PJN
ISSN 1680-5194

Pakistan Journal of Nutrition

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2016.1042.1048

Research Article

Nutritional Importance of Three Potamidid Snails from Bhavanapadu Mangrove Creek: Lipid Profiling by GC-MS: A Relative Exploration

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Abstract

Background: Seafood is one of the most nutritionally balanced foods. Studies on fatty acid composition of commercial seafood in India are limited. This might be due to lack of awareness on the benefits of these nutrients particularly from molluscan meat. **Objective:** The present comparative study aims on providing qualitatively the fatty acid composition in total tissue of three potamidid snails specifically *Telescopium telescopium*, *Cerithidea obtusa* and *Cerithidea cingulata* of Tekkali Creek, Srikakulam district, Andhra Pradesh. **Methodology:** The total fatty acid compositional studies were performed by using FAME and GC-MS analysis. **Results:** The results showed that *Telescopium telescopium* was a complex mixture of numerous compounds, among them, palmitic acid (C16:0), lauric acid (C12:0), myristic acid (C14:0) and oleic acid (18:1) appears to be the significant ones along with essential fatty acid γ-linolenic acid (C18:3 n-6). *Cerithidea obtusa* was amalgamated mixture of copious compounds, among them essential fatty acid methyl linoleate (C19:2 n-6), margaric acid methyl ester (C18:0), 11-hexadecenoic acid methyl ester (C17:1) and 1-eicosanol (C20:0) were distinctive fatty acids, oleic acid (18:1) and palmitic acid (C16:0) appears to be the noteworthy one along with essential fatty acid γ-linoleic acid (C18:3 n-6). *Cerithidea cingulata* was a complex combination of mix, among them tridecanoic acid methyl ester (C14:0), palmitoleic acid methyl ester (C17:1) and 1-pentadecane (C15:0) were unique fatty acids, palmitic acid (C16:0) appears to be the striking one along with essential fatty acid γ-linolenic acid (C18:3 n-6). **Conclusion:** These findings concluded that these can be utilized as potential fishery resource for nutrition, particularly in developing countries by being constituents in food to prevent malnutrition.

Key words: Gastropods, *Cerithidea obtusa*, *Cerithidea cingulata*, *Telescopium telescopium*, FAME, GC-MS, saturated fatty acids, unsaturated fatty acids

Received: August 27, 2016 Accepted: October 18, 2016 Published: November 15, 2016

Citation: T. Joseph Uday Ranjan and K. Ramesh Babu, 2016. Nutritional importance of three potamidid snails from Bhavanapadu Mangrove Creek: Lipid profiling by GC-MS: A relative exploration. Pak. J. Nutr., 15: 1042-1048.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Foods can be regarded as functional if they help in reducing the risk of disease and promote good health[1]. They may provide means to reduce the increasing burden on the health care system. Although there is no authoritative definition of functional lipids, one can informally define them as a subset of functional foods, which are considered to be similar in appearance to conventional foods consumed as part of a usual diet, but they have been demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions[2]. New research has linked functional lipids to the prevention and treatment of many diseases. Functional lipids such as omega-3 and omega-6 fatty acids, conjugated linoleic acids, medium chain triglycerides and phytosterols have many beneficial effects on human health such as in obesity, bone health and in treating and managing depression, blood pressure, cardiovascular health, etc. The ratio of omega-3 and omega-6 polyunsaturated fatty acids regulates the production of eicosanoids, which are the metabolites of these series of fatty acids. Scientific evidence has shown encouraging improvements in patients and beneficial effects in healthy persons with the use of supplemental and dietary forms of functional lipids[3].

Seafood is one of the most nutritionally balanced foods. The seafood diet helps to control weight and goes a long way towards preventing heart diseases. The nutritional values of bivalve do not bring the limelight so far, so consumption of these nutrient rich molluscs has not attracted attention. In recent years, the significance of polyunsaturated fatty acids analysis has gained much attention because of their various biological activities in health and disease, especially the n-3 and n-6 fatty acids. These fatty acids play an important role in the prevention and treatment of cardiovascular diseases, autoimmune diseases, eye sight and the improvement of learning ability[4].

The present comparative cram aspires on providing qualitatively the fatty acid composition in total tissue of three potamidid snails specifically *Telescopium telescopium*, *Cerithidea obtusa* and *Cerithidea cingulata* of Bhavanapadu Mangrove Creek, Srikakulam district, Andhra Pradesh.

MATERIALS AND METHODS

Sample collection: Three gastropods representing two genera namely *Telescopium telescopium*, *Cerithidea obtusa* and *Cerithidea cingulata* within the family of Potamididae were collected during the low tide from tidal mudflats of Bhavanapadu Mangrove Creek, Srikakulam district, Andhra Pradesh province, India. These gastropod molluscs were identified up to species level following the appropriate field guides of Antony and Olivia[5] and Dey[6] based on the morphology. These samples were brought to the laboratory in zip pouches and then washed thoroughly with distilled water to remove the unwanted dirt particles and the soft tissue was separated from the hard shells with the help of bone cutter and a small hammer and finally stored in refrigerator at 4°C for further use.

Lipid extraction: Lipid extraction was carried out as per Folch *et al.*[7]. In brief, frozen tissues were homogenized (using a motor pestle) in the organic solvent mixture (chloroform-methanol, 2:1), keeping the solvent/tissue ratio 20:1 and washed by centrifugation. Washing was repeated 5 times with fresh solvent mixture. The chloroform fractions, enriched with lipids were collected, pooled and dried in a rotary evaporator (Heidolph Germany, 50°C). The dried lipids were weighed, dissolved in chloroform and stored in small amber glass laboratory bottles at -20°C.

Determination of fatty acid composition by GC-MS: Fatty acid composition in three test samples namely *Telescopium telescopium*, *Cerithidea obtusa* and *Cerithidea cingulata* were determined using GC-MS according to the method described by Lepage and Roy[8]. Test dried lipid extracts (20 mg) were separately mixed with 20 mL of methanol and acetyl chloride (20:1, v/v) solution and to this 20 mL hexane was added. Mixture was heated at 100°C for 30 min under continuous stirring. After cooling to room temperature, 20 mL of water was added and using separating funnel, the fatty acid methyl esters were extracted in hexanic layer. Three more extractions with hexane were made to ensure complete removal of methyl esters (REMI centrifuge, R-8C, India). The clear supernatant (2 mL) was transferred to an auto sampler vial and injected with auto injector (AOC-20 i) into GC-MS for analysis. The GC-MS analysis carried out in a Shimadzu GC-MS-QP2010SE, equipped with a KRATOS mass detector model MS25RF (sector instrument) and a capillary column of DB×LB (30 m×0.32 mm, 0.50 µm film thickness), carrier gas helium, constant pressure 90 kPa, split 1:10. The oven was programmed initially from 70°C with 2 min hold up time to the final temperature of 250°C with 5°C min^{-1} ramp. The final temperature hold time was 20 min. The inlet and GC-MS interface temperatures were kept at 250°C and 280°C respectively. The temperature of EI 70 eV source was 200°C with full scan (25-450 m/z), scan time 0.3 sec. The mass spectra of essential oil components were identified by comparing the mass spectra of the analytes with those of authentic standards from the mass spectra of Wiley 229.LIB and mass spectra library NIST 05.LIB as well as on comparison of their retention indices of literature.

RESULTS AND DISCUSSION

The GC-MS analysis of three test samples namely *Telescopium telescopium*, *Cerithidea obtusa* and *Cerithidea cingulata* and their free fatty acid methyl ester fractions showed the presence of 8 (four saturated and four unsaturated), 7 (three saturated and four unsaturated) and 7 (three saturated and four unsaturated) fatty acid constituents proceedingly, the identified compounds possess many biological properties. The test sample *Telescopium telescopium* contained the following fatty acids in varying amounts, palmitoleic acid methyl ester ($C_{17}H_{32}O_2$:C17:1), mono-unsaturated fatty acids are components of the cellular membranes of autotrophic bacteria. To date, however, this fatty acid has only been detected in strains of the genus *Nitrospira*, which are nitrite oxidizing autotrophic bacteria. Myristic acid ($C_{14}H_{28}O_2$:C14:0), the salt of this compound is known as myristate. It is used as ingredient in soaps, cosmetic and shaving creams, often in the form of the ester isopropyl myristate. The γ-linolenic acid (GLA) ($C_{18}H_{30}O_2$:C18:3 n-6), known as omega-6 fatty acid. Unsaturated fatty acids are essential components of cell membranes and can influence receptors, enzymes, ion channels and signal transduction pathways. They can influence numerous inflammatory and immunological processes[9]. Methyl palmitate ($C_{17}H_{34}O_2$:C17:0), this compound displays the ability to inhibit phagocytic activity and Nitric Oxide (NO) production of certain cells. Additionally, palmitic acid methyl ester appears to reduce levels of TNF-α, PGE2 and IL-10 without affecting ATP levels[10]. Oleic acid, methyl ester ($C_{19}H_{36}O_2$:C19:1), the trans isomer of oleic acid is called elaidic acid. It used as a defoamer, solvent for inks and coatings and as a tar remover. Methyl oleate also used as raw material of emulsifiers or oiling agent for foods, spin finishes, surfactant and base material for perfume and as oil carrier in agricultural industry. Lauric acid methyl ester ($C_{13}H_{26}O_2$:C13:0), in foods, lauric acid is used as a vegetable shortening. In manufacturing, it is used to make soap and shampoos. It is found in many vegetable fats, particularly in coconut and palm kernel oils. Lauric acid increases total serum cholesterol more than many other fatty acids. But most of the increase is attributable to an increase in high-density lipoprotein (HDL) (the "good" blood cholesterol). As a result, lauric acid has been characterized as having a more favorable effect on total HDL cholesterol than any other fatty acid, either saturated or unsaturated[11]. 16-octadecenoic acid ($C_{18}H_{34}O_2$:C18:1), this acidic compound don't have biological significance and myristic acid methyl ester ($C_{15}H_{30}O_2$:C15:0), esterified form of myristic acid, worth was conferred above. Results demonstrated that *Telescopium telescopium* extract was complex mixture of numerous compounds, among them, palmitic acid (C16:0), lauric acid (C12:0), myristic acid (C14:0) and oleic acid (18:1) appears to be the significant ones along with essential fatty acid γ-linolenic acid (C18:3 n-6). The GC-MS chromatogram and list of fatty acids were displayed in Fig. 1 and Table 1.

The second test sample *Cerithidea obtusa* contained the subsequent fatty acids in varying amounts, γ-linolenic acid (GLA) ($C_{18}H_{30}O_2$:C18:3 n-6), oleic acid, methyl ester ($C_{19}H_{36}O_2$:C19:1) and methyl palmitate ($C_{17}H_{34}O_2$:C17:0), significance of these were bestowed above. Margaric acid methyl ester ($C_{18}H_{36}O_2$:C18:0), it occurs as a trace component of the fat and milk fat of ruminants, but it does not occur in any natural animal or vegetable fat at high concentrations. However, it was found to be a major constituent of the foot and mantle lipidome of the bivalve[12]. Methyl linoleate ($C_{19}H_{34}O_2$:C19:2 n-6), esterified form of linoleic acid is an essential omega-6 fatty acid. The main physiological requirement for omega-6 fatty acid is attributed to arachidonic acid. It is the major precursor of prostaglandins, leukotrienes and anandamides that play a vital role in cell signaling[9]. It is

Table 1: Total ionic chromatogram (GC-MS) of *Telescopium telescopium* obtained with temperature of EI 70 eV using a capillary column of DB×LB with He gas as the carrier

RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
13.03	7-hexadecenoic acid methyl ester/palmitoleic acid methyl ester	$C_{17}H_{32}O_2$	268.44	23.48
14.22	Myristic acid/tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	6.57
15.18	Gamma linolenic acid/γ-linolenic acid	$C_{18}H_{30}O_2$	278.43	39.81
16.4	Methyl palmitate/methyl hexadecanoate	$C_{17}H_{34}O_2$	270.45	5.69
17.1	9-octadecenoic acid methyl ester/oleic acid methyl ester	$C_{19}H_{36}O_2$	296.49	11.27
18.47	Dodecanoic acid methyl ester/lauric acid methyl ester	$C_{13}H_{26}O_2$	214.34	3.54
19.78	16 octadecenoic acid/octadec-16-enoic acid	$C_{18}H_{34}O_2$	282.46	4.95
21.38	Tetradecanoic acid methyl ester/myristic acid methyl ester	$C_{15}H_{30}O_2$	242.40	4.12
	Total			99.43

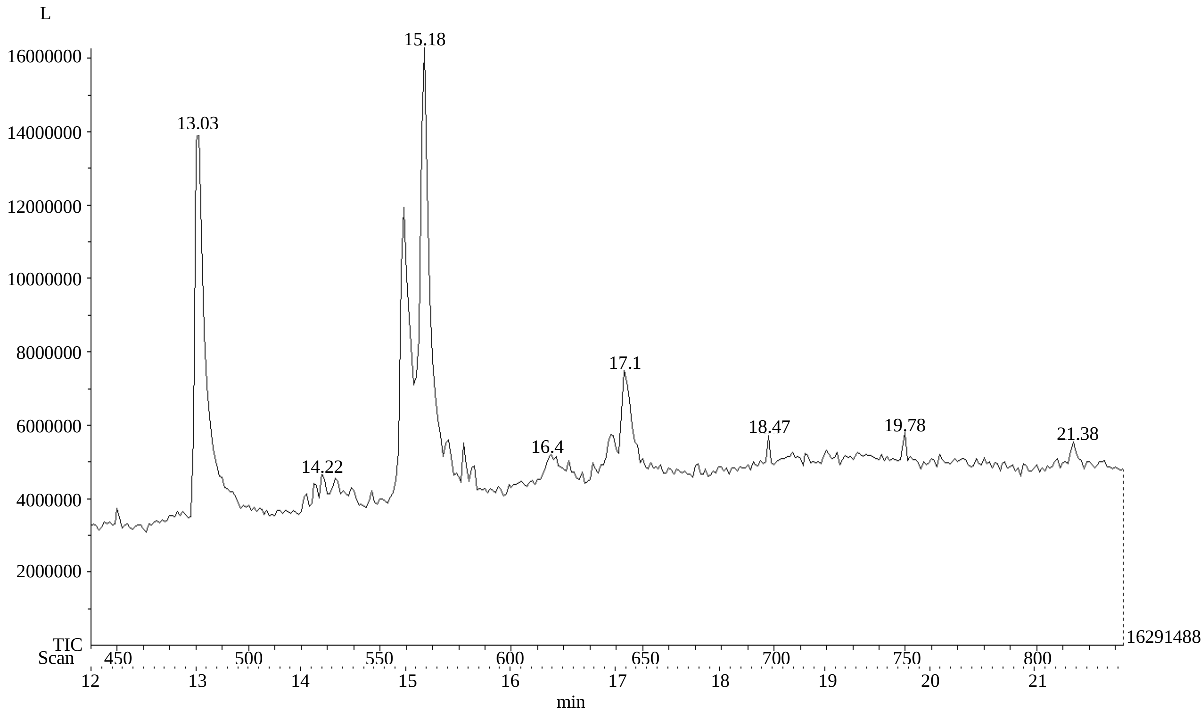

Fig. 1: GC-MS chromatogram of *Telescopium telescopium*

Table 2: Total ionic chromatogram (GC-MS) of *Cerithidea obtusa* obtained with temperature of EI 70 eV using a capillary column of DB×LB with He gas as the carrier

RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
15.3	Gamma linolenic acid/γ-linolenic acid	$C_{18}H_{30}O_2$	278.43	14.25
17.24	9-octadecenoic acid methyl ester/oleic acid methyl ester	$C_{19}H_{36}O_2$	296.49	18.21
18.53	Methyl palmitate/methyl hexadecanoate	$C_{17}H_{34}O_2$	270.45	10.96
19.84	Heptadecanoic acid methyl ester/margaric acid methyl ester	$C_{18}H_{36}O_2$	284.48	39.72
21.49	Methyl linoleate/linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294.47	5.67
23.66	11-hexadecenoic acid methyl ester/methyl 11-hexadecenoate	$C_{17}H_{32}O_2$	268.44	4.68
26.26	1-eicosanol/arachidic alcohol	$C_{20}H_{42}O$	298.55	6.49
	Total			99.98

also used in the manufacture of detergents, emulsifiers, lubricants, textile treatments and in medical research[13]. 11-Hexadecenoic acid methyl ester ($C_{17}H_{32}O_2$:C17:1), the fatty acyl analog of the major pheromone component (Z)-11-hexadecenal. 1-Eicosanol ($C_{20}H_{42}O$:C20:0) also entitled as arachidyl alcohol is a waxy substance used as an emollient in cosmetics. It is a straight-chain fatty alcohol. Exceeding results showed that the *Cerithidea obtusa* was amalgamated mixture of copious compounds, among them essential fatty acid methyl linoleate (C19:2 n-6), margaric acid methyl ester (C18:0), 11-hexadecenoic acid methyl ester (C17:1) and 1-eicosanol (C20:0) were distinctive fatty acids, oleic acid (18:1) and palmitic acid (C16:0) appears to be the noteworthy one along with essential fatty acid γ-linoleic acid (C18:3 n-6). The GC-MS chromatogram and list of fatty acids were displayed in Fig. 2 and Table 2.

In the same way, *Cerithidea cingulata* sample too contained the following fatty acids in varying amounts, Tridecanoic acid methyl ester ($C_{14}H_{28}O_2$:C14:0), commonly found in dairy products. 1-pentadecane ($C_{15}H_{32}$:C15:0), alkanes from nonane to hexadecane (an alkane with 16 carbon atoms) are liquids of higher viscosity, less and less suitable for use in gasoline. They form instead the major part of diesel and aviation fuel. Palmitoleic acid methyl ester ($C_{17}H_{32}O_2$:C17:1), methyl palmitate ($C_{17}H_{34}O_2$:C17:0), 16-octadecenoic acid ($C_{18}H_{34}O_2$:C18:1), myristic acid methyl ester ($C_{15}H_{30}O_2$:C15:0) and γ-linolenic acid (GLA) ($C_{18}H_{30}O_2$:C18:3 n-6), value of all were conversed above. End results showed that the *Cerithidea cingulata* was complex combination of mix, among them tridecanoic acid methyl ester (C14:0) and 1-pentadecane (C15:0) were unique fatty acids. Palmitoleic acid methyl ester (C17:1), palmitic acid (C16:0) and

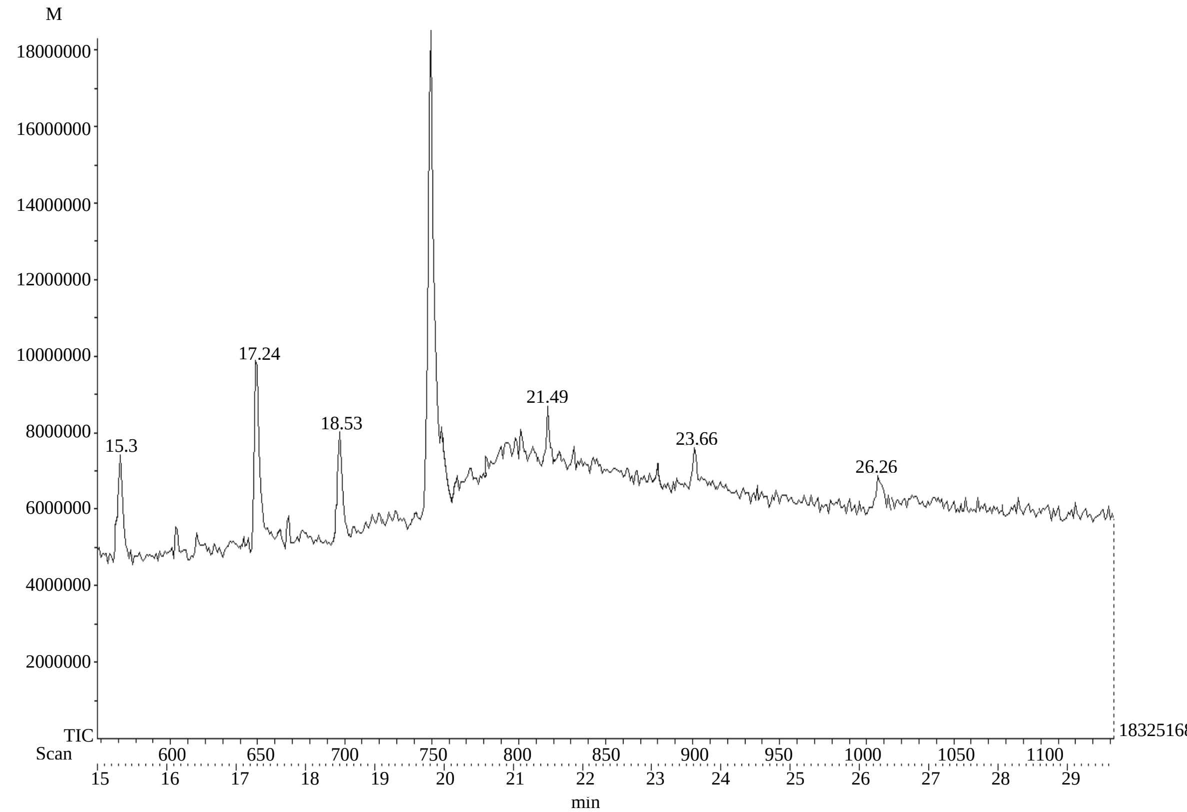

Fig. 2: GC-MS chromatogram of *Cerithidea obtusa*

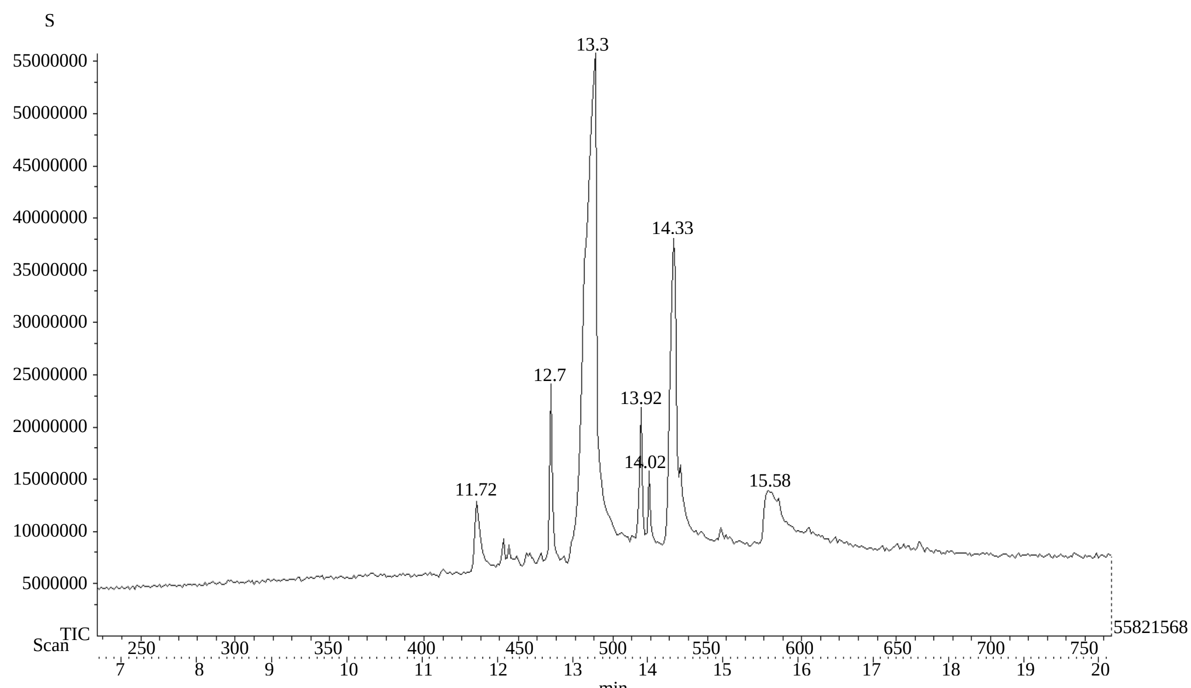

Fig. 3: GC-MS chromatogram of *Cerithidea cingulata*

16 octadecenoic acid (C18:1) appears to be the striking one along with essential fatty acid γ-linolenic acid (C18:3 n-6). The GC-MS chromatogram and list of fatty acids displayed in Fig. 3 and Table 3. The above outcomes evidently indicate that

Table 3: Total ionic chromatogram (GC-MS) of *Cerithidea cingulata* obtained with temperature of EI 70 eV using a capillary column of DB×LB with He gas as the carrier

RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
11.72	Tridecanoic acid methyl ester/methyl tridecanoate	$C_{14}H_{28}O_2$	228.37	8.25
12.7	1-pentadecane	$C_{15}H_{32}$	212.41	6.12
13.3	7-hexadecenoic acid methyl ester/palmitoleic acid methyl ester	$C_{17}H_{32}O_2$	268.44	34.21
13.92	Hexadecanoic acid methyl ester/palmitic acid methyl ester	$C_{17}H_{34}O_2$	270.45	7.46
14.02	16 octadecenoic acid/ octadec-16-enoic acid	$C_{18}H_{34}O_2$	282.46	6.43
14.33	Tetradecanoic acid methyl ester/myristic acid methyl ester	$C_{15}H_{30}O_2$	242.40	24.87
15.58	Gamma linolenic acid/γ-linolenic acid	$C_{18}H_{30}O_2$	278.43	12.24
	Total			99.58

the fatty acid composition of three experimental organisms principally depends on their habitate and nourish.

Palmitic acid was found to be the most abundant fatty acid in all the three potamidid snails. The presence of essential fatty acids, γ-linolenic acid, methyl linoleate and polyunsaturated fatty acids in tested species, specifically *T. telescopium*, *C. obtusa* and *C. cingulata* established that these can be utilized as potential fishery resource for nutrition, particularly in developing countries. The lipid composition of the mollusks can be affected by external (exogenous) factors, such as fluctuations in the environmental conditions (temperature and food availability) or by internal (endogenous) factors, such as metabolic and physiological activities[14]. Abad *et al.*[15] stated that lipids from marine sources are heterogenous and can contain unusual fatty acids such as non-methylene interrupted (NMI) fatty acids and fatty aldehydes. Numerous earlier studies reported that the algal diet could influence fatty acid composition of gastropods[16,17]. In view of the fact that marine molluscs cannot synthesize essential fatty acids *de novo*, the quality and the quantity of algal lipids is very important in the diet of marine animals and algae are the main sources of these fatty acids[18]. Conaway *et al.*[19] demonstrated the dietary influence on sterol, triacylglycerols and neutral lipids with gastropod *Biomphalaria glabrata* by means of restricted diet. Zhukova and Svetashev[20] revealed that the marine molluscs are generally characterized by the predominance of essential n-3 PUFA, mainly 20:5 n-3 and 22:6 n-3, which constitute usually almost half of the total fatty acids. Babu *et al.*[21] on the lipid and fatty acid composition of gastropod species exposed that, these appear to be an excellent source of palmitic acid (16:0), oleic acid (18:1 n-9), arachidonic acid (20:4 n-6) eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). Formerly various investigators worked out on diverse group of molluscs species for their fatty acid profiles by means of GC-MS. Ekin *et al.*[22] studied concerning lipids and fatty acids of fresh water mussels namely *Anodonta piscinalis* and *Corbicula fluminalis*, living in Tigris river. Jarzębski *et al.*[23] studied concerning fatty acids in marine bivalves *Macoma balthica* particularly because they serve as important source of polyunsaturated fatty acids (PUFAs) that are important from the standpoint of human nutrition and health. Similarly Anand *et al.*[24] carried out experiment on fatty acid profile of *Pleuroploca trapezium* meat. All the exceeding declaration ropes our experimental fallouts.

CONCLUSION

The GC-MS scrutiny of total tissues of three potamidid snails specifically *T. telescopium*, *C. obtusa* and *C. cingulata* showed the presence of therapeutic constituents, which can be expanded into top value-added substances of high-grade spice, cosmetic, food and industrial chemical and solvent etc., our end results additionally promote the development of novel food sources and for the formulation of food supplements for the revival from malnutrition.

SIGNIFICANT STATEMENT

Polyunsaturated fatty acids analysis has gained much attention because of their various biological activities in health and disease. Studies on fatty acid composition from the molluscan species are limited although they are nutritionally balanced. The significance of this study is to provide information regarding the fatty acids present in nutritionally important gastropods. This may create awareness on the nutritional and health benefits of these species.

ACKNOWLEDGMENT

The authors would like to acknowledge UGC-MANF, New Delhi, India for providing financial support to carry out this work.

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