep disturbances and inflammation^{5,6}. The objective of the present study was to detect the active constituents from aerial parts of *Stephania wightii* and to confirm their traditional uses.

MATERIALS AND METHODS:

Sample prepared by cold methanolic extraction:

The aerial parts of *Stephania wightii* was collected from Wyanad, Kerala state. The plant was identified and authenticated by Dr. K. Kalidass, Taxonomist, Botanical Survey of India, Coimbatore, Tamilnadu. The plants were shaded dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to conical flask, and treated with methanol until the powder is fully immersed and shaken in mechanical shaker. This process was repeated for 1 week and then the extract was filtered. The filtrate was collected and evaporated to dryness. The final residue thus obtained was then subjected to LC-MS analysis.

LC-MS Analysis:

LC-MS analyses were done on a Shimadzu liquid chromatography modular system consisting of two LC- 10AD pumps coupled to an LC/MS QP 8000 quadruple detector. The data were processed using Shimadzu's Class-8000 system. Analyses using the APCI (atmospheric pressure chemical ionization) interface were performed in a reverse phase C-18 column (25cmx205mm) fitted with a guard column (C-18, 20 mm x 4.0 mm x 5 µm), both Supelco, at an oven temperature of 35°C. The samples were introduced using a Rheodyne injection valve fitted with a 20 µL loop. The mobile phase consisted of aqueous and methanol (50:50) at a flow rate of 2mL

min⁻¹. The mass detector was operated in both positive and negative mode with nitrogen as the nebulizer gas at a flow rate of 2ml/ min, under the following conditions: the capillary temperature was 230°C; deflector voltage, + 47 V; CDL voltage and temperature, - 28 V and 250°C; probe voltage and temperature, +3.5 V and 400°C; acquisition range, 100 -700 m/z at 2.0 scan s⁻¹. Analyses using ESI (electrospray ionization) interface were done under the same chromatographic conditions as described for the APCI analysis, except for the guard column, which was not used in the ESI analysis. The mass detector conditions were: nitrogen as the nebulizer gas at a flow rate of 4.5 L min⁻¹; deflector voltage, + 54 V; CDL voltage and temperature, - 10 V and 230°C; probe voltage, +4.5 V; acquisition range, 50-800 m/z for negative and 50-950 m/z for positive at 2.0 scan s⁻¹.

RESULT AND DISCUSSION:

Methanolic extract of *Stephania wightii* aerial parts was analyzed for the presence of non-volatile polar compounds by Liquid chromatography-Mass spectroscopy (LC-MS) technique. The results of bioactive compounds identified by LC-MS analysis are presented in a Table 1 and Figure. 1. Mass spectroscopy analysis using Metwin 2.0 Library and retention time programme confirmed the presence of these 18 compounds. The pharmacological activities of the identified compounds were shown in Table 2.

Isolation and identification of bioactive compounds present in a crude extract sample, has emerged as the major path of new drug development from natural products. There is an enormous bioactive compound diversity of a plants crude extract to be covered. This presents a considerable challenge for the isolation and identification of bioactive compounds. During the last decade, LC-MS techniques were developed employing soft ionization methods like electrospray (ESI) or photoionization (APPI) and, simultaneously, mass spectrometers have become both more sophisticated and more robust for daily use. More recently, achievements in separation sciences propose much better solutions for the separation of the complex mixtures than it was attainable before ⁷. Already, thirteen compounds were identified in the ethanol extract from tuber of *Stephania wightii* by Gas Chromatography -Mass Spectrometry (GC-MS) analysis. The major components present in the tuber of *S. wightii* were (1H) Indolo(2,1-a) isoquinoline, 5,6,11,12-tetrahydro-2,3,8,9-tetramethoxy (59.98%), 6H Dibenzo (a,g) quinolizine, 5, 8, 13, 13a-tetrahydro-2, 3, 9,10-tetramethoxy-, (ñ)- (34.86%) and 1, 3-propanediol, 2-(hydroxymethyl) -2-nitro-(2.89%)⁸.

LC-MS analysis of methanolic extract of *Stephania wightii*.

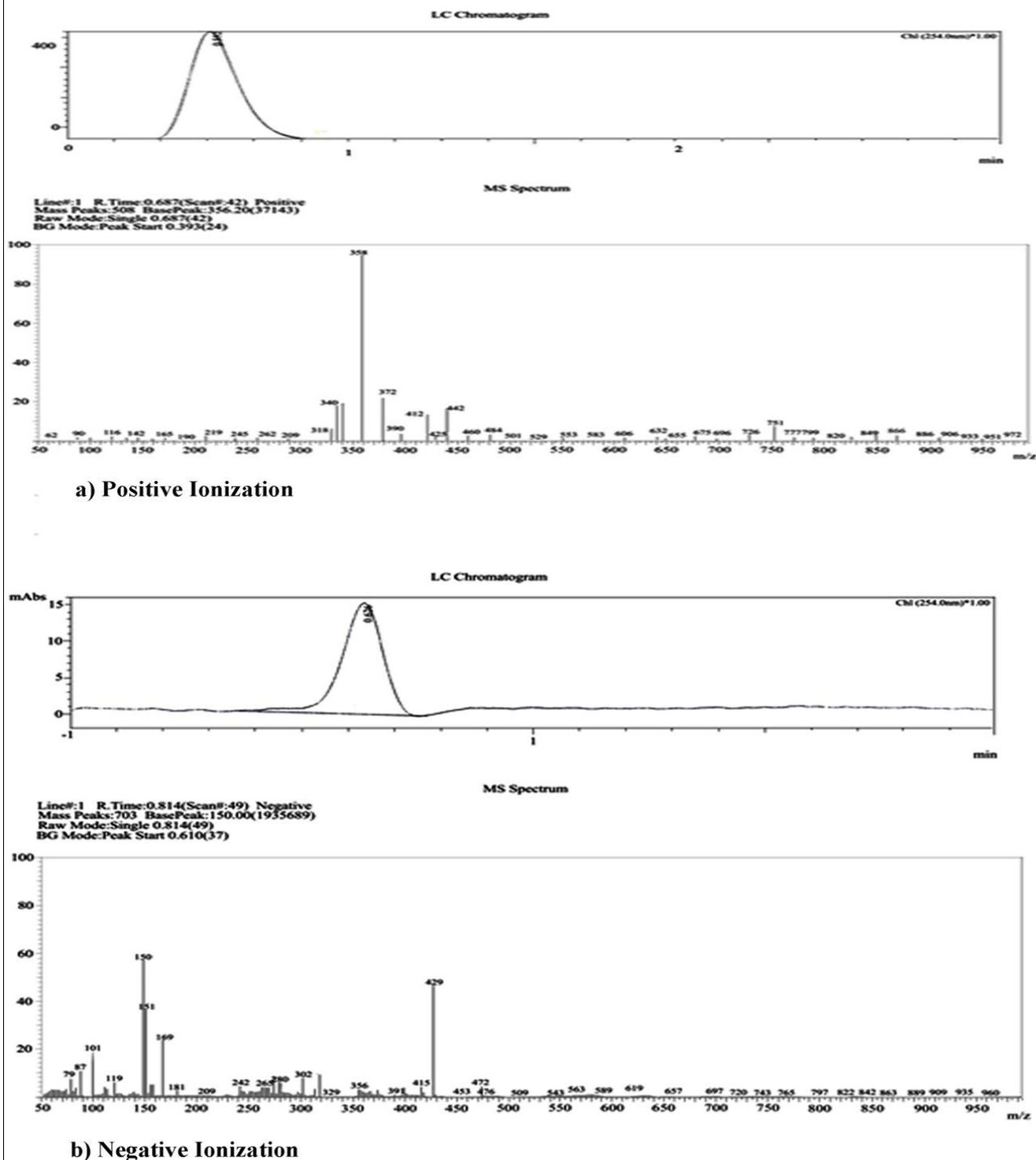
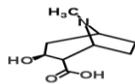
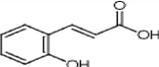
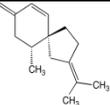
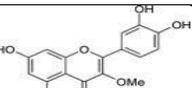
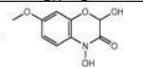
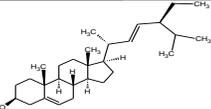
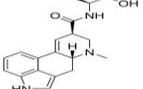
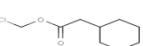
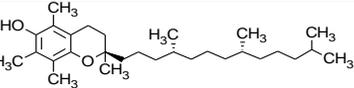
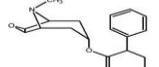
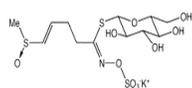
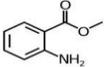


Figure 1: LC-ESI-MS analysis of methanolic extract of *Stephania wightii* under Positive and Negative Ionization mode

Table 1: Phytochemical components identified in the methanolic extract (aerial parts) of *S. wightii* by LC-ESI-MS technique.

Sr.	Compound name	Molecular mass	Structures
1	Hydroxy Tropane	141.22	
2	Trans-o-Coumaric acid	164.16	
3	Beta-Vetivone	218.34	
4	Cryptophorine	261.41	
5	Quercetin Tetramethylether	3,3',4',7- 358.35	
6	Dimboa Glucoside	371.31	
7	Stigmasterol	412.70	
8	Ergometrine maleate	441.49	
9	Hederin	750.97	
10	Butanoic acid	102.32	
11	1,3-propanediol, (hydroxymethyl)-2-nitro-	2- 151.24	
12	Cyclohexaneacetic acid	170.34	
13	Vitamine	430.25	
14	Scopolamine	303.36	
15	Glucoraphenin potassium salt	473.58	
16	Methyl anthranilate	151.17	

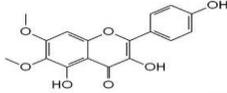
17	Eupalitin	330.29	
18	Methyl allyl disulphide	120.24	

Table 2: Activity of phyto components identified in the methanol extract of aerial parts of *Stephania wightii*.

Sr.	Compound name	Compound nature	Pharmacological activities
1	Hydroxy tropane	Alkaloid	Antioxidant activity, Antitumor activity, Cardioprotective activity
2	Trans-o-coumaric acid	Coumarin	Antioxidant activity, Anticancer activity
3	Beta-vetivone	Terpenoid	Antioxidant activity
4	Quercetin 3,3',4',7 tetramethylether	Flavonoid	Antioxidant activity
5	Dimboa glucoside	Glucoside	Antialgal activity, Antifungal activity
6	Stigmasterol	Triterpenoid	Anti-inflammatory activity
7	Hederin	Saponin	Antidiabetic activity, Anti-inflammatory activity, Antimutagenic activity,
8	Phenyl butanoic acid	Alkaloid	Anti tumor activity, Anti-inflammatory activity, Anti nociceptive activity
9	1,3-propanediol,2 (hydroxymethyl)-2-nitro-	Nitrogen compound	Antimicrobial activity
10	Cyclohexaneacetic acid	Acidic compound	Antimicrobial activity
11	Vitamin e	Vitamin compound	Antidiabetic activity, Antitumor activity, Antioxidant activity, Anti-inflammatory activity, Antiaging activity
12	Glucoraphenin potassium salt	Nitrogen and sulfur combined compound	Antagonistic activity, Anticancer activity
13	Eupalitin	Flavonol glycoside	Anti-cancer activity, Anti-osteoporosis activity, Anti-inflammatory activity
14	Methyl allyl disulphide	Terpenoid	Antifeedant activity, Anticancer activity, Antibiotic activity, Hypoglycemic activity, Hepatoprotective activity

Separation and analysis of polar compounds from the polar fraction of *Stephania yunnanensis* was established. Both the major components and minor components were analyzed by counter-current chromatography combined with liquid chromatography tandem mass spectrometry (LC-MS). From 50 mg polar fraction of crude extract, 15.2 mg corydine and 4.8 mg stepharine with purities over 90% were successfully separated via a polar solvent system n-butanol: methanol: water (4:1:5, v/v/v) with 10 mM NaOH as an additive in the lower phase, in one step operation⁹. Aristolochic acid I (AAI), tetrandrine and fangchinoline was isolated from the roots of *Stephania tetrandra* confirmed using LC-MS/MS¹⁰. The amount of obtained tetrandrine and fangchinoline were 0.26 and 0.27 $\mu\text{mol/L}$, respectively by liquid chromatography with electrochemical

detection¹¹.

CONCLUSION:

The present results confirm that LC-MS is a convenient and reliable method for the direct profiling active constituents in plant extracts. The different types of phytochemicals could be easily identified from the mass spectral fragmentation pattern. The technique is of immense value in the search for pharmaceutically important drugs. This study has identified as many as 18 compounds from this plant. The detection and identification of phytoconstituents may give a new direction to the analysis of new pharmaceutically important drug.

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