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Investigation Extraction of the Garden Snail's Shells using GC-MS Spectrometry

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Abstract

This study focused on the powder of the shell of the local garden snail in Iraq to identify the chemical compounds and determine the diversity and biological contents that might be applied for other applications and studies. Thus, snails shell was collected from local rivers in Al Diwaniya city, middle of Iraq; 500 grams of the shell powder of the garden snail was smashed and grinded and analysed during this study. The shell powder was extracted by ethanol, and 250mg of the yield was injected in GC-Mass spectrometry and analyzed. The detection of chemical compounds in the garden snail shell was identified by comparison with the mass spectra library of the GC-Mass data system. Results exhibited that six chemical compounds were largely predominant among other elements of the shell powder, including hexadecanoic acid, ethyl ester, (E)-9-Octadecenoic acid ethyl ester, pentadecanoic acid, heptadecanoic acid, ethyl ester, and 9-Octadecenamide, (Z)-. Also, the analysis profiled more than 23 chemical compounds that constituted the structure of the garden snail shell with a low percentage area. These substances were classified into fatty acids, saturated fatty acids, organic compounds, natural products, and methyl and ethyl esters. Consequently, these natural materials are essential active compounds that could be used for more medical and research applications studies. However, the yield of chemical compounds was a low amount. To sum up, GC-Mass spectrometry has displayed a high biological diversity of chemical compounds substances in the shell of garden snails, which can help researchers to investigate more for different natural chemical resources in the shell of a garden snail.

Introduction

The Garden snail (syn. Cryptomphalus aspersus) is considered one of the group terrestrial molluscs or land molluscs (mollusks) and is classified as a land snail and under the family Helicidae (Blacket, 2016; Geiger, 2011; Cowie, 2011). The Garden Snail (*Cornu aspersum*) species usually has sexual reproduction and has two genders, male and female, for the same species and producing both male and female gametes (Koene, 2017; Shackelford & Goetz, 2012).

However, self-fertilization sometimes has been recorded in *Cornu aspersum* (Cvetkovska, 2019; Kramarenko, 2013). Also, the male and female of *Cornu aspersum* are reached sexual maturity after one to two years and exchange sperm after sexual mating (Dahirel & Madec, 2016; Schärer et al., 2015). Garden snail has sheltered about 80 spherical pearly-white eggs under a stone or other locations in the garden.

Cornu aspersum feeds on different types of flowers, cereals, vegetable crops, rose bushes, leaves, and fruit trees (Rogers, 2015). On the other hand, *Cornu aspersum* is a good food source

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for birds, omnivorous scavengers, worms, lizards, frogs, centipedes, small mammals, frogs, and predatory insects (Fisher et al., 1980).

Anatomically, the external anatomy of the snail garden consists of a spiral shell firmly attached to muscles. These muscles help the snail to withdraw its body inside its shell and protect it from enemies and predators (Davies, 1975). Grossly snail has a spiral shell, and two openings represent the anal pore of the digestive system and the pneumostome for the respiratory system (Walker, 1962). Also, the foot and head, comprised of upper tentacles, are extended with eyes, and the lower tentacles are short for smelling and tasting food, in addition to the mouth. Internally, the circulatory system has a heart with two chambers, one atrium, and one ventricle (Zwart, 2015). The heart continues with the aorta, which is branched into smaller arteries for all body organs. In the same way, the pulmonary vein drains from the lung to the heart (Zwart, 2015).

The nervous system consists of cerebral ganglia, which are simple types of bipolar and multipolar nerve cells endings with epidermis or skin. The nervous system assists snails in feeling a sense of touch and pain (Fernandez, 1966). The digestive system is comprised of the mouth, crop, salivary gland, oversized liver, stomach, and ended with anal pore (Fernandez, 1966). The respiratory system has large lungs attached via pulmonary veins to the heart, receiving oxygen from the external environment by pneumostome open (Zwart, 2015; Martins, 2005). The reproductive tract consists of the vagina and penis, which are continued with the oviduct, mucous gland, dart sac, and vas deferens attached to the kidney (Dummalod & Dama, 2019). Also, the reproductive tract is externally opened by the genital pore (Gómez, 2001).

The extractions and ingredients of *Cornu aspersum* were used in different preparation for medical purposes, such as creams and gels for skin diseases or reducing dry skin, acne, wrinkles, and therapy for wounds and scars of some injuries or infections. Different types of proteins have been purified from the mucus of the Cornu aspersum against various bacterial strains as an antibacterial agent.

Many studies reported the garden snail's extensive biological composition and nutritive value. They found the main contains, which detected many fatty acids, crude proteins, salts, and other materials (Kermedchiev, 2021; Di Filippo, 2020; Howard, 1981; Sogbesan, 2006; Adeyeye & Afolabi, 2004). Therefore, the research aims to analyze and see biodiversity compositions in the shell powder of garden snails, which might be used in different medical applications or biological purposes.

Methods

Shells of the dead garden snail were collected from local rivers in Al Diwaniya city/Iraq, and all shells were cleaned and dried and then grinded by Pestle and Mortar. Five hundred grams of the shells of the garden snail was the yield of shells, then snail's shells were soaked in 70% ethanol, and the volume was 1ml for each 5gms and left for ten days; afterward, the mixture was filtered using Whatman® filter papers (33mm), next, filtered solution was evaporated and dried using incubation (370) under vision, following, the yield was collected approximately 2 grams after completing extraction process. 250 grams was taken from the yield, dissolved in ml ethanol, and injected in GC-MS spectrometry (C20675502379, LG, Shimadzu). Finally, GC-MS spectrometry was detected and profiled all elements and substances of the garden snail. GC-MS analysis profiles were settled following (Ghazi et al., 2019; Labyad, 2016; Benhanifia, 2014) in table (1).

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Table 1. This table showed the GC-MS conditions that were settled during this study.

System Part1	Conditions values	
GC properties	Injector temperature	280^0 C
	Split	Split inlet
	Split ratio	10:1
	Column property	Capillary column(length 60mm, internal diameter 0.25mm, film thick 0.25 µm
	Oven tem. program	40^0 C for 5 minutes, increased to 280^0 C for 15 minutes
	Carriers gas	Helium(99.99%)
	Carriers gas flow rate	1ml/minute
Massive selective Detector	Electron impact ionization	70e V
	Ion source temperature	200^0 C
	Detector temperature	230^0 C
	Ms scan range spectra	40 to 600 m/z
	Minimum scan rate	0.5 scan/sec.
	Scan speed	500 u/sec.

Results and Discussion

This study displayed that powder shells of garden snails have about 29 chemical compounds that constitute the shell of snails. GC-MS spectrometry was used to analyse the main compositions of snail shells, and conditions were settled according to table (1), GC-MS analysis profile detected 29 chemical compounds constituted the shell of the garden snail with different retention times and predicted percentage area for each component in the shell of a snail, figure (1).

As a result, the retention time for all elements was extended from 3.026 to 45.182 minutes, and the percentage area was between 0.7 to 17.65 %, also Hexadecanoic acid, and ethyl ester was a high area of 17.65, while the smallest area was 0.51 for Cyclopropane, nonyl-, table (1). Also, the highest peak was recorded at 35.016 minutes for Hexadecanoic acid, ethyl ester. Our study was distinguished many chemical compounds in the shells of the garden snail; these chemical substances were fatty acids, saturated fatty acids, organic compounds, alcohol, and natural products.

Therefore, this result distinguished the following chemical compounds, including hexanal ($C_6H_{12}O$) and heptanal ($C_7H_{14}O$) are an alkyl aldehyde; nonanal($C_9H_{18}O$) is an oily aldehyde liquid, and a component of perfumes, the cyclopropane; nonyl- or nonyl cyclopropane ($C_{12}H_{24}$) is cycloalkanes; 2-Tridecenal, (E)- ($C_{13}H_{24}O$) is a fatty aldehyde; 1-Tridecene ($C_{13}H_{26}$) is an unsaturated aliphatic hydrocarbons; dodecanoic acid (Lauric acid) ($C_{12}H_{24}O_2$) is saturated fatty acid with a 12-carbon atom chain; 9-Eicosene, (E)- ($C_{20}H_{40}$) is a natural product which has a limited data with different names; 1,2-cyclohexanediol, 1-methyl-4-(1-methylethenyl)- ($C_{10}H_{18}O_2$) might be referred to decalactones(δ-Decalactone, γ-Decalactone) ; cis-2-Decenoic acid, 8-Hydroxygeraniol, multistriatin, 1-Octen-3-yl acetate, and sobrerol, tetradecanoic acid or myristic acid ($C_{14}H_{28}O_2$) is a common saturated fatty acid.

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Moreover, GC-MS was identified: caffeine ($C_8H_{10}N_4O_2$) is a bitter substance that occurs naturally plants including coffee beans and tea leaves; 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione($C_{17}H_{24}O_3$) may refer to cyclandelate, a vasodilator, onchidal, a naturally occurring neurotoxin, shogaols, pungent constituents of ginger, pentadecanoic (pentadecylic) acid ($C_{15}H_{30}O_2$) is an odd-chain saturated fatty acid; hexadecanoic acid, ethyl ester or ethyl palmitate($C_{18}H_{36}O_2$) is an organic compound; cis-Vaccenic acid or oleic acid ($C_{18}H_{34}O_2$) is a fatty acid found in various animal and vegetable fats and oils; n-Propyl 9,12-octadecadienoate ($C_{21}H_{38}O_2$) is a natural product occurred in Zea mays with data available; octadecanoic acid or stearic acid ($C_{18}H_{36}O_2$) is a saturated fatty acid with an 18-carbon chain; (E)-9-Octadecenoic acid ethyl ester ($C_{20}H_{38}O2$) is fatty acid found in a variety of plant oils and nuts; 9-Octadecenoic acid (Z)-, tetradecyl ester ($C_{32}H_{62}O_2$) is wax monoesters; octadecanamide ($C_{18}H_{37}NO$) is a fatty amide of stearic acid, heptadecanoic acid, ethyl ester ($C_{19}H_{38}O_2$) is a saturated methyl ester and extracted from vegetable oils; Eicosane ($C_{20}H_{42}$) is an alkane, 9-Octadecenamide; (Z)- or Oleamide ($C_{18}H_{35}NO$) is an organic compound, and it is an amide derived from the fatty acid oleic acid; E,E,Z-1,3,12-Nonadecatriene-5,14-diol or sterculic acid ($C_{19}H_{34}O_2$) is a cyclopropene fatty acid; 9-Eicosyne ($C_{20}H_{38}$) is a chemical compound, no more information, octadecanamide($C_{18}H_{37}NO$) is a fatty amide of stearic acid; 2-Dodecen-1-yl(-)succinic anhydride ($C_{16}H_{26}O_3$) is the methyl ester of todomatuic acid; heneicosane ($C_{21}H_{44}$) is a white wax, diisooctyl phthalate has consisted of phthalate ester and diester, table(2).

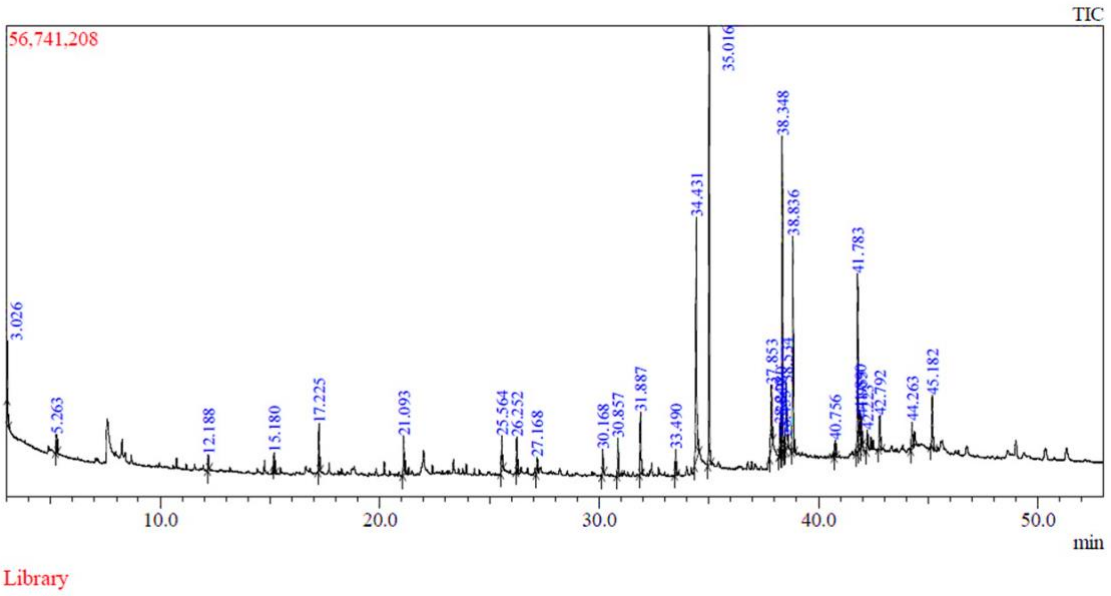

Figure 1. illustrates different peaks and curves of varying chemical materials detected by GC-MS.

Table 2. This table shows extracted predicted materials from shells of the snail

Compounds	**Retention Time**	**Area**	**Area%**	**Molecular Formula**	**Molecular Weight** (g/mol.)
Hexanal	3.026	15609254	2.01	$C_6H_{12}O$	100.16
Heptanal	5.263	5447602	0.7	$C_7H_{14}O$	114.18
Nonanal	12.188	4165564	0.54	$C_9H_{18}O$	142.24
Cyclopropane, nonyl-	15.18	3983951	0.51	$C_{12}H_{24}$	168.32
2-Tridecenal, (E)-	17.225	13601398	1.75	$C_{13}H_{24}O$	196.33
1-Tridecene	21.093	13567491	1.75	$C_{13}H_{26}$	182.34
Dodecanoic acid	25.564	11647607	1.5	$C_{12}H_{24}O_2$	

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1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)-	27.168	10310430	0.65	$C_{10}H_{18}O_2$	200.3178
Tetradecanoic acid	30.168	5035852	1.06	$C_{14}H_{28}O_2$	228.37
9-Eicosene, (E)-	30.857	8200988	1.55	$C_{20}H_{40}$	280.5
Caffeine	31.887	12056544	2.91	$C_8H_{10}N_4O_2$	194.19
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	33.49	22611148	1.01	$C_{17}H_{24}O_3$	276.38
Pentadecanoic acid	34.431	7866619	12.92	$C_{15}H_{30}O_2$	242.3975
Hexadecanoic acid, ethyl ester	35.016	137094479	17.65	$C_{18}H_{36}O_2$	284.48
cis-Vaccenic acid	37.853	26987917	3.47	$C_{18}H_{34}O_2$	282.47
n-Propyl 9,12-octadecadienoate	38.24	26987917	1.28	$C_{21}H_{38}O_2$	322.5
Octadecanoic acid	38.28	9944738	1.46	$C_{18}H_{36}O_2$	284.48
(E)-9-Octadecenoic acid ethyl ester	38.348	11306731	13.73	$C_{20}H_{38}O_2$	310.51
9-Octadecenoic acid (Z)-, tetradecyl ester	38.455	5785083	0.74	$C_{32}H_{62}O_2$	478.8
Octadecanamide	38.534	5785083	3.84	$C_{18}H_{37}NO$	283.5
Heptadecanoic acid, ethyl ester	38.836	29843998	8.84	$C_{19}H_{38}O_2$	298.50
Eicosane	40.756	68658266	0.62	$C_{20}H_{42}$	282.5475
9-Octadecenamide, (Z)-	41.783	4844247	7.94	$C_{18}H_{35}NO$	281.477
E,E,Z-1,3,12-Nonadecatriene-5,14-diol	41.89	61650543	1.98	$C_{19}H_{34}O_2$	294.5
9-Eicosyne	41.955	15396200	1.3	$C_{20}H_{38}$	278.5
Octadecanamide	42.223	10127069	0.8	$C_{18}H_{37}NO$	283.5
2-Dodecen-1-yl(-)succinic anhydride	42.792	6235541	2.02	$C_{16}H_{26}O_3$	266.381
Heneicosane	44.263	15726194	1.27	$C_{21}H_{44}$	296.57
Diisooctyl phthalate	45.182	9901916	2.85	$C_{24}H_{38}O_4$	390.5561

Recently, there have been many studies that investigated the extraction effects of the different species of snails in Vitro and in Vivo and employed on various diseases involved with cancer and skin diseases (Matusiewicz, 2018; Burton, nd). Thus, this study has been conducted on the shell of garden snails to identify different compositions and chemical compounds shell part of garden snails.

GC-Mass spectrometry was used to analyse the chemical compounds of a garden snail and other species of snails which was a very effective technique to detect the main compositions of the snails (Weinert et al., 1993; Kramarz, 2009; Murakami, 1992), and our study found that there are 29 chemical compounds in the shell of the garden snail using GC-Mass spectrometry.

Our finding identified chemical compounds in the powder of the shell of garden snail which there are predominant chemical compounds which constituted the shell of the garden snail and included, ethyl ester, (E)-9-Octadecenoic acid ethyl ester, pentadecanoic acid, heptadecanoic

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acid, ethyl ester, and 9-Octadecenamide, (Z)-, and hexadecanoic acid. However, these chemical compounds did not detect in garden snails except saturated fatty acids involved in pentadecanoic and pentadecanoic acid detected in garden snails by other researchers (Maćkowiak, 2020).

Also, this study harvested 23 chemical compounds that constituted the structure of the shell of a garden snail with a low percentage. So, four saturated fatty acids were detected in the powder of the shell of the garden snail and included dodecanoic acid (Lauric acid), myristic acid, pentadecanoic (pentadecylic) acid, and octadecanoic acid or stearic acid, as a result, these chemical substances were detected by other researchers in the garden snails and other species (Maćkowiak 2020; Sverdrup-Thygeson et al., 2019; Ballard, 2021; Belitz, 2004; Pandirajan, 2022; Odesanya, 2011; Galluzzo, 2019).

Also, our analysis reported many fatty acids. It derived the fatty acids in the shell powder of the garden snails included cis-Vaccenic acid or oleic acid, (E)-9-Octadecenoic acid ethyl ester, 9-Octadecenoic acid (Z)-, tetradecyl ester, octadecanamide, so, all these chemical compounds were distinguished by (Odesanya, 2011; Galluzzo, 2019; Kowalczy & Puchalski, 2008; Dawidar, 2012; Lim, & Iris 2016;) in garden snails and other species of snails. In addition, we found more fatty acids in the shell of the garden snail for the first time, which were 9-Octadecenamide, (Z)- or Oleamide, 14-diol or sterculic acid, and 2-Tridecenal, (E)-. Other studies might not detect these fatty acids because they did not cover all parts of garden snails, or methods for extraction and GC-Mass spectrometry conditions were different, which could be given other chemical compounds.

There are many other products detected in the shell powder of garden snails during this study; some products identified by other researchers in the snails comprised hexanal and heptanal, hexadecanoic acid, ethyl ester, or ethyl palmitate, nonanal heptadecanoic acid, ethyl ester 1-methyl-4-(1-methylethenyl)- (Sverdrup-Thygeson et al., 2019; Ballard, 2021; Belitz, 2004; Pandirajan, 2022; Odesanya, 2011; Kubisa, 1980; Lasekan, 2018; Pissia et al., 2021; Harborne, 1997; Iannino, 2020).

Taking all results, this study highlighted more compound substances in the garden snail shell than previous studies. GC-mass spectrometry could be different according to conditions and type of extraction methods of the garden snail.

In conclusion, GC-Mass spectrometry has uncovered many chemical compounds in the shell powder of the garden snail, including saturated and unsaturated fatty acids, in addition to many other natural products. However, six chemical substances have a high percentage area, and some of these compounds were detected for the first time in the garden snail. These findings confirmed that the shell of a garden snail has a high biological diversity substance which can allow for further studies to confirm these results and investigate more about different natural chemical substances sources in the shell of a garden snail.

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Conflict of Interest

This study has been conducted in the labs of the College of Veterinary Medicine-University of Al-Qadisiyah and has no conflict of interest.

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