Advances in Bioresearch
Adv. Biores., Vol 12 (1) January 2021: 170-176
©2021 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.12.1.170176

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ORIGINAL ARTICLE

Investigation of Bioactive Compounds from Medicinally Important Herb *Byttneria herbacea* Extract Using GC-MS.

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ABSTRACT
The present study carried out the screening of the bioactive compounds present in Byttneria herbacea whole plant extract using Gas Chromatography–Mass Spectrometry (GC-MS). The study, confirms presence of tannin, saponin, flavonoids, steroids, terpenoids, triterpenoids, anthroquinone, polyphenol, glycoside and coumarins. In the GC-MS investigation, twenty four compounds were identified and they were capable of various pharmacological activities like anti-inflammatory, anticancer, anti-arthritic, antiulcer and antimicrobial activity.
Key words: *GC-MS, Bioactive compound, pharmacological activities, Byttneria herbacea*

Received 29.11.2020 Revised 22.12.2020 Accepted 07.01.2021

How to cite this article:
M. Sathish, P.K. Siva, T.Parvathi, and M. Arumugam. Investigation of Bioactive Compounds from Medicinally Important Herb *Byttneria herbacea* Extract Using GC-MS.. Adv. Biores., Vol 12 (1) January 2021: 170-176

INTRODUCTION

Plants are rich source of traditional medicine systems, food supplements, modern medicines, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs due to presence of numerous bioactive phytochemicals, essential mineral elements and other pharmacological properties [1]. A small part of the higher plants that yields around 120 remedial measures of known chemical structure have been identified the major plants derived drugs arevinblastine, podophyllotoxin, camptothecin, vincristine, taxol, digitoxigenin, gitoxigenin, digoxigenin, capscicine, allicin, curcumin, artemesinin, tubocurarine, morphine, codeine, headache medicine, atropine, pilocarpine, capscicine, allicin, curcumin, artemesinin and ephedrine [2].

The family "Malvaceae" consist of approximately 244 genera and 4225 species [3]. It is one of the biggest families among the Angiosperms. Many plants of this family have been used in traditional systems of medicines from their ancient times. Still, there are certain medicinal plants whose medicinal properties have not been explored properly. Phytochemical screening is crucial for validating the traditional use of these medicinal plants.

GC-MS technique was used to measure of the active principles in plants [4]. GC-MS analysis technique can be used to investigate traditional medicine and to characterize the compounds [5]. The present study aimed to make a preliminary phytochemical and GC-MS analysis of the bioactive compounds present in the *Byttneria herbacea*.

MATERIAL AND METHODS

Collection and Preparation of Sample

The aerial part of *B. herbacea* was collected from Narthamalai Hills of Pudukkottai District, Tamil Nadu. The collected plant-parts were washed in running tap-water to remove the adhering soil particles. They were later washed with sterile distilled water. The cleaned plant-parts were shade-dried, powdered with electric blender and preserved for further investigations. The powder sample was extracted by soxhlet apparatus with different solvents such as Methanol, Acetone, Chloroform, Ethanol and Water.

Preliminary Phytochemical Screening

Preliminary phytochemical analysis such as tannin, saponin, flavonoids, steroids, terpenoids, triterpnoids, alkaloid, carbohydrate, polyphenol and glycoside were carried out based on the standard procedure [6],[7], [8], [9].

GC-MS Analysis of *B. herbacea*

GC-MS analysis of the extracts was performed using a GC-MS (Model; Thermo Trace GC Ultra Ver.5.0) equipped with a DB-35MS fused-silica capillary column (30m length X outside diameter 0.25 mm X internal diameter 0.25 µm) and gas chromatograph interfaced to a Mass Selective Detector (MS-DSQ-II) with XCALIBUR software. For GC-MS detection, an electron ionization system with ionization energy of -70eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1ml/min and the sample was 1µl injected; Injector temperature at 250°C; Ion source temperature at 200°C. The oven temperature was programmed from 70° to 200°C at the rate of 10°C/min, held isothermal for 1minutes and finally raised to 250°C at 10°C/min. Interface temperature was kept at 250°C. Total run time was 40mins. The comparative percentage of each extract constituent was expressed as percentage with peak area normalization. Identification of compounds interpretation of mass spectrum of GC-MS was conducted using the mass spectral database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown constituent was compared with the spectra of the known components stored in the NIST library.

RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis

The presence of phytoconstituents make the plant useful for treating different diseases and have a possible of providing useful drugs to cure human diseases. The present study has found that most of the biologically active phytochemicals were present in various extract of *B. herbacea*. The present investigation showed the availability in the extract of the phytochemical in less concentration (Table 1). The present study confirmed the presence of phytochemical are tannin, saponin, flavonoids, steroids, terpenoids, triterpnoids, anthroquinone, polyphenol, glycoside and coumarins in the *B. herbacea* plant. Preliminary phytochemical analysis was performed on total extract of *Berberis goudotii* in phenol, polyphenol, flavonoid, and proanthocyanidin, and the tannin content was quantified [10].

Preliminary phytochemical screening has shown the presence of alkaloids, steriods, flavonoids, phenols, terpenoids, and cardiac glycosides. The aqueous leaf extract of *S. acuta* have shown moderate anti-bacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [11]. From this study it was concluded that, *Sida acuta* has rich phytochemical compounds. The presence of these secondary metabolites indicated they can be used to treat various diseases.

The qualitative phytochemical analysis has showed that the extract is positive for saponins, flavonoids, alkaloids, phenols and same extract is negative for carbohydrate, tannins, glycosides, cardiac glycosides,terpenoids, coumarins, steroid,phytosteroids, phlobutanins, anthraquinones. Quantitative analysis of phytochemicals includes the estimation of flavonoid, tannin and total content of phenol [12]. Phytoconstituents of Flavonoids, Saponin,Steroids, Terpenoids, Triterpnoids, Anthroquinones, Polyphenols, Glycosides and Coumarins indicate that the leaves of *Abutilon hirtum* [13].

Table 1: Preliminary qualitative analysis of *Byttneria herbacea*

S.No.	Name of the Test	Phytochemical constituents	Ethanol	Methanol	Chloroform	Acetone	Aqueous
1.	Ferric chloride test	Tannin	+	++	++	++	++
2.	Test for Saponins	Saponin	++	++	+	+	++
3.	Ammonia	Flavonoids	++	++	++	++	++
4.	Libermann's	Steroids	++	++	+	+	-
5.	Salkowaski	Terpenoids	++	++	++	++	++
6.	Chloroform, acetic anhydrate and H2So4	Triterpnoids	++	++	+	+	++
7.	Mayer's reagent	Alkaloid	-	-	+	+	+
8.	Molisch's reagent	Carbohydrate	++	++	++	+	-
9.	10% Nacl	Coumarin	++	++	++	+	++
10.	keller-killani	Glycoside	++	+	+	-	++

(-) Indicates Absence; (+) Indicates Presence; (++) High concentrations

GC-MS analysis

The GC-MS Chromatogram shows (Table-2: & Figure -1) the presence of 24 different peaks which confirm the presence of 24 phytocompounds with their respective RT; (Retention Time) in the extract of the whole plant of *B.herbacea.* The prevailing compounds in 1-1-Diethoxy-Ethane, 3-buten-2-one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl), Octadecanoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis- (CAS), Benzene, 1-ethyl-2,3-dimethyl- (CAS), à-L-Galactopyranose, 6-deoxy-, cyclic 1,2:3,4-bis(methylboronate), 1-Hexadecanol (CAS), 11-Octadecenal (spectrum disagrees) (CAS), 2-Hexadecanol (CAS), 7-Methoxy-2,2-dimethyl-2H-1-benzothiopyran, 1-Octadecene (CAS), Lucenin 2, 1,3-diformyl-2-chloro-5-isopropylbenzene, 6-Octadecenoic acid, 1-Octadecene (CAS), Pentadecanoic acid, 14-methyl-, methyl ester CAS), Hexadecanoic acid, ethyl ester (CAS), 6-Octadecenoic acid, 9-Octadecenoic Acid (Z)-, Ethyl Ester, Bacteriochlorophyll-c-stearyl, Heptaethylene glycol monododecyl ether, 1H-Cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol,3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-,9,9a - diacetate, [1aR 1aa, 1ba, 4aa, 5a, 7aa, 7ba, 8a, 9a,9aa], Stigmasterol, Methyl 7-ethyl-10-hydroxy-11-hydroxy(18o)-3,11-dimethyl-2,6-tridecadienoate, Stigmast-5-en-3-ol, (3a)- (CAS),. GC-MS analysis of mucilage showed presence of glucose, fructose, sucrose, maltose and xylose and showed it's scope to be of scientific relevance particularly as plant polymer based excipient and coating material in pharmaceutical products [14].

GC-MS analysis revealed the presence in phytocompounds. Most of them like n-Hexadicanoic acid, Phytol, Vitamin E, Lupeol, 2,3 Dihydrobenzofuran, Stigmasterol, Ergost-5-en-3-ol,(3.beta.,24R),gamma–Sitosterol, Neophytadien, Naphthalene, Tocopherols such as alpha Tocospiro A and alpha Tocospiro B are reported to have medicinal utility[15]. GC–MS analysis discovered the presence of the following phyto-compounds Methyl tetradecanoate, 9-dodeoenoic acid methyl ester E, Hexadecanoic acid methyl ester, 9, 12-octadecadienoic acid methyl ester, Methyl stearate and Methyl 18-nonadecanoate [16].

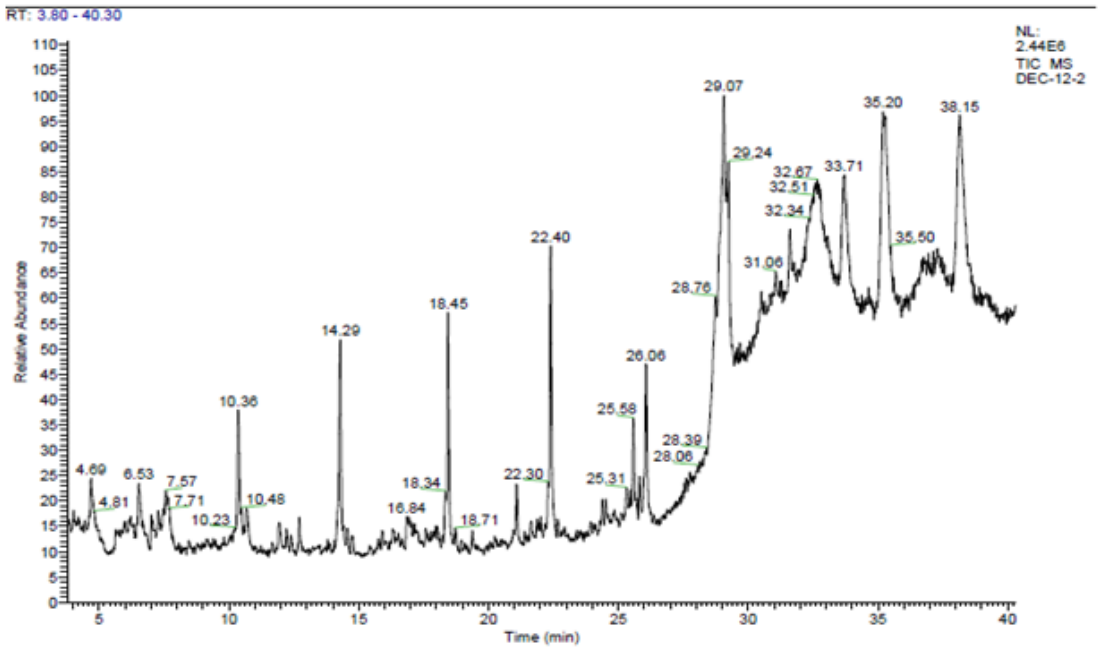
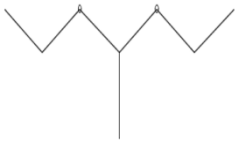
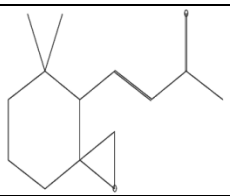
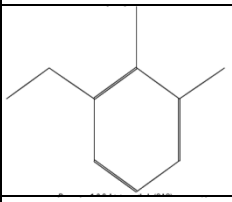
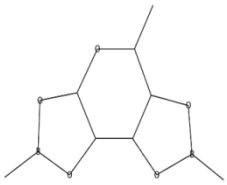
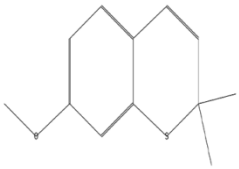
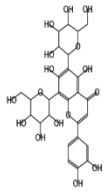
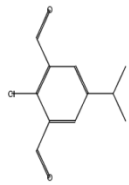
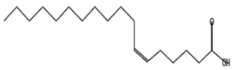
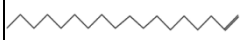
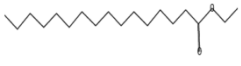
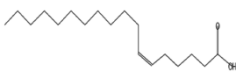
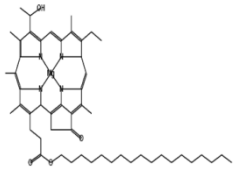
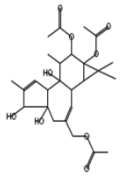
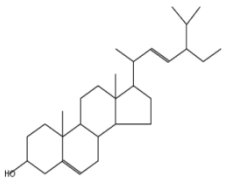
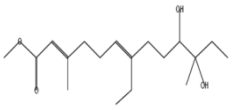
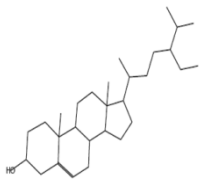


Figure 1: Chromatogram of Phytoconstituents from *Byttneria herbacea* using GC-MS

Table 2: Phytoconstituents present in *B. herbacea* using GC-MS

Peak	R. Time (min)	Area %	Molecular formula	Mol. wt.	Molecular Structure	Name of compounds
1.	3.04	4.50	$C_6H_{14}O_2$	118		1-1-Diethoxy-Ethane
2.	4.71	1.61	$C_{13}H_{20}O_2$	208		3-buten-2-one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl)
3.	6.20	0.99	$C_{28}H_{46}O_4$	446		Octadecanoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis- (CAS)
4.	6.55	1.69	$C_{10}H_{14}$	134		Benzene, 1-ethyl-2,3-dimethyl- (CAS)
5.	7.61	2.07	$C_8H_{14}B_2O_5$	212		à-L-Galactopyranose, 6-deoxy-, cyclic 1,2:3,4-bis(methylboronate)
6.	10.36	3.12	$C_{16}H_{34}O$	242		1-Hexadecanol (CAS)
7.	11.95	0.84	$C_{18}H_{34}O$	266		11-Octadecenal (spectrum disagrees) (CAS)
8.	12.23	0.74	$C_{16}H_{34}O$	242		2-Hexadecanol (CAS)

9.	12.72	0.76	$C_{12}H_{14}OS$	206		7-Methoxy-2,2-dimethyl-2H-1-benzothiopyran
10.	14.29	5.09	$C_{18}H_{36}$	252		1-Octadecene (CAS)
11.	15.92	0.84	$C_{27}H_{30}O_{16}$	610		Lucenin 2
12.	16.33	1.00	$C_{11}H_{11}ClO_2$	210		1,3-diformyl-2-chloro-5-isopropylbenzene
13.	16.90	0.97	$C_{18}H_{34}O_2$	282		6-Octadecenoic acid
14.	18.45	5.37	$C_{18}H_{36}$	252		1-Octadecene (CAS)
15.	21.09	1.43	$C_{17}H_{34}O_2$	270		Pentadecanoic acid, 14-methyl-, methyl ester CAS)
16.	22.40	7.04	$C_{18}H_{36}O_2$	284		Hexadecanoic acid, ethyl ester (CAS)
17.	24.39	1.18	$C_{18}H_{34}O_2$	282		6-Octadecenoic acid

18.	25.58	1.88	$C_{20}H_{38}O_2$	310		9-Octadecenoic Acid (Z)-, Ethyl Ester
19.	26.06	3.84	$C_{52}H_{72}MgN_4O_4$	840		Bacteriochlorophyll-c-stearyl
20.	29.07	15.55	$C_{26}H_{54}O_8$	494		Heptaethylene glycol monododecyl ether
21.	32.61	7.28	$C_{26}H_{36}O_9$	492		1H-Cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol,3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-, 9,9a-diacetate,[1aR-(1aa,1ba,4aa,5a,7aa,7ba,8a,9a,9aà)]-
22.	35.24	10.88	$C_{29}H_{48}O$	412		Stigmasterol
23.	37.30	1.73	$C_{18}H_{32}O_4$	312		Methyl 7-ethyl-10-hydroxy-11-hydroxy(18o)-3,11 -dimethyl-2,6-tridecadienoate
24.	38.15	8.05	$C_{29}H_{50}O$	414		Stigmast-5-en-3-ol, (3á)- (CAS)

CONCLUSION

The current investigation reveals the potential of *B. herbacea* whole plant extract as a good sources of bioactive compounds such as 17-Pentatriacontene, 9,12,15Octadecatrienoic Acid, Stigmasterol, Hexadecanoic acid, methyl ester, Stigmast 5 en 3 ol and 9-Octadecenoic Acid (Z)-, Ethyl Ester that bioactive compounds are used as various ailments through traditional medicines. Study of these bioactive compounds may yield Anti-inflammatory, Anticancer, anti-arthritic, antiulcer and antibacterial drugs.

ACKNOWLEDGEMENT

The authors are grateful to the financial support (F.No:4-4/2015-16/MRP-SERO) of the UGC, SERO, Hyderabad. We are also sincere thanks to Dr. S. Soosairaj, Assistant Professor, Department of Botany, St. Joseph College, Tiruchirappalli for plant identification and authentication.

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