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GC-MS analysis of bioactive compounds from Freshwater mussels of *Parreysia corrugata* (Muller 1774) and their pharmacological activities

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ABSTRACT

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. The freshwater mussels *Parreysia corrugata* was analyzed using Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. Gas chromatography mass spectrometry (GC-MS) analysis revealed the presence of 26 compounds. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. The first compound identified with less retention time (30.236 min) was 2,6-Difluorobenzoic acid, tridec-2-ynyl ester, whereas gamma.-Tocopherol was the last compound which took longest retention time (29.84min) to identify. Many of them are used in industry for various applications like flavor, antioxidant, anti-inflammatory, antimicrobial, pesticide and cancer preventive.

Keywords: Freshwater mussels, *Parreysia corrugata*, GC-MS, Bioactive components

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INTRODUCTION

Freshwater mussels are ecologically important group of burrowing organisms in aquatic ecosystems. The mussels are ecologically important because of their widespread distribution and biological filtration activity and also economically used as food and in the production of freshwater pearls. The knowledge of biochemical composition of any edible organism is extremely important since the nutritive value is reflected in its biochemical contents. The mussels provide high quality protein with all the dietary amino acids for maintenance and growth of human body. Freshwater mussels contain significant amount of omega 3 fatty acids in particular Docosahexaenoic acid (DHA) [1].The freshwater molluscs form an important part of the aquatic ecosystems. These sentinel organisms are used in aquatic pollution monitoring programs as they accumulate toxic environmental contaminants to levels well above those present in the surrounding environment thus providing information on the spatio-temporal pollution trends [2-3].

Parreysia corrugata is an important constituent of freshwater fauna of the Indian sub-continent. In India, this species is reported to be widely distributed in the states like Punjab, Bihar, Madhya Pradesh, Maharashtra, Orissa and Karnataka [4]. Recently it has been found to be a potential candidate species for freshwater pearl production [5]. Further, *Parreysia* spp. is known to have medicinal importance [6]. GC-MS is a method that combines the features of gas liquid chromatography and mass spectrometry to identify different substances within a test sample. It is a sensitive analytical technique that is used in a wide range of applications such as environment monitoring, flavor and fragrance analysis [7], pesticide analysis, metabolite analysis [8], forensic and criminal cases etc [9]. Considering all these facts, the present study was designed to investigate the presence of various bioactive compounds in Freshwater mussels of *Parreysia corrugata* using Gas chromatography and mass spectrometry.

MATERIAL AND METHODS

Parreysia corrugata species was collected in June 2018 from coleroon river, Lower Anaicut Reservoir, Thanjavur district, Tamilnadu, India. The Live specimens of freshwater gastropod *P.corrugata* was randomly collected by hand picking method. The collected fresh molluscs were

preserved with ice and transported to the laboratory and identified by the standard literature of [10]. The ethanolic extract of flesh was prepared by [11]. The specimen was brought to the laboratory and their soft bodies were removed by breaking the shell. The flesh sample was dried using hot air oven at 600C and powdered. 25grams sample was soaked in methanol and maintained for 3 days. The extract was filtered through Whatman No.1 filter paper. The resultant extract was concentrated by using rotary vaccum evaporator with reduced pressure. The resultant extract were then kept in airtight container and stored at 40C for further analysis.

GC–MS

This technical process was done in Periyar Maniammai Institute of Science & Technology, Thanjavur, Tamil Nadu, India. Chromatographic separation was performed using a column of GC-MS-QP 2010 (SHIMADZU) column Db 30.0 (0.25 µm in diameter, 0.25 µm thick). The oven temperature is raised to 10°C/min to 200°C and then programmed to 5°C/min to 280°C and 70°C (isothermal 5 min) ending to 35° isothermal. Obtained at 70 in excess of 62,000 models.

Identification of Compounds

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials was ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version (2005), software, Turbomas 5.2.

RESULTS AND DISCUSSION

GC-MS is a powerful tool that is being increasingly used in biomarker discovery. It can be used for analysis of wide range of biological compounds including Fatty acids, essential oils, eicoscanoids, wax, esters by the selection of suitable columns. The GC-MS analysis of Freshwater mussels *P.corrugata* revealed the presence of twenty six compounds that could contribute the medicinal quality of the animal. The identification of the biological active compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in Table 1 and Fig 1. The first compound identified with less retention time (30.236 min) was 2,6-Difluorobenzoic acid, tridec-2-ynyl ester, whereas gamma.-Tocopherol was the last compound which took longest retention time (29.84min) to identify. The bioactive compounds identified through GC-MS analysis showed many biological activities relevant to this study are listed in Table 1. The biological activities listed are based on Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA. The compound Hexadecanoic Acid methyl ester, showed pharmacological activity as reported in the freshwater gastropod of *Pila virens* [12]. Similarly Octadecanoic acid, Tetradecanoic acid and gamma.-Tocopherol also showed the various biological activities. However, isolation and characterization of individual compounds may proceed to discover the novel drugs and their pharmacological activities.

Table 1: GC-MS analysis of Freshwater mussels *Parreysia corrugata*

Peak	Retention time	Molecular Formula	Bioactive Compounds	Biological Activities
1	14.279	$C_{20}H_{26}F_2O_2$	2,6-Difluorobenzoic acid, tridec-2-ynyl ester	Anti-oxidant, Hypocholesterolemic, Nematicide, Anti-androgenic, Hemolytic, Pesticide, Lubricant, 5-Alpha Reductase inhibitor, antipsychotic
2	15.541	$C_{16}H_{22}O_4$	1,2-Benzenedicarboxylic acid, die	Aromatic Compound
3	17.410	$C_{14}H_{28}O_2$	Tetradecanoic acid	Antifungal, Antioxidant, cancer preventive, nematicide, hypercholesterolemic, Lubricant
4	19.160		Hexadecanoic acid, methyl ester	Antifungal, Anti-tumour, Antibacterial
5	19.402	$C_{16}H_3O_2$	cis-9-Hexadecenoic acid	No activity report
6	19.557	$C_{17}H_{34}O_2$	n-Hexadecanoic acid	Antioxidant, Hypocholesterolemic, Nematicide, Anti-androgenic, Flavor, Hemolytic
7	20.495	$C_{22}H_{40}O_2$	Butyl 9,12-octadecadienoate	No activity report
8	20.960	$C_{17}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Anti-inflammatory, Nematicide, Insectifuge, Hypocholesterolemic, cancer preventive, Heptaoprotective, Antistaminic, Antiacne, Anti arthritic, Antieczemic,5-Alpha reductase inhibitor, Antiandrogenic, Anticoronary. Anti coronary, Anti cancer,
9	21.235	$C_{19}H_{38}O_2$	Octadecanoic acid, methyl ester	No active reported.
10	21.448	$C_{16}H_{30}O$	cis-9-Hexadecenal	Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, anti-asthama, diuretic.
11	21.677	$C_{18}H_{36}O_2$	Octadecanoic acid	Antioxidant, hypoglycemic and thyroid inhibiting properties
12	22.262	C_2H_4O	Oxirane, [(dodecyloxy)methyl]-	precursor of progesterone, antimicrobial,
13	22.667	$C_{20}H_{40}O_2$	Eicosanoic acid, 2-hydroxyethyl	anticancer, anti-arthritic, anti-asthama,

			ester	
14	22.794	$C_{16}H_{30}O_2$	Z-10-Methyl-11-tetradecen-1-ol propionate	5-Alpha reductase inhibitor, antipsychotic.
15	23.042	$C_9H_{11}O$	2-(Dimethylamino)ethyl 1-adama	Antimicrobial, Antifouling
16	23.150	$C_{22}H_{32}O_2$	4,7,10,13,16,19-Docosahexaenoic acid, methyl	antiinflammatory, diuretic.
17	23.247	$C_8H_{14}O_2$	3-Cyclopentylpropionic acid, 2-dimethylamino	No activity report
18	23.484	$C_{19}H_{38}O4$	Hexadecanoic acid, 2-hydroxy-1,3	Hemolytic, pesticide, flavour, antioxidant.
19	25.715	$C_{35}H_{68}O_5$	2,2-Dimethylpropanoic acid, heptadecyl ester	No activity report
20	26.532	$C_{24}H_{50}$	2,6,10,14,18-pentamethyl-2,6,10,14,18-i	Cancer Preventive, Choleretic, Dermatitigenic Flavor, Hypocholesterolemic.
21	27.773	$C_{57}H_{98}O_6$	Trilinolein	Insectifuge Irritant, Percutaneostimulant, Perfumery, propecic.
22	27.313	$C_{63}H_{122}O_6$	Triarachine	Act as lipid anchor in bio membranes, Anti xonotic.
23	27.668	$C_{17}H_{34}O_3$	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl	Antioxidant, Flavor, Anti fibrinolytic, Hypocholesterolemic, Antiandrogenic, Lubricant, Hemolytic, 5-Alpha reductase inhibitor, Nematicide, Antialopecic
24	28.669	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate	Antimicrobial.
25	29.004	$C_{14}H_{30}O_2$	Decane, 1,1-diethoxy-	Hemolytic, pesticide, flavour, antioxidant.
26	30.236	$C_{28}H_{48}O_2$	.gamma.-Tocopherol	Cancer Preventive.

Source of reference: Dr. Duke's Phytochemical and Ethnobotanical Databases, 1992-2016

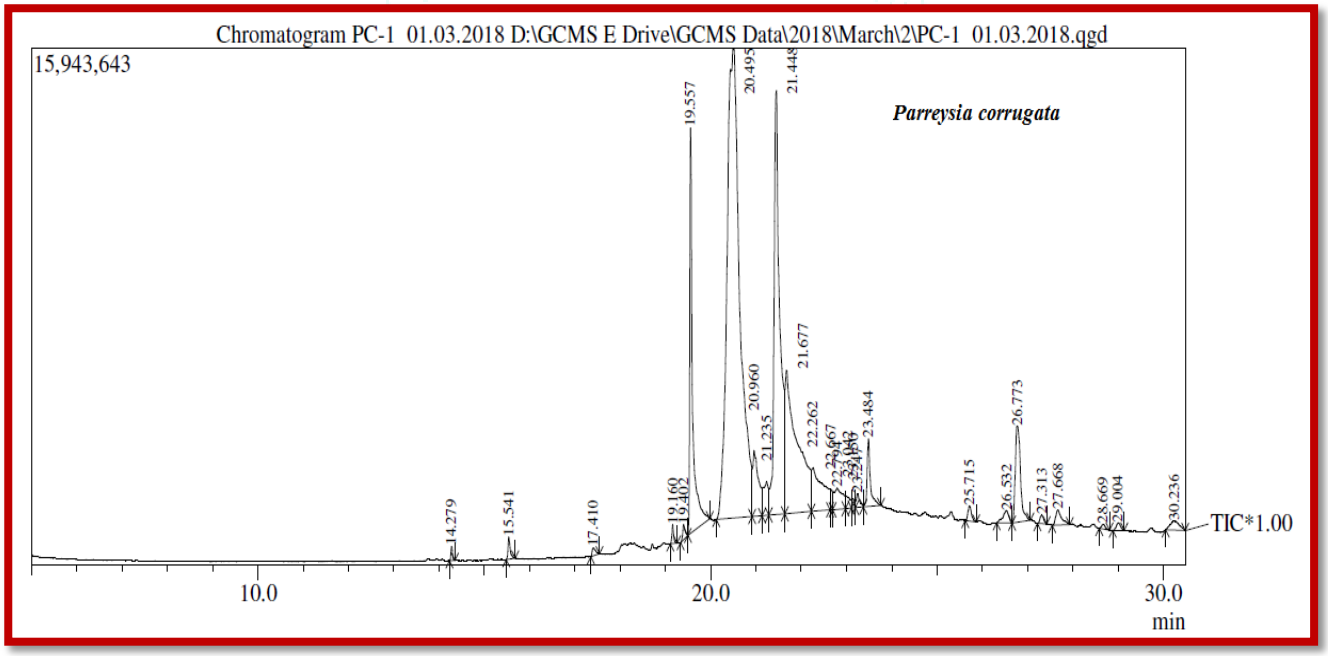

Fig 1: GC-MS Chromatogram of Freshwater mussels *Parreysia corrugata*

CONCLUSION

GC-MS method is a direct and fast analytical approach for identification of bioactive compounds. The Freshwater mussels *Parreysia corrugata* has 26 different chemical compounds and they have different pharmacological activities. Each chemical compound can be extracted individually and can be used in clinical trials to check efficacy, and to develop a novel drug from a crude drug. The GC-MS analysis Freshwater mussels will also be a part of database of bioactive products of natural drugs.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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