

GCMS ANALYSIS IN THE ETHANOLIC LEAF EXTRACT OF *PSYCHOTRIA BISULCATA* WIGHT & ARN., (RUBIACEAE).

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ABSTRACT

Plants are the source of many bioactive compounds. In the world from the Ancient times, the plant based drugs have been widely used for the treatment of various diseases. Compounds of plant origin have been and still are an important source of compounds for the production of novel drugs. The present study has been carried out to identify the phytochemicals present in the ethanolic leaf extract of *Psychotria bisulcata* Wight & Arn., (Rubiaceae) through GC-MS analysis. The GC-MS analysis revealed the presence of 22 compounds from the leaf of *P. bisulcata*. The compounds that are identified and found predominantly were Hydroxymethylfurfural, Triethyl citrate, Oleic acid Eicosyl ester, Lidocaine and 3-ethyl-5-(2-ethylbutyl). Interpretation of these compounds was identified by using the database of National Institute standard and Technology (NIST).

KEYWORDS: *Psychotria bisulcata*, Rubiaceae, GCMS, Ethanolic extract, Hydroxymethylfurfural

INTRODUCTION

One of the two main kingdoms of living things is the plant kingdom. They are the only living things that can use solar energy to make their own energy^[1]. Out of the two life forms prevailing plants play the major role in the life of Human beings. They are the only life forms that can produce their own food using energy from sunlight^[2]. For the manufacturing of drugs, the medicinal plants either as a whole or the parts such as leaf, stem, roots, inflorescence etc are used widely depends upon the curative properties. They can be either used directly or made with formulations. *Psychotria bisulcata* belongs to the family Rubiaceae. Herbal remedies frequently use plants from the genus *Psychotria* (leaves, roots, barks, and rhizomes) to cure bronchial and digestive disorders such as cough, bronchitis, ulcer, and stomachache. Infections of the female reproductive system are treated with them as well^[3,4,5].

MATERIALS AND METHODS

Identification and Collection of the Plant Samples:

The plant *P. bisulcata* belonging to the family Rubiaceae was collected in the Kinnakorai region of Kundha Taluk in The Nilgiris, Tamil Nadu. The authenticity of the plant has been done by referring the deposited specimen in the Botanical survey of India, Southern Regional Circle, Coimbatore. The voucher number of the specimen is BSI/SRC/5/23/2021/Tech-204. The leaves of the plant under study were collected washed and shade dried. The dried leaves were then powdered and stored in air tight container for further successive extraction.

Solvent Extraction:

The powdered plant sample was extracted successively with petroleum ether, chloroform, acetone and ethanol using Soxhlet apparatus at 55- 85°C for 8 to 10 hours and the aqueous extract was extracted using cold maceration technique order to extract the nonpolar and polar compounds. The solvent of respective extracts were reduced at room temperature and stored under 4°C in refrigerator for further use.

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 Whatman 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 260-900 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.

GCMS Analysis:

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0.32mm, column length is 30 m, column thickness 0.50 μ m), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73ml /min and an injection volume of 0.5 μ l was employed (split ratio of 10:1) injector temperature 270 $^{\circ}$ C; ion-source temperature 200 $^{\circ}$ C. The oven temperature was programmed from 40 $^{\circ}$ C (isothermal for 2 min), with an increase of 8 $^{\circ}$ C/min, to 150 $^{\circ}$ C, then 8 $^{\circ}$ C/min to 250 $^{\circ}$ C, ending with a 20 min isothermal at 280 $^{\circ}$ C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0 [6].

RESULTS

The present study has been carried out to find out the bioactive compounds present in the leaf of *P.bisulcata*. The pharmacological aspect of any plant is due to the presence of the secondary metabolites. Plants consist of active substances such as alkaloids, flavonoids, phenols, tannins, terpenoids, and steroids. These compounds are utilised extensively in medicine to treat a wide range of ailments.

Functional Groups Identification

The FTIR spectrum was used to identify functional groups of the active components present in plant samples, based on the peaks values in the region of IR radiation. The results of FTIR analysis have confirmed the presence of alcohol, phenol, alkanes, aldehyde, aromatic compound, carboxylic acids, amines and Nitro group of compound. (Fig-1 and Table-1)

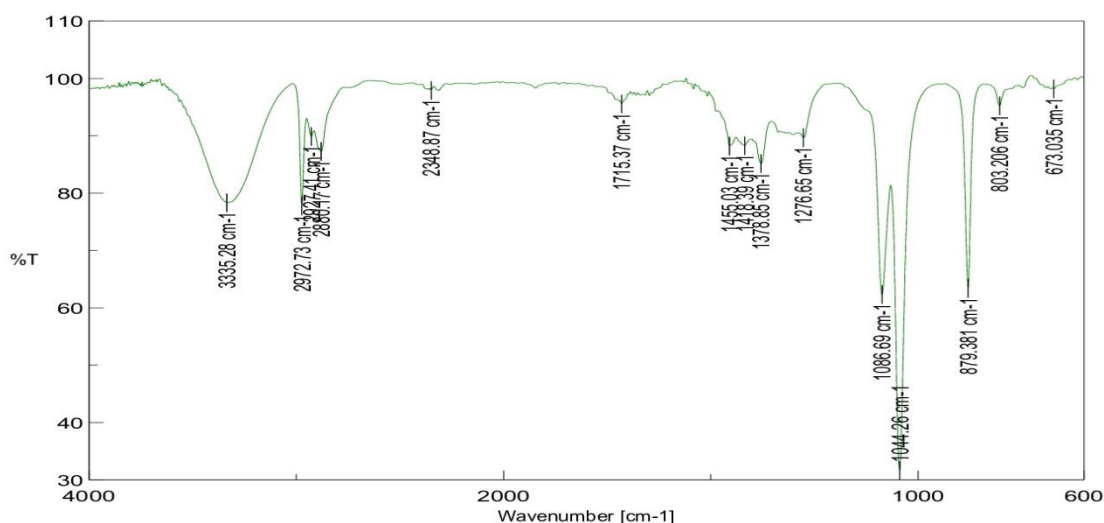


Fig 1: FTIR spectrum of ethanol

Table-1 Functional groups in *P.bisulcata* ethanolic leaf extract

S.NO	FUNCTIONAL GROUP/ASSINGMENT	INTENSITY	CHARACTERISTIC ABSORPTION (cm-1)
1	Phenols & Alcohols		3335.28
	O-H (free), usually sharp	Stretching Vibration,	
	O-H (H-Bonded), usually broad	Strong	
2	Alkanes		2972.73
	CH ₃ , CH ₂ & CH	Stretching Vibration, strong	
3	Aldehydes		2348.87
	C-H	Stretching Vibration Medium	
4	Carboxylic Acids & Derivatives		1715.37
	C=O (H-bonded) Acids	Stretching Vibration, Strong	
5	Aromatic Alkanes compounds		1455.03
	CH ₂ & CH ₃ deformation	Bending Vibration, Medium	
6	Alkanes		1378.85
	CH ₃ deformation	Bending Vibration, Medium	
7	Carboxylic Acids & Derivatives		1276.65
	O-C (sometimes 2-peaks)	Stretching Vibration Medium-Strong	
8	Amines		1086.69
	C-N	Stretching Vibration, Medium Strong	
9	Arenes		879.381
	C-H bending & ring puckering	Bending Vibration, strong-Medium	

Determination of compounds in GCMS analysis

The phytocomponents found in the ethanolic leaf extract of *Psychotria bisulcata* were identified by using GC-MS analysis and the running time is 35min. The GC- MS chromatogram (Fig:1) of ethanolic leaf extract of *Psychotria bisulcata* and the active components with their molecular formula, molecular weight (MW), retention time (RT), and concentration (% peak area) are given in Table:1 and the secondary metabolites and their medicinal activities are presented in Table: 2. In the GC-MS analysis 22 compounds were identified. The prevailing compounds were α-D-Glucopyranose (0.057), 1-Butanol, 3- methyl-, acetate (0.065), dl-Glyceraldehyde dimer (0.063), 2-Furancarboxaldehyde, 5-methyl- (0.059), Hexanoic acid, ethyl ester (0.038), Propane, 1,1,3- triethoxy- (0.046), Methyl 2-furoate (0.121), 4H-Pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6-methyl-5- (0.216), Hydroxymethylfurfural (4.124), Maltol (0.069), 1H-Pyrrolizine-1- carboxylic acid,hexahydro-,methyl ester (0.125), Cetene (0.047), D-Fucose (0.040), Melezitose (0.093), D-Allose (0.128), d-Gala-l-ido-octonic amide (0.156), Triethyl citrate (0.816), Lidocaine (0.130), l-(+)-Ascorbic acid 2,6-dihexadecanoate (0.268), cis-Vaccenic acid (0.220), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (0.081), Oleic acid, eicosyl ester (0.092). The most dominant compounds are Hydroxymethylfurfural (4.124), Triethyl citrate (0.816), L-(+)-Ascorbic acid 2,6-dihexadecanoate (0.268) followed by cis-Vaccenic acid (0.220). Lidocaine is a crystalline compound which is used as a local anesthetic. It is also habituated to produce infiltration anesthesia and various nerve blocks. Lidocaine inhibits nerve conduction by way of blocking sodium ion flux across the nerve membranes. This creates an anesthetic property by preventing pain signals from procreating to the brain but by ceasing them before they begin^[7].

Hydroxymethylfurfural (HMF) which is also known as 5-(hydroxymethyl) furfural, is an organic compound that is formed by the dehydration of the reducing sugars^[8,9]. 5-hydroxymethyl-2-furoic acid (HMFA) is a major metabolite in humans which is also known as Sumiki's acid, that is excreted in urine. HMF binds intracellular sickle hemoglobin (HbS). *In vivo* examination on transgenic sickle mice manifested that orally administered 5HMF hampered the formation of sickle cells in the blood^[10]. Under the development

code Aes-103, HMF contemplated for the treatment of sickle cell disease^[11]. Oleic acid, eicosyl ester has activities such as anti inflammatory, cancer preventive, dermatitigenic hypocholesterolemic, anemiagenic, insectifuge. (Fig -2and Table 2)

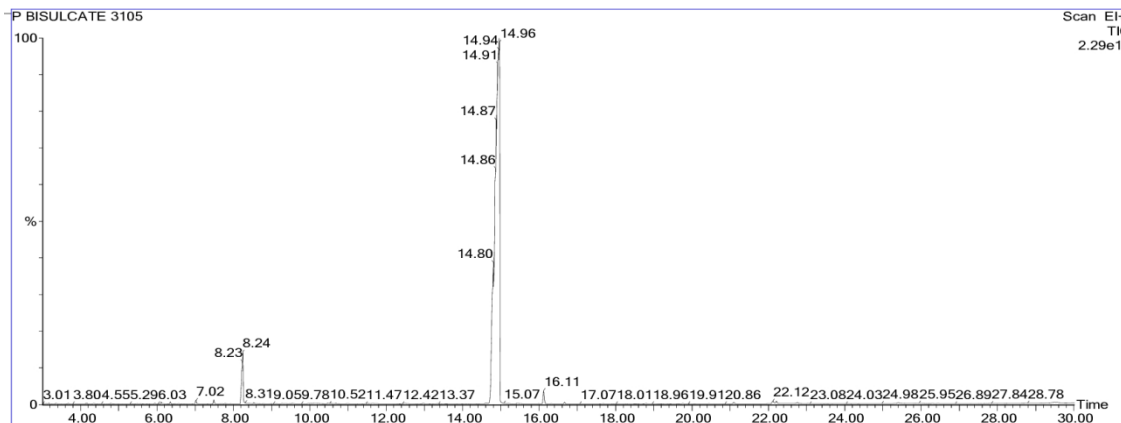


Fig: 2 GCMS analysis of *P.bisulcata* leaf extract

Table:2 GCMS analysis of *P. bisulcata* leaf extract

S.NO	RT	NAME OF THE COMPOUND	MOLICULAR FORMULA	MOLICULAR WEIGHT	PROBABILITY	PEAK AREA
1	3.108	á-D-Glucopyranose	C ₆ H ₁₂ O ₆	180.16	20.1	0.057
2	3.173	1-Butanol, 3-methyl-, acetate	C ₇ H ₁₂ O ₂	128.17	82.2	0.065
3	3.349	dl-Glyceraldehyde dimer	C ₆ H ₁₂ O ₆	180.16	56.3	0.063
4	4.339	2-Furancarboxaldehyde, 5-methyl-	C ₇ H ₆ O ₄	154.12	89.8	0.059
5	4.789	Hexanoic acid, ethyl ester	C ₁₀ H ₁₈ O ₄	202.25	67.3	0.038
6	5.945	Propane, 1,1,3-triethoxy-	C ₉ H ₂₀ O ₃	176.25	84.1	0.046
7	6.110	Methyl 2-furoate	C ₆ H ₆ O ₃	126.11	12.1	0.121
8	7.020	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	144.12	95.4	0.216
9	8.240	5-Hydroxymethylfurfural	C ₅ H ₆ N ₂ O ₃	142.11	96.6	4.124
10	8.531	Maltol	C ₆ H ₆ O ₃	126.11	15.4	0.069
11	10.106	1H-Pyrrolizine-1-carboxylic acid, hexahydro-, methyl ester	C ₈ H ₁₃ NO ₂	155.19	62.1	0.125

12	10.551	Cetene	$C_{20}H_{42}O_3$	330.5	2.6	0.047
13	11.007	D-Fucose	$C_6H_{12}O_6$	180.16	3.3	0.040
14	11.537	Melezitose	$C_{18}H_{32}O_{16}$	504.4	34.2	0.093
15	12.477	D-Allose	$C_6H_{12}O_6$	180.16	36.6	0.128
16	15.408	d-Gala-l-ido-octonic amide	$C_8H_{17}NO_8$	255.22	19.5	0.156
17	16.114	Triethyl citrate	$C_{26}H_{48}O_9$	504.7	89.1	0.816
18	21.090	Lidocaine	$C_{16}H_{27}BrN_2O$	343.30	51.6	0.130
19	22.116	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652.9	12.4	0.268
20	25.392	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5	11.9	0.220
21	29.489	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7	21.5	0.081
22	29.554	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	562.99	7.7	0.092

CONCLUSION

In conclusion, the present study has revealed 22 components from *P. bisulcata* ethanolic leaf extract using GC-MS analysis. Traditional practitioners have been using this plant for a variety of diseases, which is justified by the presence of several bioactive components. In order to use it in the creation of traditional medicines, more research is required to extract unique active components from the medicinal plants, which could lead to the discovery of a new treatment for a variety of incurable illnesses.

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