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RESEARCH ARTICLE

GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS IN AQUEOUS HOT EXTRACT OF *Eugenia uniflora* (L.) LEAVES

***Dr. Geedhu Daniel**

Assistant Professor, Pazhassiraja College, Pulpally, Wayanad, Kerala-673591, India

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****Corresponding author:*** **Dr. Geedhu Daniel**

ABSTRACT

The present study was designed to determine the bioactive components in aqueous hot extract of *Eugenia uniflora* leaves. Phytochmical screening of the extract revealed the presence of various metabolites like alkaloids, flavanoids, cardiac glycosides, phenols, tannins etc., GC-MS analysis in aquous extract was carried out using Perkin Elmer GC Clarus 500 system comprising AOC-20i Auto-sampler and a Gas chromatography interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-5MS (5% Diphenyl 95% Dimethyl Poly Siloxane) Fused capillary column (30× 0.25m 1D × 0.25m dF). GC-MS analysis has shown the presence of 39 biomolecules corresponding to 39 peaks. From the result it is evident that the aqupous leaf extract of *Eugenia uniflora* leaves contains various phytocomponents and is recommended as a plant of phyto pharmaceutical importance.

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INTRODUCTION

From ancient time onwards, natural products have been considered as source of medicines for preventing and treating human diseases (Cragg *et al.,* 1997). Natural products have used as antibacterial, anticancer, anticoagulant, antiparasitic and immunosuppressant for treating 87% of all human diseases (Newman *et al.,* 2003). India is the largest producer of medicinal herb and India is called as botanical garden of the world (Ahmedull and Nayar, 1999). Large number of medicinal plants and their phytochemical constituents has shown several therapeutic potentials. In order to promote these medicinal plants as source of a drug, it is important to investigate their composition and activity thoroughly. Structural elucidation provides an idea to select a crude plant extract with useful properties for further chemical and pharmacologic investigation. Since few years gas chromatography and mass spectrometry has become a key technological platform for profiling various metabolites in various plant species .Analyses of small amount of phytoconstituents have become easier and most cost effective by GC-MS analysis. GC-MS can identify pure compounds that are present at less than 1gm. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. In the last few years, gas chromatography-mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species (Robertson, 2005; Fernie *et al.,* 2004; Kell *et al.,* 2005). Gas- Chromatography-Mass Spectrometry (GC-MS) is a helpful technique for reliable profiling of secondary meta bolites (Mariswamy Y, Gnaraj, 2011). GC-MS can also be used in airport security to detect substances in luggage or on human beings. However, fewer reports are available with respect to the pharmacological properties of the plant (Cowan, 1999). GC-MS is one of the best techniques to identify the bioactive constituents of long-chain branched chain hydrocarbons, ester, acids, alcohols etc.

Eugenia uniflora L. (Myrtaceae) is a tropical and subtropical shrub widely distributed in American countries (Rotman, 1995). It is commonly referred to as Pitanga cherry or Brazilian cherry. Regarding their effects on human health, both fruit and leaves are used as folk medicine to treat similar diseases, although the leaves show the advantage of being perennial and continuously available, while the fruits are available during a short period of the year (Kanazawa *et al.,* 2000). The fresh or dried leaves have been used empirically as medicine, since the 15th century, for treating inflammatory and stomach diseases, rheumatism, fever, and hypertension (Alonso, 1998; Adebajo *et al.,* 1989). Some studies have confirmed that *Eugenia uniflora* possesses anti-inflammatory, antimicrobial, and antifungal properties (Adebajo *et al.,* 1989; Consolini and Sarubbio, 2012; Schapoval *et al.,* 1994; Holetz *et al.,* 2002). These benefits are usually attributed to the presence of many secondary metabolites present in the leaves, which includes many volatile terpenoid oils, flavonoids, and condensed and hydrolysable tannins, leucoanthocyanidins, and steroids and/or triterpenoids (Lima *et al.,* 1993). Considering

all the pharmaceutical importance of the extract, the present study aims for the GC-MS analysis of bioactive compounds in aqueous hot extract of Eugenia uniflora leaves.

MATERIALS AND METHODS

Collection and authentication of plant sample: Fresh leaves of *Eugenia uniflora* were collected from Wayanad district, Kerala, India during the month of February 2014. Taxonomic authentication was done by Dr. V.S Ramachandran, Taxonomist, Department of Botany, Bharathiar University, Coimbatore, Tamilnadu, India.

Processing of plant sample: Fresh leaves were cleaned, washed and rinsed with distilled water. Leaves were then shade dried at room temperature. The dried sample was powdered using a mechanical grinding mortar for effective extraction with solvents. Accurately 10 grams of the dried powdered material was tied in a muslin cloth. The material was put in a soxhlet apparatus and then extracted with 100 ml of seven different solvents such as methanol, ethanol, chloroform, petroleum ether, ethyl acetate, acetone, aqueous cold and hot extract, based on increasing polarity. Extraction was carried out for 8 hours. The residue was then collected and concentrated using rotary evaporator to remove the solvent. The residue was collected and kept at room temperature for 16 hours for complete evaporation of solvent. The extract was stored in cold room at temperature of 4˚ C for further use.

Hot Water Decoction: 10g of the powdered sample was dissolved in 100ml of distilled water which was boiled for one and half an hour and filtered. The decoction was stored at 4°C for further usage.

GC-MS analysis of the aqueous extract: GC-MS analysis of the extract of *Eugenia uniflora* was performed using a Perkin Elmer GC Clarus 500 system comprising AOC-20i Auto-sampler and a Gas chromatography interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-5MS (5% Diphenyl 95% Dimethyl Poly Siloxane) Fused capillary column (30× 0.25m 1D × 0.25m dF). An electron ionization system was operated in electron impact mode with ionization energy of 70ev for GC-MS detection. Carrier gas used is Helium gas (99.999%) at a constant flow rate of 1ml/min, and an injection volume of 2 1 was employed (split ratio of 10:1). The injector temperature was maintained at 250^{o}C, the ion-source temperature was 200^{o}C, the oven temperature was programmed at 110^{o}C (isothermal for 2 min), with an increase of 10^{o}C/min to 200^{o}C, then 5^{o}C/min to 280^{o}C. Mass spectra were taken at 70ev at a scan interval of 0.5 seconds and fragments from 45-450Da. The solvent delay was 0 to 2 min and 36 minutes was the total GC-MS running time. The relative percentage amount of each component was calculated by comparing its average peak areas. Turbo-Mass Gold-Perkin Elmer was the mass detector used in GC-MS analysis. Turbo-Mass ver 5.2 was the software adopted to handle mass spectra and chromatograms.

Identification of phytocomponents: Using the database of central electrochemical research institute characterization and measurement laboratory having more than 62,000 patterns, interpretation on mass-spectrum GC-MS was conducted. The spectrum of the unknown components was compared with the spectrum of known components stored in the central electrochemical research institute characterization and measurement laboratory. The name, molecular weight and structure of the components of the test materials were determined.

RESULTS AND DISCUSSION

The results pertaining to GC-MS analysis of aqueous extract of *Eugenia uniflora* leaves leads to the identification of number of compounds. These compounds were identified through mass spectroscopy attached with gas chromatography. The mass spectrometer analyses compounds eluted at different time in order to identify the structure and nature of the compounds. Large compounds get fragmented to smaller ones giving rise to the appearance of peaks at different m/z ratio (mass to charge ratio). These spectra are the fingerprints of the bioactive compounds which can be further identified from the data library. The present investigation identified 39 biomolecules corresponding to 39 peaks. The active principles identified with greater peak area are presented along with retention time (RT), molecular formula, molecular weight and peak area (%) in table 1 and figure 1. The peak area is proportional to the quality of the compound present in the extract under study. The presence of various bioactive compounds detected after GC-MS analysis using aqueous extract of *Eugenia uniflora* justifies the use of this plant for various purposes by traditional

Table 1. Compounds identified in aqueous extract of Eugenia uniflora leaves by GC-MS analysis

S.no	Peak no.	RT	Peak area (%)	Compound identified	Molecular formula	M.wt (g/mol)
1	Peak 1	5.424	1.00	2-hydroxy-2-methyl-4-pentanone	$C_6H_{12}O_2$	100.16
2	Peak 2	5.490	2.25	Dimethyl sulfoxide	$(CH_3)_2SO$	78.129
3	Peak 3	6.363	1.90	Cyclohexanone	$C_6H_{10}O$	98.145
4	Peak 6	15.517	4.41	Germacrene b	$C_{15}H_{24}$	204.357
5	Peak 9	17.071	6.68	Isofuranogermacrene	$C_{15}H_{20}O$	216.318
6	Peak 10	19.744	3.73	Spatulenol	$C_{15}H_{24}O$	220.35
7	Peak 12	21.812	21.67	1-(3-methyl1.2-butadienyl)cyclopentane-1-ol	$C_6H_{16}O$	152.237
8	Peak 15	24.488	1.12	3-methyl-5-(2,6,6-trimethyl-1-cyclohexene-1-yl)-1-pentyn-3-ol	$C_{15}H_{24}O$	220.18
9	Peak 17	25.123	21.63	Perillaldehyde	$C_{10}H_{15}NO$	165.23
10	Peak 19	25.634	1.10	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronapthalene-2,3-diol	$C_{15}H_{24}O_2$	236.355
11	Peak 21	26.076	1.50	1-nonadecene	$C_{19}H_{38}$	266.513
12	Peak 25	29.155	1.58	Palmitic acid	$C_{16}H_{32}O_2$	256.43
13	Peak 28	29.531	1.01	1-heptatriacotanol	$C_{37}H_{76}O$	537.014
14	Peak 29	29.603	2.98	1-nonadecene	$C_{19}H_{38}$	266.513
15	Peak 30	29.742	5.25	1-Oxaspiro[2.5] octane,5,5-dimethyl4-(3-methyl 1-3-butadienyl)	$C_{14}H_{22}$	206.324
16	Peak 33	31.293	1.10	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.535
17	Peak 36	32.277	1.85	Behenic alcohol	$C_{22}H_{44}O_2$	340.59
18	Peak 38	39.968	1.68	Stigmast-5-en-3-ol	$C_{29}H_{50}O$	414.718

RT- Retention time, M.wt- Molecular weight

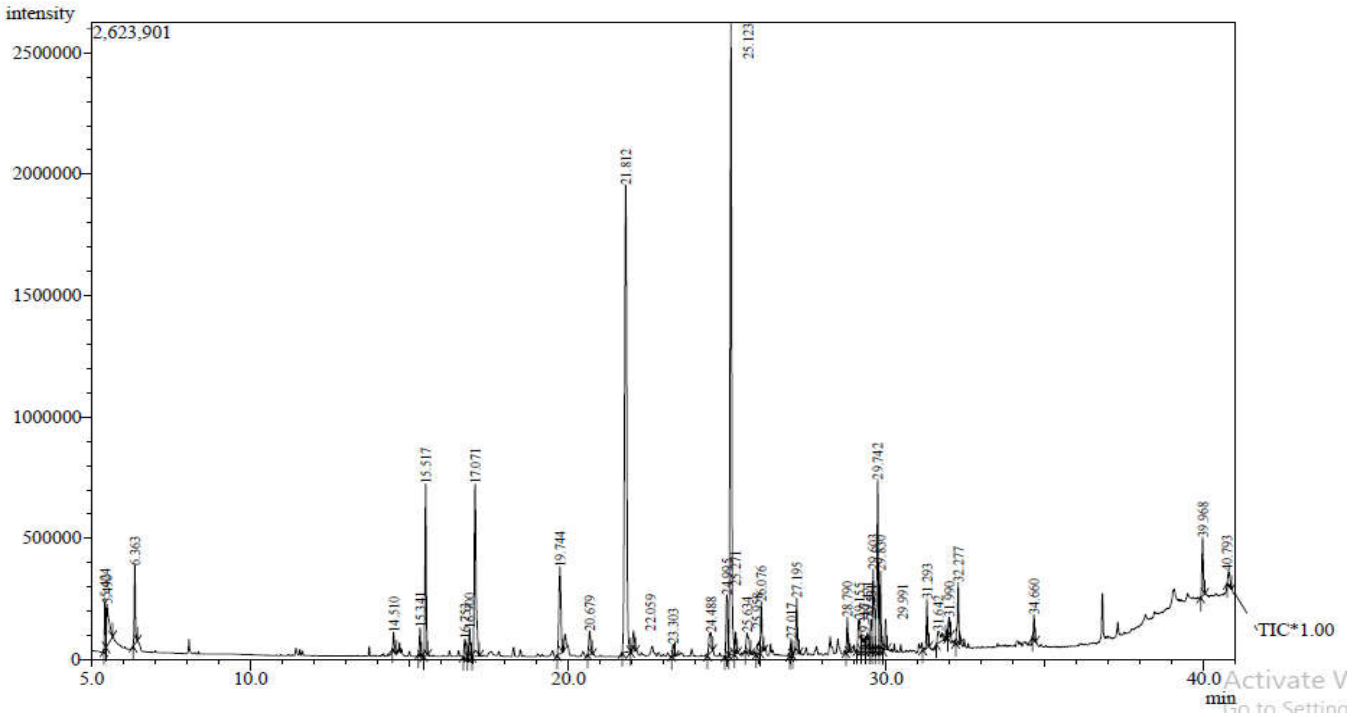

Figure 1. GC-MS chromatogram of aqueous extract of *Eugenia uniflora* leaves

practioners. However further investigation and isolation of individual phytochemical constituents, their structural elucidation and identifying biological activities will give fruitful results of their pharmacological activity and will open a new pathway for further drug development process.

Conclusion

The presence of various bio-active compounds detected after GC-MS analysis using the aqueous extract of *Eugenia uniflora* justifies the use of whole plant for various elements by traditional practitioner. However, isolation of individual phytochemical constituents and subjecting it to the biological activity will be definitely gives fruitful results and will open a new area of investigation of individual components and their pharmacological potency. Evaluation of pharmacological activity is under progress. Therefore, it is recommended as a plant of phytopharmaceutical importance. 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 be helpful for further detailed study.

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