WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 12, 1791-1798. Research Article ISSN 2277– 7105

BIOACTIVE COMPONENTS OF STEVIA REBAUDIANA LEAF USING ETHANOL EXTRACT

Sunitha V.* **and Irene Wilsy J.**

*Research Scholar, Assistant professor in Botany Department of Botany & Research centre, Scott Christian College (Autonomous) Nagercoil, Kanyakumari -629 003, Tamilnadu., India.

Article Received on
19 Oct 2015,

Revised on 09 Nov 2015,
Accepted on 29 Nov 2015,

***Correspondence for Author**
Sunitha V.
Research Scholar, Assistant professor in Botany Department of Botany & Research centre, Scott Christian College (Autonomous) Nagercoil, Kanyakumari - 629 003, Tamilnadu., India.

ABSTRACT

The present investigation was carried out to determine the possible bioactive compounds from Stevia rebaudiana leaf by GC-MS Technigue. This analysis revealed that Stevia rebaudiana contain Benzo(b)thiophene, 6 methyl, 1 H-Pyrrol (2,3-C Pyridine propanoic acid 5(4H)-oxo-6,7-dihydromethyl ester, Pentadecanoic acid, 13 methyl;methyl ester, Hexahydropyridine, 4- (4,5-dimethoxy pheny,Hexadecenoic acid,14-ethyl; methyl ester, 9-Octa decanoic acid,(E), 5- Octadecanoic acid Heptadecanoic acid,15-methyl; ethyl ester, Pregna-4, 6-diene 3, 20-dione, 17, hydroxy -6, 16a- dimethyl, 5a-Pregna 16-en 3, 20-one, 3a, 12a di hydroxyl diacetate, 3,4 Dimethyl-5 oxo-2,5-dihydro-1H-pyrrol-2-y–(4-4 dimethyl 5(2,3,3-trimethyl-5 methylthio-3,4-dihydro-2H-pyrrol-2ylmethylene)pyrrolidin-2yelene) thio acetic acid,5 –(tert-butyl)ester, Propanoic acid, and 2-(3 acetoxy-4,4,14 trimethyl and rost-8-en-17yl).

KEYWORDS: Stevia rebaudiana, GC-MS, Bioactive Components.

INTRODUCTION

Medicinal plants have occupied an important position in the socio-cultural, development of rural people of India. Plants and leaves are as considered one of the main sources of biologically active compounds. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in manydeveloping countries.[1] Plant-based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc.[2] Screening active compounds from plants has lead to the invention of newmedicinal drugs which have different protection and treatment roles against various

diseases, including cancer[3] and Alzheimer's disease.[4] The modern methods describing the identification and quantification of active constituents in plant material may be useful for proper standardization of herbal and its formulations. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc.[5] In recent years there has been increasing demand for natural non-nutritive high intensity sweeteners with low-calorie value as an alternative to sucrose. Ex- tracts of the leaves of Stevia rebaudiana(Bertoni), have been known for their sweet taste. Steviosides and rebaudioside-A are the two major diterpenoidglucosides components present in the leaf extracts of the stevia, these glycosides are 300 times sweeter than sugar and also exhibits wide therapeutic activity.

MATERIALS AND METHODS

Extraction of plant materials

The plant material was shade dried and pulverized in to powder, using a mixer grinder. Required quantity of the powder were weighed,transferred to the flask,treated with ethanol until the powder was fully immersed,incubated overnight and filtered through a Whatmann No.1 filter paper along with sodium sulphate to remove the sediments and traces of water in the filter paper.Before filtering, the filter paper along with sodium sulphate was wetted with absoluted alcohol. The filtrate is then concentrated to 1ml by bubbling nitrogen gas in to the solution.

The ethanol extract contains polar component of the plant material and 2ml of sample of solution was employed in GC-MS for analysis of different compounds.

Gas Chromatography-Mass Spectrometry (GC-MS Analysis)

GC-MS Analysis of the ethanol extract of the selected plant was performed using a Perkin Elmer GC Clarus 500 system comprising AOC-20iAuto sampler and a Gas chromatograph interfaced to a mass spectrometer(GC-MS)equipped with a Elite-5MS(5%Diphenyl/Dimethyl poly siloxane) fused silica capillary column (30×0.25mm×1D×0.25mmdF). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70ev.Helium gas (99.999%)was used as carrier gas at a constant flow rate of 1ml/mi, and an injection volume of 2ml was employed (split ratio of 10:1).The injector temperature was maintained at 250^{0}c, the ion-source temperature was 200°c, the oven temperature was programmed at 110°c (isothermal for 2 min)with an increase of 100c/min to

200°c then, 5°c/min to 280°c ending with 9min isothermal at 280°c. Mass spectra was taken at 70ev;a scan interval of 0.5 seconds and fragments from 45-450Da.The solvent delay was 0 to 2 min. The relative percentage of each component was calculated by comparing its average peak area to the total areas. The mass detector used in this analysis was Turbo-Mass Gold-Perkin Elmer and the software adopted to handle mass spectra and chromatograms was a Turbomass.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National institute of standard technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknowncomponentwas compared with the spectrum of the known components stored in the NIST library. The name, Retention time, and structure of the components of the test materials were ascertained.

RESULT

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the ethanolic extract of stevia rebaudiana. These compounds were identified through mass spectrometry attached with GC. The results of the present study were tabulated in Table 1. The results revealed that the presence of Benzo(b)thiophene, 6 methyl, 1 H-Pyrrol (2,3-C Pyridine propanoic acid 5(4H)-oxo-6,7-dihydromethyl ester, Pentadecanoic acid, 13 methyl;methyl ester, Hexahydropyridine, 4- (4,5-dimethoxy pheny,Hexadecenoic acid,14-ethyl; methyl ester, 9-Octa decanoic acid,(E), 5- Octadecanoic acid Heptadecanoic acid,15-methyl; ethyl ester, Pregna-4, 6-diene 3, 20-dione, 17, hydroxy - 6, 16a- dimethyl, 5a-Pregna 16-en 3, 20-one, 3a, 12a di hydroxyl diacetate, 3,4 Dimethyl-5 oxo-2,5-dihydro-1H-pyrrol-2-y –(4-4 dimethyl 5(2,3,3-trimethyl-5 methylthio-3,4-dihydro-2H-pyrrol-2ylmethylene)pyrrolidin-2yelene) thio acetic acid,5 –(tert-butyl)ester, Propanoic acid, and 2-(3 acetoxy-4,4,14 trimethyl and rost-8-en-17yl). The spectrum profile of GC-MS confirmed the presence of 14 compounds with retention time 14.2, 16, 17.38, 17.62, 17.98, 19.1, 19.67, 19.82, 20.43, 23.28, 23.28, and 21.7 respectively. The individual fragmentation of the components is illustrated in Figure.1

Table 1.GC-MS Analysis of active fraction using ethanol extract of Stevia rebaudiana leaf

No.	Retention time	Name of the compounds	Peak area (%)	Molecular weight	Molecular formula
1	14.2	Benzo(b)thiophene, 6 methyl	32	148.225	C_9H_8S
2	16	1 H-Pyrrol (2,3-C Pyridine propanoic acid 5(4H)-oxo-6,7-dihydromethyl ester	26.3	222.24	$C_{11}H_{14}N_2O_3$
3	17.38	Pentadecanoic acid, 13 methyl;methyl ester	10.1	270.450	$C_{17}H_{34}O_2$
4	17.62	Hexahydropyridine, 4- (4,5-dimethoxy pheny	13.2	221.295	$C_{13}H_{19}NO_2$
5	17.98	Hexadecenoic acid,14-ethyl; methyl ester	12.9	284.477	$C_{18}H_{36}O_2$
6	19.1	9-Octa decanoic acid,(E)	15	282.461	$C_{18}H_{34}O_2$
7	19.67	5- Octadecanoic acid	8.7	296.487	$C_{18}H_{34}O_2$
8	19.82	Heptadecanoic acid,15-methyl; ethyl ester	12.4	298.503	$C_{19}H_{34}O_2$
9	20.43	Pregna-4, 6-diene 3, 20-dione, 17, hydroxy -6, 16a-dimethyl	19.4	416.937	$C_{24}H_{29}C/O_4$
10	23.28	5a-Pregna 16-en 3, 20-one, 3a, 12a di hydroxyl diacetate	17.6	278.928	$C_{23}H_{34}O_4$
11	23.28	3,4 Dimethyl-5 oxo-2,5-dihydro-1H-pyrrol-2-y –(4-4 dimethyl 5(2,3,3-trimethyl-5 methylthio-3,4-dihydro-2H-pyrrol-2ylmethylene) pyrrolidin-2yelene) thio acetic acid,5 –(tert-butyl) ester	12.3	503.765	$C_{27}H_{41}N_{302}S_2$
12	21.7	Propanoic acid, 2-(3 acetoxy-4,4,14 trimethyl and rost-8-en-17yl)	17.7	430.619	$C_{27}H_{42}O_4$

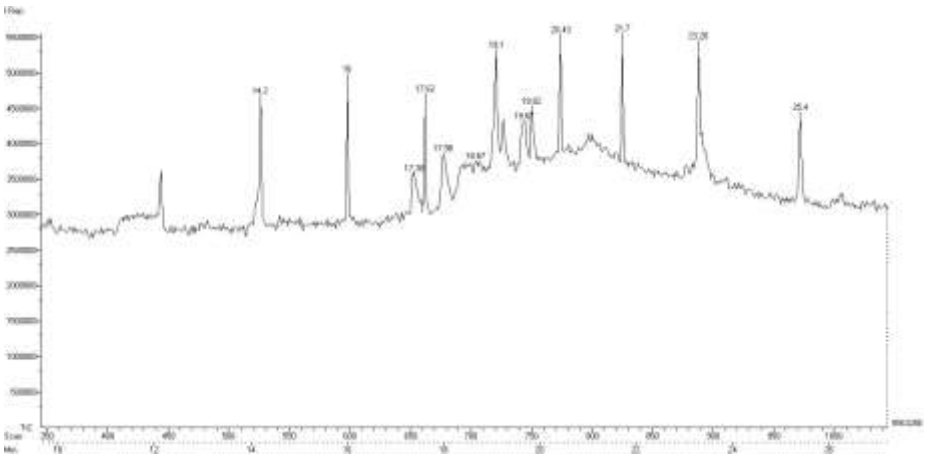
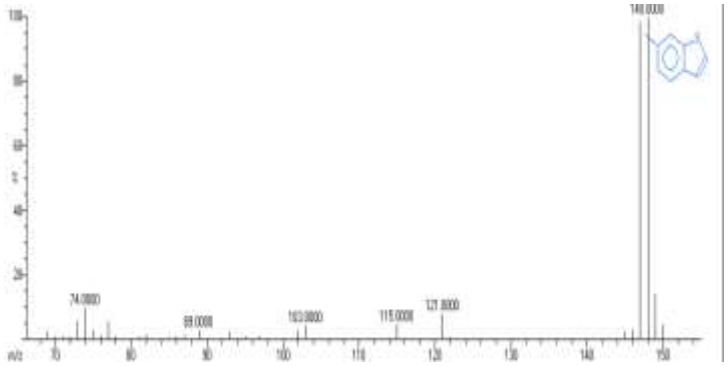
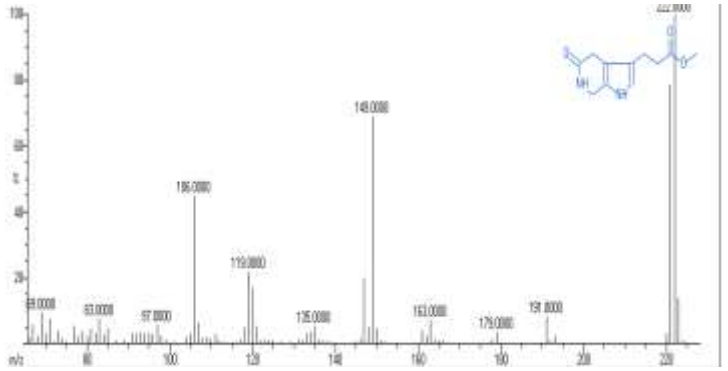


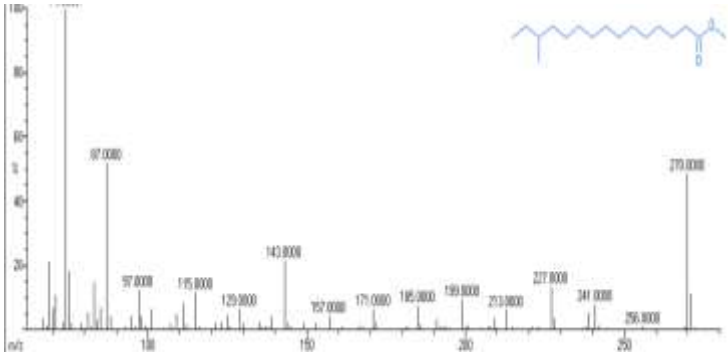
Figure.1. GC-MS Chromatogram of ethanol extract in Stevia rebaudiana leaf



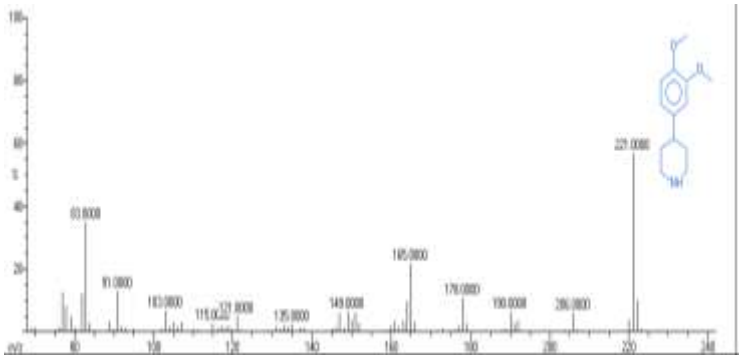
Mass spectrum of Benzo(b)thiophene, 6 methyl



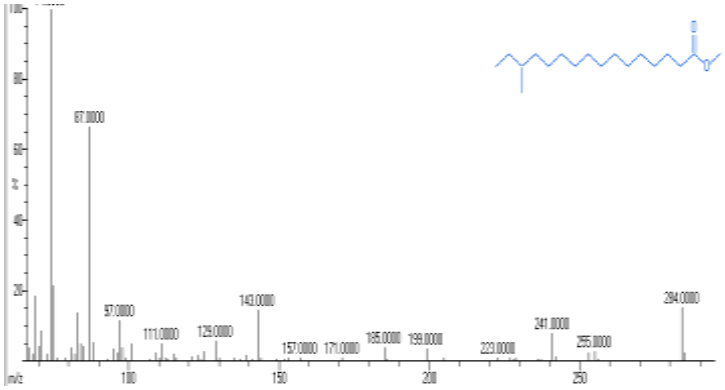
Mass spectrum of 1H-Pyrrol (2,3-C Pyridine propanoic acid 5(4H)-oxo6,7-dihydromethyl ester



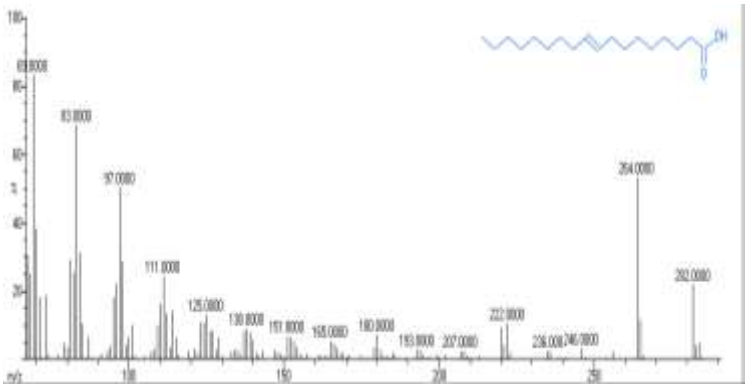
Mass spectrum of Pentadecanoic acid, 13 methyl; methyl ester



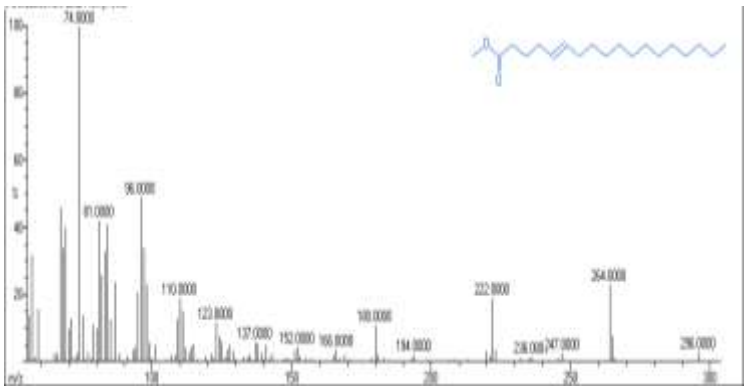
Mass spectrum of Hexahydropyridine, 4-(4,5-dimethoxy pheny)



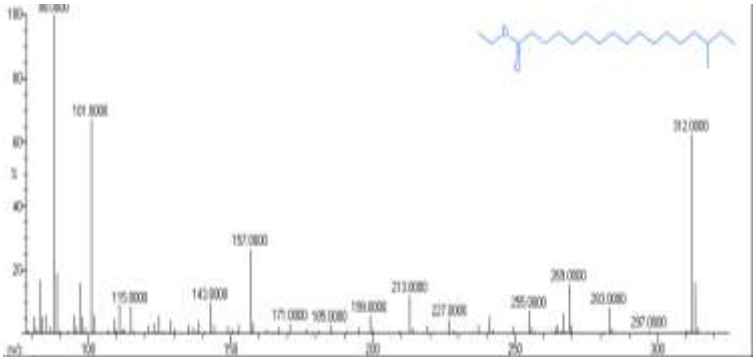
Mass spectrum of Hedadecenoic acid, 14-ethyl; methyl ester



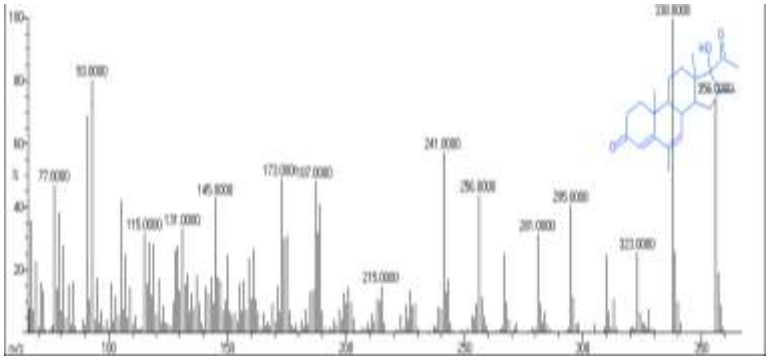
Mass spectrum of 9-Cota decanoic acid, (E)



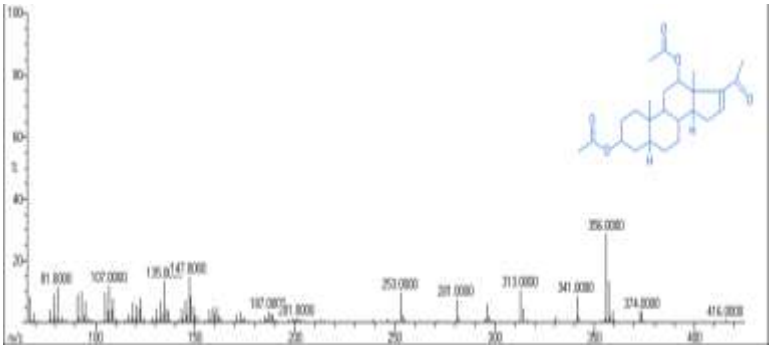
Mass spectrum of 5-Octadecanoic acid



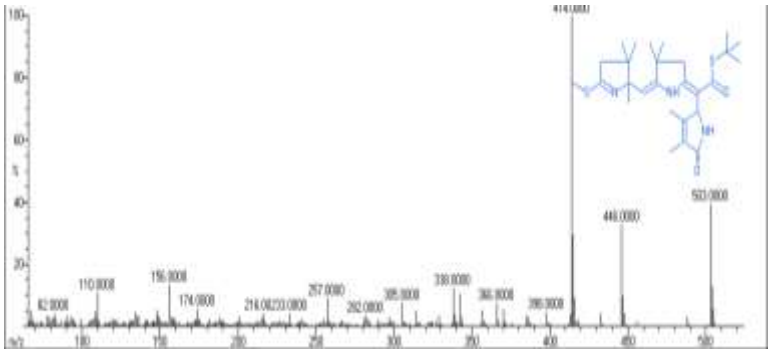
Mass spectrum of Heptadecanoic acid, 15-methyl; ethyl ester



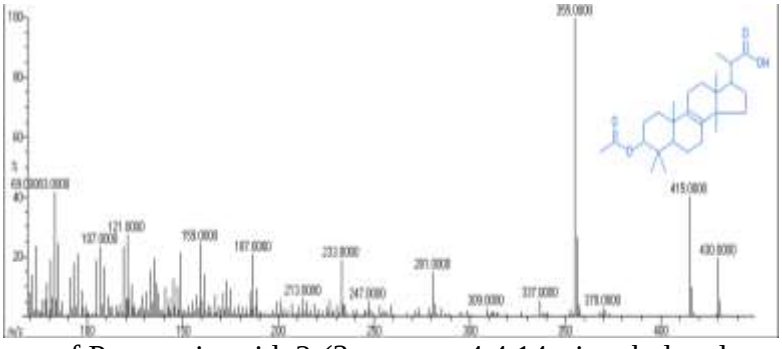
Mass spectrum of Pregna-4, 6-diene 3, 20-dione, 17, hydroxyl -6, 16a dimethyl



Mass spectrum of 5a-Pregna 16-en 3, 20-one, 3a, 12a di hydroxyl; diacetate



Mass spectrum of 3,4 Dimethyl-5 oxo-2,5-dihydro-1H-pyroll-2-y – (4-4 dimethyl 5 (2,3,3-trimethyl-5 methylthio-3,4-dihydro-211-pyrrol-2ylmethylene) pyrrolidin-2yelene) thio acetic acid, 5-(tert-butyl) ester



Mass spectrum of Propanoic acid, 2-(3 acetoxy-4,4,14 trimethyl and rost-8-en-17yl)

DISCUSSION

In the present study, the GC-MS analysis of the ethanolic extract of plant of Stevia rebaudiana leaves showed the presence of twelve compounds. The major compounds have all shown to have cancer preventive, antioxidant and antimicrobial are shown by pentadecanoic acid-, 14-methyl-, methylester and octadecanoic acid, 7-hydroxy-, methyl ester. There is growing awareness in correlating the phytochemical compounds and their biological activities.[6,7] We report the presence of some of the important components resolved by GC-MS analysis and their biological activities. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will helpful for further detailed study.

CONCLUSION

In the present investigation twelve bioactive compounds were identified using ethanol extract in Stevia rebaudiana leaf. Stevia's extract from leaves has been used for a few decades to sweeten soft drinks, soju, soya sauce, yogurt and other benificial effects on human health.

REFERENCE

1. Bobbarala V, Bramhachari PV, Ravichand J, Reddy YHK, Kotresha D, Chaitanya KV. J Pharm Res, 2011; 4(1): 252-255.
2. Gordon DM. Microbiology 2001; 147:1079-1085.
3. Sheeja K, Kuttan G. ImmunopharmacolImmunotoxicol, 2007; 29: 81-93.
4. Mukherjee PK, Kumar V, Houghton PJ. Phytother Res, 2007; 21: 1142-1145.
5. Muthulakshmi A, Joshibhi Margret R,Mohan V R. App. pharmac.sci, 2012; 2:69-74.
6. Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L. Nat Rev Mol Cell Biol, 2004; 5: 763 - 769.
7. Sumner LW, Mendes P, Dixon RA. Phytochem, 2003; 62(6): 817-836.