Shetty and Biradar
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RESEARCH

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Analysis of different bioactive compounds in the tissue of the epigeic earthworm, *Eisenia fetida*

Aishwarya Shetty[1] and Pulikeshi M. Biradar[1*]

Abstract

Background *Eisenia fetida* is the epigeic earthworm renowned for organic waste management in vermitechnology. The medicinal properties of earthworm biomass is gaining much more importance in extracting various biomolecules. Therefore, the present study was carried out to analyze the bioactive compounds of *Eisenia fetida* by using gas chromatography and mass spectroscopy analysis of four different solvent extract and highlighting their biological activities.

Results The analysis showed the presence of 17, 22, 21 and 18 bioactive compounds in chloroform, ethyl acetate, methanol and distilled water solvent extract, respectively. Each compound were analyzed based on their peak number, R-time and Base m/z values. The molecular formula, molecular weight, compound nature, their structure and biological activities were tabulated.

Conclusion The presence of a wide range of bioactive compounds in the epigeic earthworm, *Eisenia fetida*, justifies the pharmacological use in curing various diseases.

Keywords Bioactive compounds, *Eisenia fetida*, Epigeic earthworm, Gas chromatography and mass spectroscopy and phytochemicals

Background

Our prehistoric ancestors sought different natural compounds to cure various ills and diseases so as to improve and enrich their lives, most of these compounds were derived from either plants or animals. Research on traditional medicines has been vital in search for organic pharmaceutical compounds. Animal-based medicines have also been extracted and isolated from different parts of the animal body, from their products (secretions and excrements) or from materials they produce like cocoons and compost in earthworm. A total of 252 essential compounds have been selected by World Health Organization (WHO), of which only 8.7% are from animal origin used as medicine (Costa-Neto, 2005). For almost 4000 years, China has a history of research into medicinal uses of earthworm.

Earthworms are well-known invertebrates, are used extensively as folk fare medicines and claim their successful use in treatment of cancer, ulcer, inflammation, diarrhea, fever, dysentery, tooth ache, etc. (Edwards & Bohlen, 1996). Various studies have shown that earthworms exhibit anti-pyretic, anti-septic, detoxifying, diuretic, anti-hypertensive, anti-allergic, anti-asthmatic, spermatocidal, anti-oxidative, anti-microbial, anti-cancer, anti-ulcer and anti-inflammatory activity (Cooper & Roch, 1984; Cooper et al., 2004; Hori et al., 1974). High protein content of earthworm makes it a desired supplementary food material to a wide range of animals such as fish, amphibians, reptiles, birds and mammals

*Correspondence:
Pulikeshi M. Biradar
pulikeshi123@gmail.com
[1] Department of Zoology, Karnatak University, Dharwad, Karnataka 580 003, India

(Edwards & Bohlen, 1996). The presence of plant and animal growth-promoting amino acids and other substances showed the therapeutic effects (Edwards & Bohlen, 1996). Presently, there is an increasing demand and interest in naturally produced medicines, accompanied by modern laboratory investigations into pharmacological properties of bioactive ingredients and their ability to treat various diseases (Smita, 2021). Further it encourages looking for other molecules of curative value.

In recent years, zootherapy (the science of treating various ailments by using therapeutic materials obtained from animals) is gaining valuable importance as it is potentially safe drugs to use. Therefore, pharmaceutical industries are much interested and are trying to use these bioactive compounds and other metabolites as potent agents in treatment of many diseases (David & Gordon, 2020). Many studies revealed that primary and secondary metabolites are used in the treatment of chronic as well as infectious diseases (Kesava & Usha, 2016). In recent years, GC–MS has become the key tool for profiling secondary metabolites which is widely used in the separation and identification of complex components, as it has high resolution of GC and high sensitivity of mass spectrometry. Hence, the present study is focused on identification of different bioactive compounds through GC–MS technique from the epigeic earthworm, *Eisenia fetida*, using different solvent system like distilled water, chloroform, methanol and ethyl acetate.

Methods

a. *Selection and collection of earthworm*: Epigeic earthworm, *Eisenia fetida* species, was selected based on their mass multiplication rate in vermiculture and was collected from the stock culture maintained in vermitechnology laboratory, Department of Zoology, Karnatak University, Dharwad, as it is voracious feeder and breeder continuously and can withstand a wide range of abiotic factors.
b. *Preparation of sample powder*: Around thirty sexually matured healthy *Eisenia fetida* earthworms were collected and washed them in distilled water and the surface debris were removed with the help of blotting paper. The cleaned earthworms were sundried for about seven days. The dried biomass was ground to get fine powder with the help of mortar and pistil.
c. *Sample extract*: 2.5 g of powdered sample was used and subjected to extraction with chloroform (40 ml), ethyl acetate (40 ml), methanol (40 ml) and distilled water (40 ml) solvents.
d. *GC–MS analysis*: Gas chromatography–mass spectroscopy analysis was carried out to evaluate various metabolites and bioactive compounds present in the powdered sample, the epigeic earthworm, *Eisenia fetida*, in four different solvent extracts. The sample was analyzed on GC 2010 with split injection mode and linear velocity flow control mode having 80^0C of column oven temperature with 65.0 kPa of pressure. The GC program was performed on GC–MS–QP 2010 with 2200C Ion source temperature and 2800C of interface temperature. The solvent cut time was 6.50 min with relative type of detector gain mode, and the detector gain is 1.03 kV + 0.20 kV at 1000 threshold. The start time was 7.00 min, and the end time was 50.00 min with scan ACQ mode. The event time is for 0.50 s with 1000 scan speed.
e. *Identification of components*: Various bioactive compounds are identified based on the retention time (R-time). Interpretation of mass spectrum of GC–MS was done by using the database of the National Institute Standard and Technology (NIST). The data reported in the present study are obtained from various online repositories, websites like MedChem Express, PubChem, ChEBI, ChemSpider, Smolecule, Cymitquimicq and Chemical Book. Based on this, the molecular weight, molecular formula, their structure, nature of compounds and their biological activity were tabulated in the results.

Results

The GC–MS profiling of powdered sample of *Eisenia fetida* in four different solvent extracts (chloroform, ethyl acetate, methanol and distilled water) gave a wide range of bioactive compounds. The GC–MS chromatogram of chloroform extract showed the peaks indicating the presence of 17 bioactive compounds and is presented in Fig. 1. Table 1 indicates the details of identified bioactive compounds such as name of the compound along with its peak number, R-time, base m/z, compound nature, chemical formula and molecular weight, whereas the structure of compounds and their biological activities are presented in Table 2. Similarly, Figs. 2, 3 and 4 present the GC–MS chromatogram of *Eisenia fetida* which showed the peaks indicating the presence of 22, 21 and 18 different bioactive compounds in ethyl acetate, methanol and distilled water extract, respectively. Tables 3, 4 and 5 present the details of the bioactive compounds such as compound's name with its peak number, R-time, base m/z, compound nature, chemical formula and molecular weight, whereas the structure of respective compounds

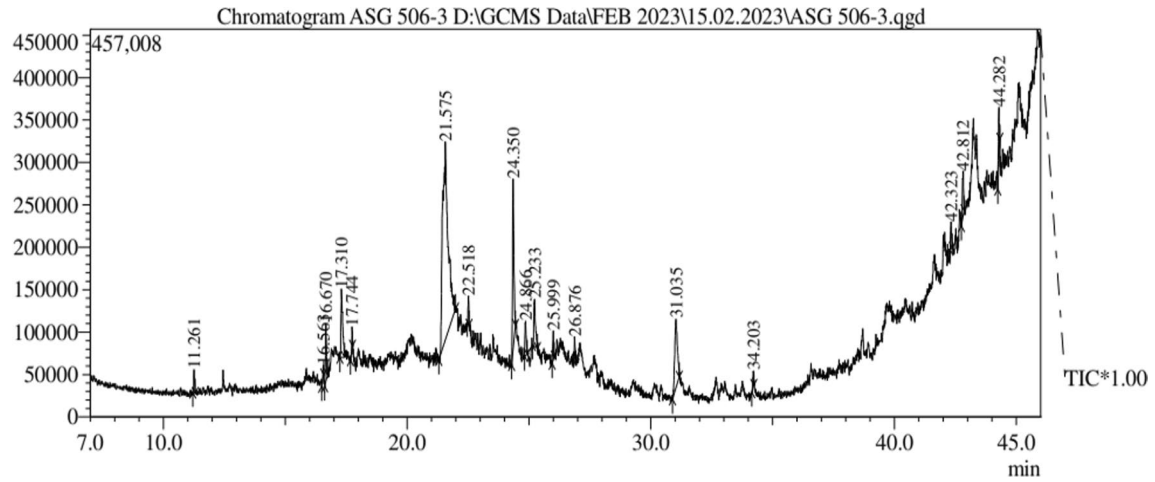


Fig. 1 Chromatogram of GC–MS in chloroform extract of the epigeic earthworm, *Eisenia fetida*

Table 1 Details of bioactive compounds analyzed in chloroform extract of the epigeic earthworm, *Eisenia fetida*, through GC–MS

Peak No	R. Time	Compound name	Base m/z	Compound nature	Molecular formula	Molecular weight (g/mol)
1	11.261	Hexane, 3,3-dimethyl-	71.05	Alkane	C_8H_{18}	114.22
2	16.563	Sulfurous acid, pentadecyl 2-propyl ester	71.10	Ester	$C_{18}H_{38}O_3S$	334.55
3	16.670	Dodecane, 2,6,11-trimethyl-	57.05	Alkane	$C_{15}H_{32}$	212.41
4	17.310	2,4-Ditert-butylphenol	191.15	phenol	$C_{14}H_{22}O$	206.32
5	17.744	Undecane, 3,8-dimethyl-	57.05	Alkane	$C_{13}H_{28}$	184.36
6	21.575	2-Propenoic acid, pentadecyl ester	55.00	Ester	$C_{18}H_{34}O_2$	282.00
7	22.518	Tetradecane	57.05	Alkane	$C_{14}H_{30}$	198.39
8	24.350	Neophytadiene	68.05	Alkene	$C_{20}H_{38}$	278.51
9	24.866	(E)-Phytol	68.10	Phytosterol	$C_{20}H_{40}O$	296.53
10	25.233	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	81.00	Acyclic monosaturated diterpene alcohol	$C_{20}H_{40}O$	296.53
11	25.999	2-Bromotetradecane	57.05	Halide	$C_{12}H_{25}Br$	249.23
12	26.876	Tetratricontane	57.05	Alkane	$C_{34}H_{70}$	478.91
13	31.035	Phytol isomer	71.05	Acyclic hydrogenated diterpene alcohol	$C_{20}H_{40}O$	296.53
14	34.203	Hexadecane, 1,1-bis(Dodecyloxy)-	57.05	Alkane	$C_{40}H_{82}O_2$	595.10
15	42.323	Acetic acid n-octadecyl ester	55.05	Ester	$C_{20}H_{40}O_2$	312.53
16	42.812	Nonadecane	57.05	Alkane	$C_{19}H_{40}$	268.50
17	44.282	4,4′-(p-Phenylene) diisopropylidene) diphenol	331.20	diphenol	$C_{24}H_{26}O_2$	346.46

and their biological activities are presented in Tables 6, 7 and 8 with respect to ethyl acetate, methanol and distilled water extracts, respectively.

GC–MS analysis of different extracts of the epigeic earthworm, *Eisenia fetida*, showed various bioactive compounds of pharmaceutical importance. There are in total 78 different bioactive compounds identified from four solvent extracts (chloroform, ethyl acetate, methanol and distilled water), which are chemically alkanes, esters, phenol, alkenes, fatty alcohol, carboxylic acid, quinoline, sulfur allotrope, fluorides, alkyl iodides, salt, alkyl benzene, dicarboxylic acid, aromatic compounds, phytosterol, acyclic monosaturated diterpene alcohol, halides, heterobicyclic compounds, cyclicalkanes, lactum

Table 2 Structure and bioactivity of the compounds obtained in the chloroform extract of the epigeic earthworm, *Eisenia fetida*, through GC–MS

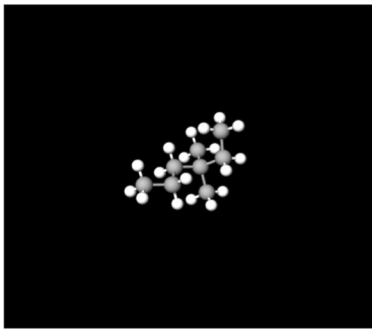
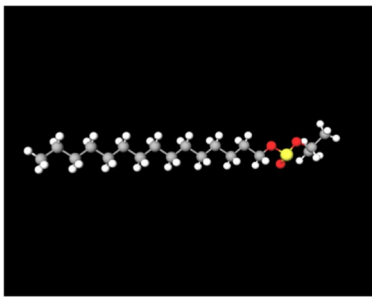
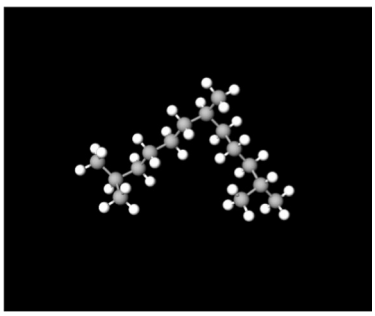
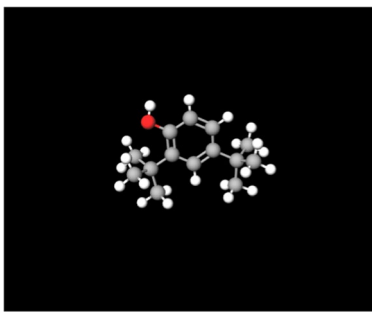
Sl. No	Compound name	Structure	Uses/biological activity	References
1	Hexane, 3,3-dimethyl-		Can be used in production of phytochemical compound effective in removal of heavy metals	Ghorbani et al. (2022)
2	Sulfurous acid, pentadecyl 2-propyl ester		Anti-oxidant, anti-microbial and anti-inflammatory	Patil et al. (2015)
3	Dodecane, 2,6,11-trimethyl-		Unreported	Unreported
4	2,4-Ditert-butylphenol		Anti-cancer	Song et al. (2018)

Table 2 (continued)

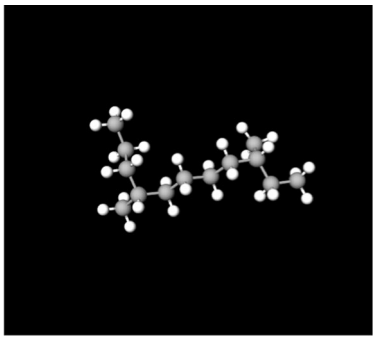
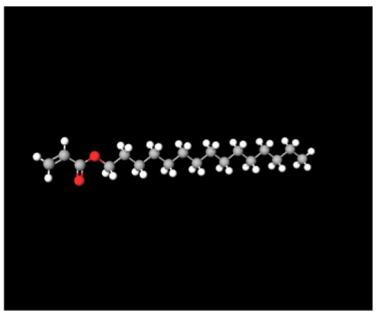
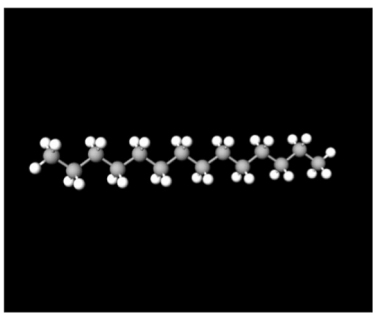
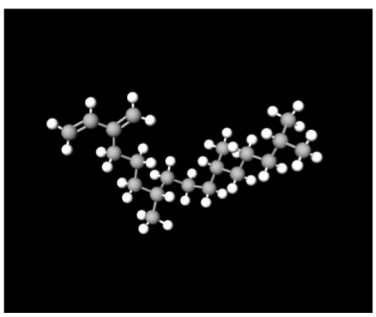
Sl. No	Compound name	Structure	Uses/biological activity	References
5	Undecane, 3,8-dimethyl-		Acts as metabolite in cancer metabolism	PubChem
6	2-Propenoic acid, pentadecyl ester		Hydroxylation of liver enzymes during phase I metabolism, hair growth promoter, inhibit production of uric acid and arachidonic acid inhibitor in human body	Shunmugapriya et al. (2017)
7	Tetradecane		Anti-microbial[a], cytotoxicity[b], anti-pyretic, anti-helmentic, tumor, bronchitis, asthma, tuberculosis, dyspepsia, constipation, anemia, throat diseases, elephantiasis, anti-diabatic, anti-inflammatory, anti-diarrheal[c]	Javidnia et al. (2008), Choo et al. (2001), Gurudeeban et al. (2010)
8	Neophytadiene		Anti-inflammatory, anti-oxidant and cardioprotective property	MCE

Table 2 (continued)

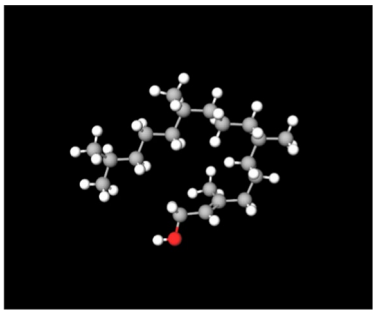
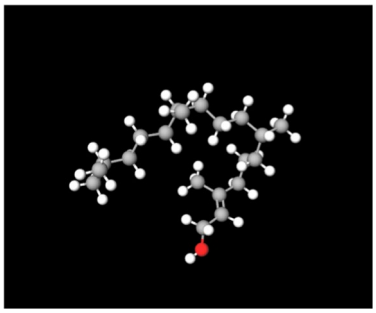
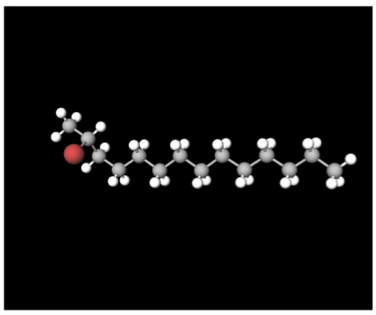
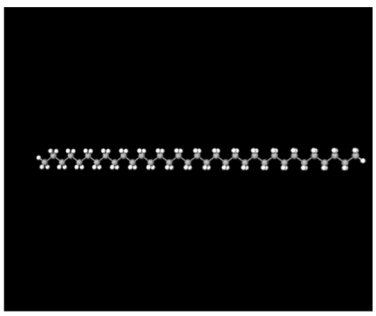
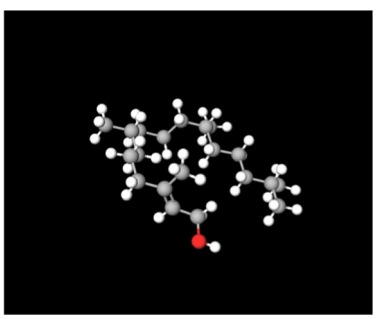
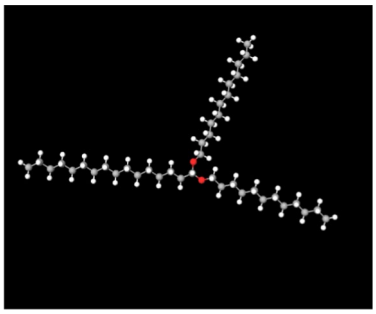
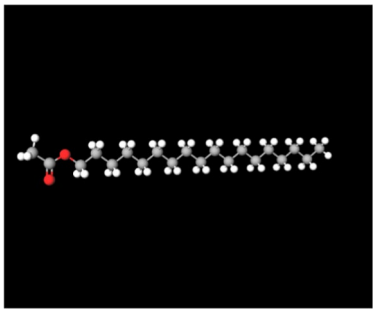
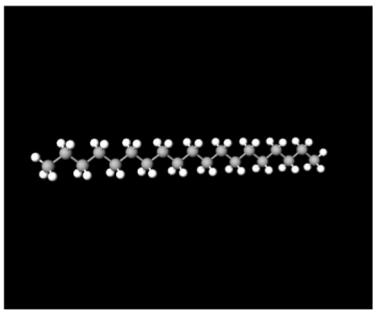
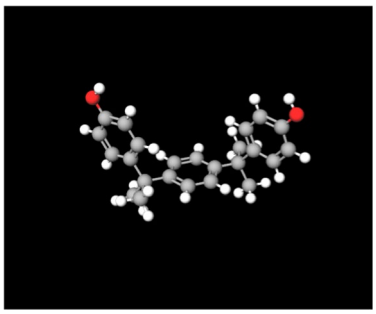
Sl. No	Compound name	Structure	Uses/biological activity	References
9	(E)-Phytol		Anti-histomal, anti-nociceptive, anti-oxidant, anti-inflammatory, anti-allergic and anti-microbial	Josué de et al. (2014)
10	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		Anti-microbial and anti-inflammatory	Rajeswari et al. (2012)
11	2-Bromotetradecane		Anti-microbial	Apoorva et al. (2018)
12	Tetratriacontane		Anti-oxidant and anti-microbial	Mustapha and Runner (2016)
13	Phytol isomer		Anti-nociceptive, anti-oxidant, anti-inflammatory, anti-allergic and anti-microbial	Josué de et al. (2014)

Table 2 (continued)

Sl. No	Compound name	Structure	Uses/biological activity	References
14	Hexadecane, 1,1-bis(dodecyloxy)-		Unreported	Unreported
15	Acetic acid n-octadecyl ester		Unreported	Unreported
16	Nonadecane		Anti-HIV, anti-oxidant, anti-bacterial, anti-microbial, cytotoxic agent and anti-malarial	Haval and Fuad (2022)
17	4,4′-((p-Phenylene)diisopropylidene)diphenol		Unreported	Unreported

and liquid alkane in nature. Out of 78 compounds identified, the biological activities of 23 compounds are not yet reported. The presence of all these bioactive compounds some or other way showed various pharmaceutical properties. Majority of the compounds obtained in the present study are chemically alkane in nature, exhibiting different biological activities like anti-fungal, anti-bacterial, anti-cancer, anti-pyretic, anti-helminthic, anti-inflammatory, etc. biological activities. Sulfurous acid, pentadecyl 2-propyl ester is chemically ester in nature, and exhibits anti-oxidant, anti-microbial and anti-inflammatory activities (Patil & Deshmukh, 2015). 2-Propenoic acid, pentadecyl ester is also chemically ester in nature helps in hydroxylation of liver enzymes, promotes hair growth, and inhibits the production of uric acid and arachidonic acid in the human

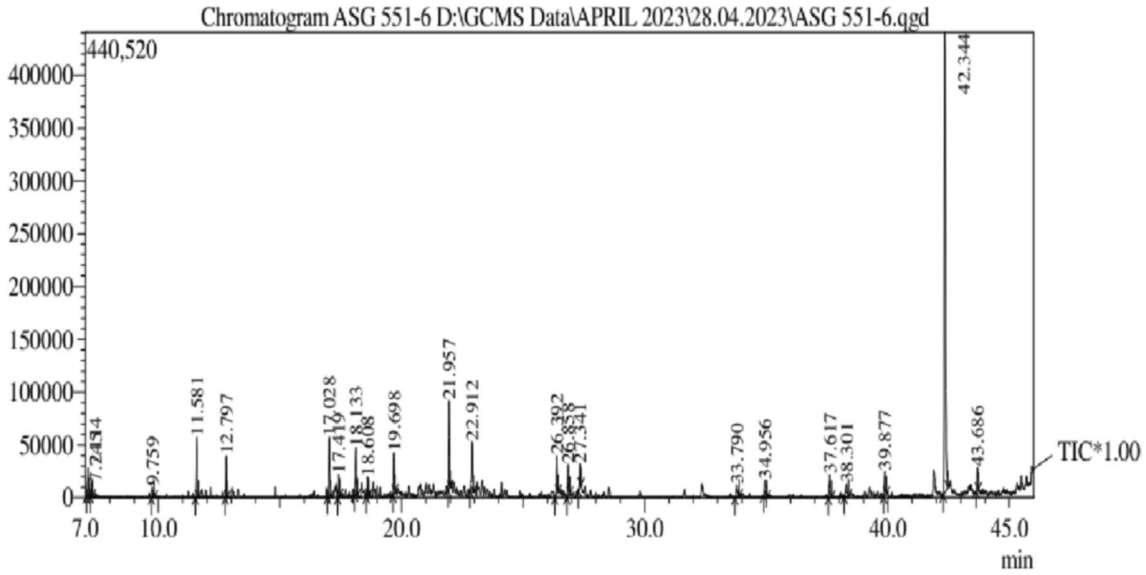


Fig. 2 Chromatogram of GC–MS in ethyl acetate extract of the epigeic earthworm, *Eisenia fetida*

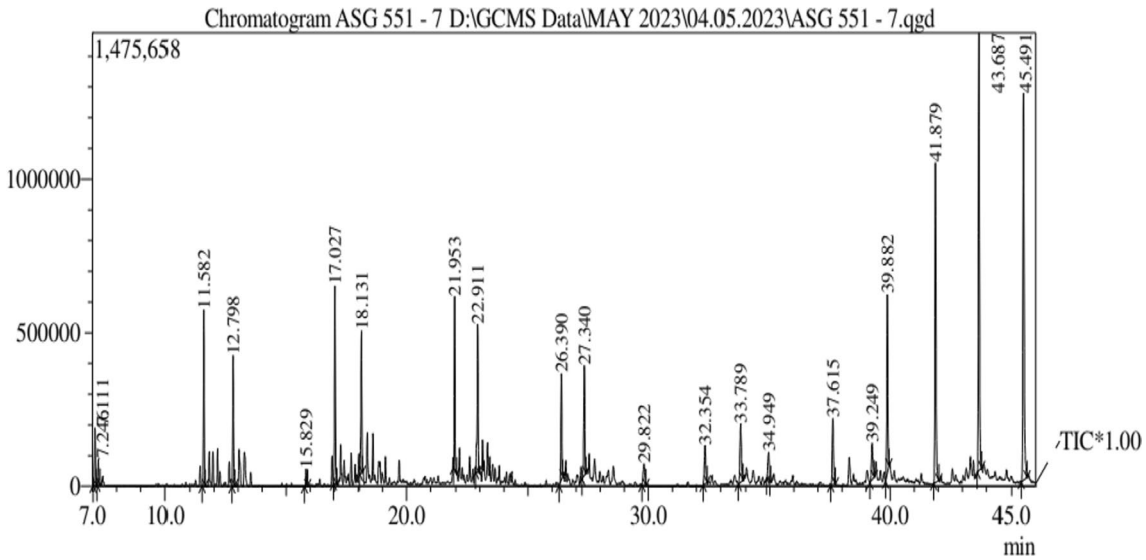


Fig. 3 Chromatogram of GC–MS in methanol extract of the epigeic earthworm, *Eisenia fetida*

body (Shunmugapriya et al., 2017). (E)-Phytol, which is chemically phytosterol in nature, exhibits anti-histomal, anti-nociceptive, anti-oxidant, anti-inflammatory, anti-allergic and anti-microbial activities (de Josué et al., 2014). 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, which is chemically acyclic monosaturated diterpene alcohol in nature, exhibits anti-microbial and anti-inflammatory activities (Rajeswari et al., 2012). The aromatic compound, phenol, 3,5-bis(1,1-dimethylethyl)-, which exhibits anti-oxidant and anti-bacterial activity (Rizvi et al., 2014). The fatty alcohol compound, 2-octyldodecan-1-ol, also exhibits anti-inflammatory, anti-oxidant and anti-microbial activities (Cymitquimicq.com). The dodecane, 1-iodo which is chemically alkyl iodide in nature exhibits anti-diabetic activity (Mahmood et al., 2020). Quinoline, 1,2-dihydro-2,2,4-trimethyl- is chemically quinoline in nature exhibits anti-oxidant property which is also used in styrene–butadiene and nitrile–butadiene rubber and latex (Chemical Book). The sulfur allotrope, octathiocane, exhibits anti-cancer, anti-oxidants and anti-microbial activities (Smolecule). The fatty alcohol, celidoniol, deoxy-, exhibits anti-bacterial (Köse et al., 2016) and

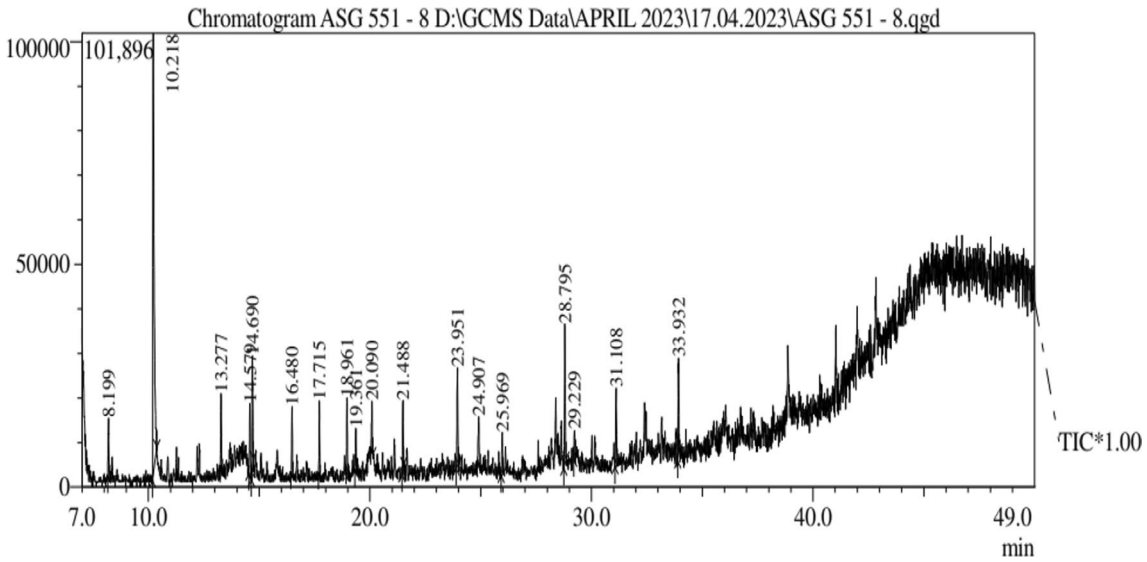


Fig. 4 Chromatogram of GC–MS in distilled water extract of the epigeic earthworm, *Eisenia fetida*

Table 3 Details of bioactive compounds in ethyl acetate extract of the epigeic earthworm, *Eisenia fetida*, through GC–MS

Peak No	R. time	Compound name	Base m/z	Compound nature	Molecular formula	Molecular weight (g/mol)
1	7.114	Undecane, 4,7-dimethyl-	57.00	Alkyl benzene	$C_{13}H_{28}$	184.36
2	7.245	Pentane, 2,3,3-trimethyl-	57.00	Alkane	C_8H_{18}	114.22
3	9.759	2,3-Heptadienylaminium hydrogen oxalate	76.95	Salt	$C_9H_{15}NO_5$	216.21
4	11.581	Undecane, 4,7-dimethyl-	57.05	Alkane	$C_{13}H_{28}$	184.36
5	12.797	Hexane, 3,3-dimethyl-	57.05	Alkane	C_8H_{18}	114.22
6	17.028	Octadecane	57.05	Alkane	$C_{18}H_{38}$	154.49
7	17.419	Phenol, 3,5-bis(1,1-dimethylethyl)-	191.05	Aromatic compound	$C_{14}H_{22}O$	206.32
8	18.133	Hexadecane	71.10	Alkane	$C_{16}H_{34}$	226.44
9	18.608	2-Octyldodecan-1-ol	71.10	Fatty alcohol	$C_{20}H_{42}O$	298.55
10	19.698	Pentadecane	57.10	Alkane	$C_{15}H_{32}$	212.41
11	21.957	Nonadecane	57.05	Alkane	$C_{19}H_{40}$	268.52
12	22.912	Tetracosane	57.05	Alkane	$C_{24}H_{50}$	338.65
13	26.392	Undecane, 4,8-dimethyl-	71.10	Alkane	$C_{13}H_{28}$	184.36
14	26.858	2-(1,3-Benzothiazol-2-ylsulfanyl)ethanol	166.95	Alcohol	$C_{11}H_{13}NO_2S_2$	255.4
15	27.341	Hexane, 2,3,4-trimethyl-	71.10	Alkane	C_9H_{20}	128.25
16	33.790	Docosane	71.10	Alkane	$C_{22}H_{46}$	310.60
17	34.956	Octadecyl flouride	57.05	Fluorides	$C_{18}H_{37}F$	272.49
18	37.617	Dodecane, 1-iodo-	57.05	Alkyl iodides	$C_{12}H_{25}I$	296.23
19	38.301	1-(2-Hydroxyethoxy)tridecane	71.15	Ether	$C_{15}H_{32}O_2$	244.42
20	39.877	Octadecane	57.00	Alkane	$C_{18}H_{38}$	254.49
21	42.344	1,2-Benzenedicarboxylic acid	149.00	Dicarboxylic acid	$C_8H_6O_4$	166.13
22	43.686	Tetracosane	57.05	Alkane	$C_{24}H_50$	338.65

anti-inflammatory activity (Zakariaa et al., 2014), which helps in chemical communication in *Anopheles stephensi* mosquito (Brei et al., 2004) and acts as pheromone of *Orgyia leucostigma* (Grant et al., 1987). The anti-inflammatory activity is also reported in undecane which is chemically liquid alkane in nature and heptadecyl acetate

Table 4 Structure and bioactivity of the compounds in the ethyl acetate extract of the epigeic earthworm, *Eisenia fetida*, through GC–MS

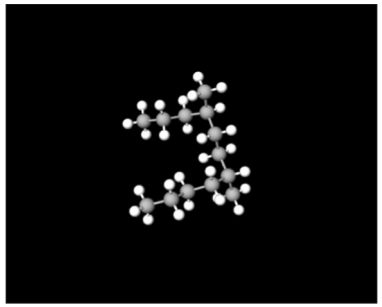
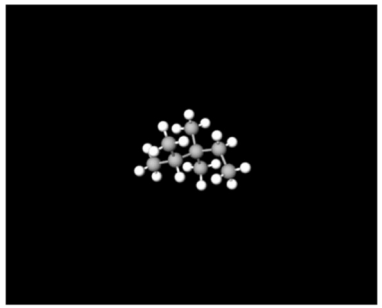
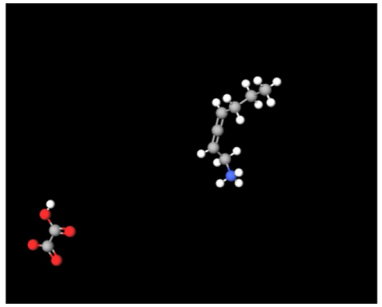
Sl. No	Compound name	Structure	Uses/biological activity	References
1	Undecane, 4,7-dimethyl-		Unreported	Unreported
2	Pentane, 2,3,3-trimethyl-		Human, bacterial and mammalian metabolite	PubChem
3	2,3- Heptadienylaminium hydrogen oxalate		Unreported	Unreported

Table 4 (continued)

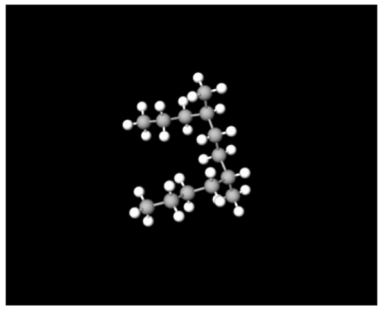
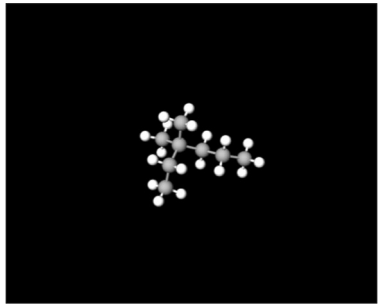
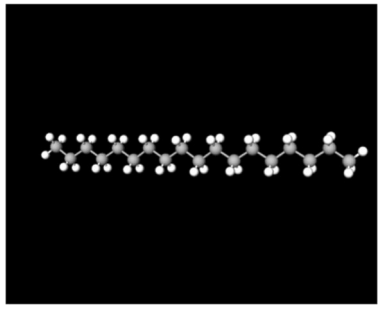
Sl. No	Compound name	Structure	Uses/biological activity	References
4	Undecane, 4,7-dimethyl-		Unreported	Unreported
5	Hexane, 3,3-dimethyl-		Metabolite	Ghorbani et al. (2022)
6	Octadecane		Anti-oxidant, anti-inflammatory[a] cough, lung diseases, cold and fever detoxification[b], anti-corrosion agent[c], anti-sepsis[d]	Marzoqi et al. (2015), Zhao et al. (2017), Jyothirmayi et al. (2014), Sowmya et al. (2015)

Table 4 (continued)

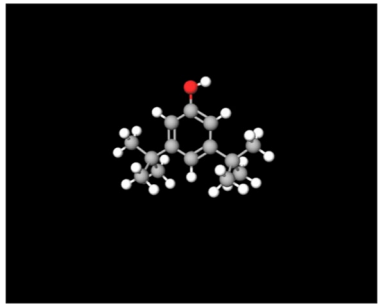
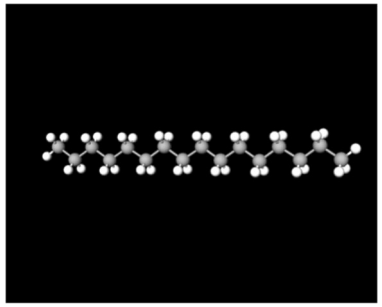
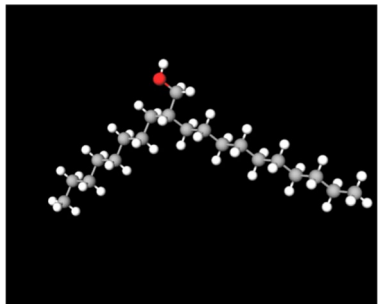
Sl. No	Compound name	Structure	Uses/biological activity	References
7	Phenol, 3,5-bis(1,1-dimethylethyl)-		Anti-oxidant and anti-bacterial	Rizvi et al. (2014)
8	Hexadecane		Cytotoxicity[a], anti-microbial, anti-oxidant[b], anti-pyretic, anti-helminthic, anti-tumor, anti-diabetic and anti-inflammatory[c]	Choo et al. (2001), Neda et al. (2004), Gnanavel and Mary (2013)
9	2-Octyldodecan-1-ol		Anti-inflammatory, anti-oxidant and anti-microbial	Cymitquimicq.com

Table 4 (continued)

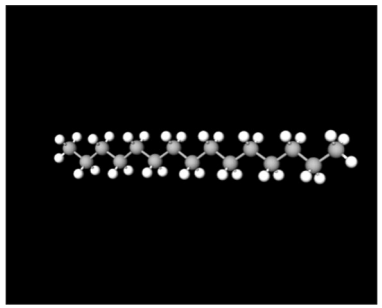
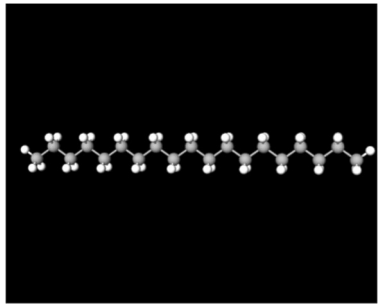
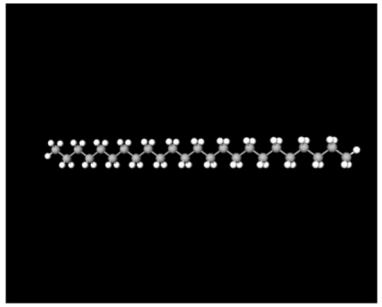
Sl. No	Compound name	Structure	Uses/biological activity	References
10	Pentadecane		Anti-bacterial	Yogeswari et al. (2012)
11	Nonadecane		Anti-HIV, anti-oxidant, anti-bacterial[a], anti-microbial[b], cytotoxic effect, anti-microbial[c,b] anti-malarial[d], unini uses like weakness of the principal organs like heart, brain, liver, general weakness, hemoptysis, palpitation, conjunctivitis, earache, stomatitis[e]	Mahmoodreza et al. (2010), Javidnia et al. (2008), Akpuaka et al. (2013), Ombito et al. (2014), Prathapa et al. (2015)
12	Tetracosane		Cytotoxic toward gastric cancer cells by induction of apoptosis	Uddin et al. (2012)

Table 4 (continued)

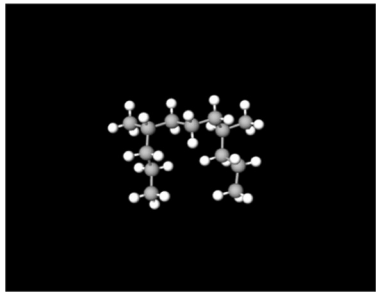
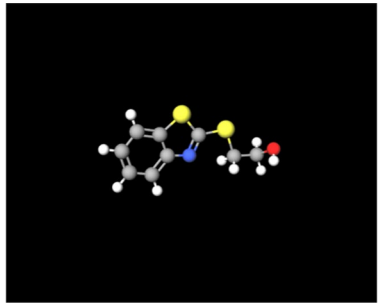
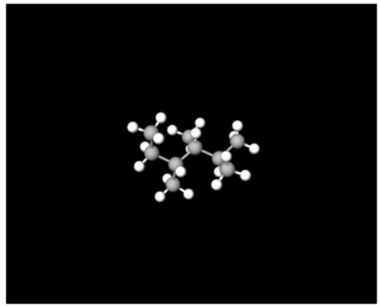
Sl. No	Compound name	Structure	Uses/biological activity	References
13	Undecane, 4,8-dimethyl-		Unreported	Unreported
14	2-(1,3-Benzothiazol-2-ylsulfanyl)ethanol		Unreported	Unreported
15	Hexane, 2,3,4-trimethyl-		Unreported	Unreported

Table 4 (continued)

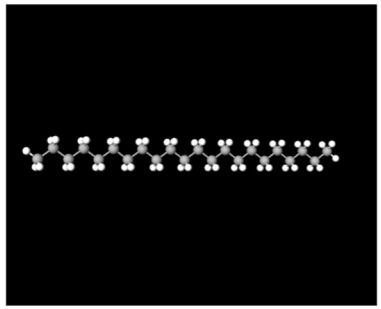
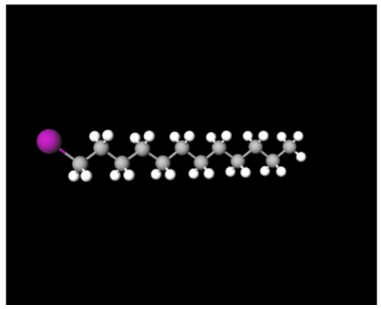
Sl. No	Compound name	Structure	Uses/biological activity	References
16	Docosane		Anti-bacterial[a] and enhances host egg parasitization[b]	Gumgumjee and Hajar (2015), Paul et al. (2002)
17	Octadecyl fluoride		Unreported	Unreported
18	Dodecane, 1-iodo-		Anti-diabetic	Mahmood et al. (2020)

Table 4 (continued)

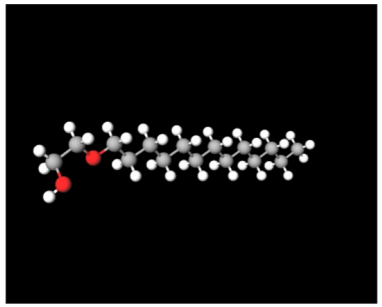
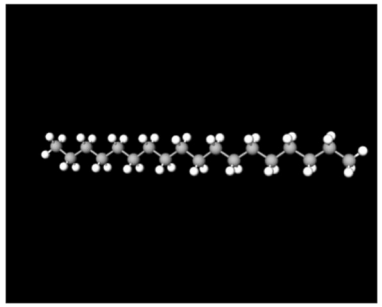
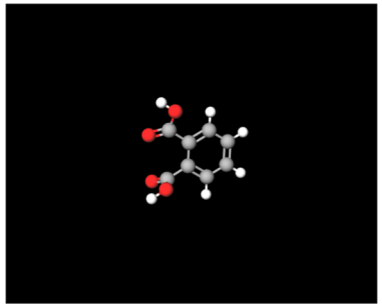
Sl. No	Compound name	Structure	Uses/biological activity	References
19	1-(2-Hydroxyethoxy)tridecane		Unreported	Unreported
20	Octadecane		Anti-oxidant, anti-inflammatory[a], cough, lung diseases, cold and fever detoxification[b], anti-corrosion agent[c], anti-sepsis[d]	Marzoqi et al. (2015), Zhao et al. (2017), Jyothirmayi et al. (2014), Sowmya et al. (2015)
21	1,2-Benzenedicarboxylic acid		Unreported	Unreported

Table 4 (continued)

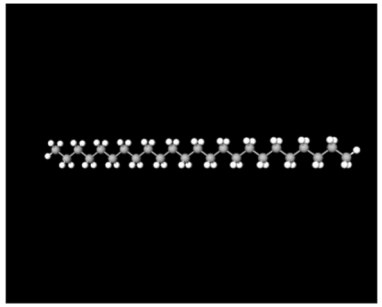
Sl. No	Compound name	Structure	Uses/biological activity	References
22	Tetracosane		Cytotoxic against cancerous cells[a], anti-diarrheal[b], anti-bacterial[c], anti-helmenthic, cardiotonic, laxative, anti-inflammatory, peptic ulcer treatment[d], anti-corrosive, anti-oxidant[e], anti-trichomonas[f]	Shaikh et al. (2012), Alqasim (2013), Thirunavukkarasu et al. (2016), Geeta et al. (2009), Dandekar et al. (2015), Naemi et al. (2014)

Table 5 Details of bioactive compounds in methanol extract of the epigeic earthworm, *Eisenia fetida*, through GC–MS

Peak No	R. Time	Compound name	Base m/z	Compound nature	Molecular formula	Molecular weight (g/mol)
1	7.111	3,7-Dimethyldecane	57.05	Alkane	$C_{12}H_{26}$	170.33
2	7.246	Nonane, 4,5-dimethyl-	57.00	Alkane	$C_{11}H_{24}$	156.30
3	11.582	Tetradecane	57.05	Alkane	$C_{14}H_{30}$	198.39
4	12.798	Dodecane, 2,6,11-trimethyl-	57.05	Alkane	$C_{15}H_{32}$	212.41
5	15.829	Quinoline, 1,2-dihydro-2,2,4-trimethyl-	158.10	Quinoline	$C_{12}H_{15}N$	173.25
6	17.027	Nonadecane	57.05	Alkane	$C_{19}H_{40}$	268.50
7	18.131	Eicosane	57.05	Alkane	$C_{20}H_{42}$	282.50
8	21.953	Octadecane	57.05	Alkane	$CH_3(CH_2)_{16}CH_3$	254.49
9	22.911	Hexadecane, 2,6,10,14-Tetramethyl-	57.00	Alkane	$C_{20}H_{42}$	282.54
10	26.390	Dotriacontane	71.10	Alkane	$CH_3(CH_2)_{30}CH_3$	450.86
11	27.340	Hexadecane, 2,6,11,15-tetramethyl-	57.05	Alkane	$C_{20}H_{42}$	282.54
12	29.822	Octathiocane	63.95	Sulfur allotrope	S_8	256.50
13	32.354	Docosane	57.05	Alkane	$C_{22}H_{46}$	310.60
14	33.789	Eicosane	71.05	Alkane	$C_{20}H_{42}$	282.50
15	34.949	Tridecanol, 2-ethyl-2-methyl-	57.05	Alcohol	$C_{14}H_{30}O$	214.38
16	37.615	Nonadecane	57.05	Alkane	$CH_3(CH_2)_{17}CH_3$	268.51
17	39.249	Docosane	71.10	Alkane	$C_{22}H_{46}$	310.60
18	39.882	Tetracosane	57.05	Alkane	$C_{24}H_{50}$	338.00
19	41.879	Pentacosane	57.05	Alkane	$C_{24}H_{50}$	338.66
20	43.687	Hexacosane	57.05	Alkane	$C_{26}H_{54}$	366.71
21	45.491	Celidoniol, deoxy-	57.05	Fatty alcohol	$C_{29}H_{60}O$	424.78

which is chemically ester in nature (PubChem). 2-Piperidinone which is chemically lactum in nature exhibits anti-microbial activity (Dawood et al., 2019). The acyclic alkane, hexane, 3,3-dimethyl-, has been used in the production of phytochemical compound effective in the removal of heavy metals (Ghorbani et al., 2022). The carboxylic acid, hydrocinnamic acid, exhibits anti-oxidant (Razzaghi-Asl et al., 2013), anti-inflammatory (Nagasaka et al., 2007) and anti-microbial activities (Taofiq et al., 2017). Cyclopentane, 1,1,3-trimethyl- is a alicyclic hydrocarbon and exhibits anti-cancer activity (Alaqeela et al., 2022). The anti-cancer, anti-microbial, anti-diabetic, anti-convulsant, anti-inflammatory, anti-viral and anti-tubercular activities are also reported in benzothiazole, 2-(2-hydroxyethylthio)-, which is a heterobicyclic compound (Ruhi & Nadeem, 2013).

Discussions

Many of these bioactive compounds obtained in the present study through GC–MS are commonly reported in various medicinal plants, which are pharmacologically important. Some of the bioactive compounds like hexadecane, tetradecane, eicosane, nonadecane, phytol and tetracosane are identified in the present study which are also identified in the leaf extract of *Waltheria indica* Linn. exhibiting similar pharmacological activities (Prabhanna & Jayaraj, 2017). Indra et al., (2018) identified several bioactive compounds in *Datura starmonium* of which docosane, 1,2-benzenedicarboxylic acid, nonadecane and eicosane are identified in the present study with similar biological activity. Tetratriaconitane, pentadecane and quinoline are also identified in the present work which are present in the fenugreek seed oil with similar biological activity reported by Sweeta et al. (2019). Delicia and Shyam (2008) reported the diverse bioactive compounds in the *Bombyx mori* gut, of which celidoniol deoxy, 2-bromotetradecane, tetracosane, pentadecane, nonadecane and eicosane were also noticed in the *Eisenia fetida* extracts of the present study exhibiting similar pharmacological activities. Many such GC–MS analyses of phytochemicals have been focused on plant extracts, and very few studies are available on animal tissue extracts. GC–MS analysis to investigate anthelminthic activity using *Corallocarpus epigaeus* extracts using four different organic solvents against *Pheretima postuma* earthworm was reported by Kalpesh and Priya (2020) where different

Table 6 Structure and bioactivity of the compounds in the methanol extract of the epigeic earthworm, *Eisenia fetida*, through GC–MS

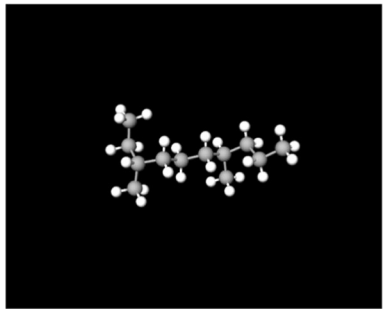
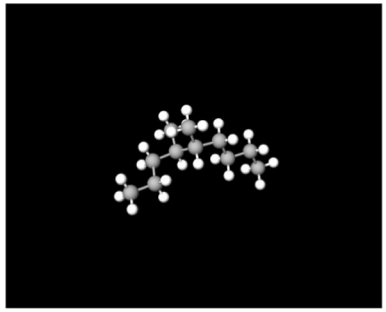
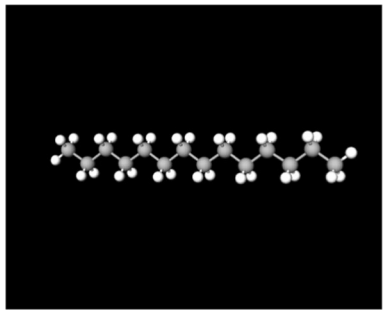
Sl. No	Compound name	Structure	Uses/biological activity	References
1	3,7-Dimethyldecane		Unreported	Unreported
2	Nonane, 4,5-dimethyl-		Unreported	Unreported
3	Tetradecane		Anti-microbial[a], cytotoxicity[b], anti-pyretic, anti-helmentic, tumor, bronchitis, asthma, tuberculosis, dyspepsia, constipation, anemia, throat diseases, elephantiasis, anti-diabetic, anti-inflammatory, anti-diarrheal[c]	Javidnia et al. (2008), Choo et al. (2001), Gurudeeban et al. (2010)

Table 6 (continued)

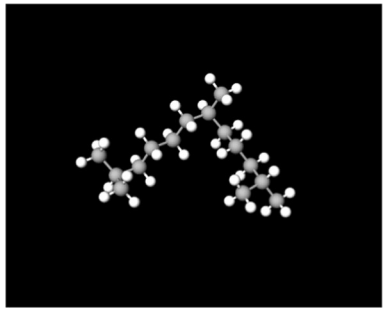
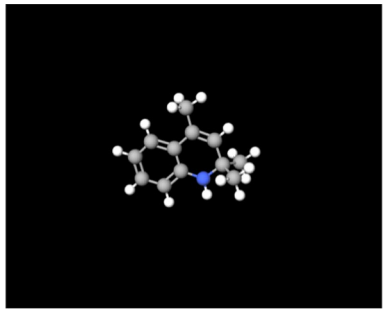
Sl. No	Compound name	Structure	Uses/biological activity	References
4	Dodecane, 2,6,11-trimethyl-		Unreported	Unreported
5	Quinoline, 1,2-dihydro-2,2,4-trimethyl-		Anti-oxidant, used in styrene–butadiene and nitrile–butadiene rubber and latex	Chemical book
6	Nonadecane		Anti-HIV, anti-oxidant, anti-bacterial[a], anti-microbial[b], cytotoxic effect, anti-microbial[c,b], anti-malarial[d], unini uses like weakness of the principal organs like heart, brain, liver, general weakness, hemoptysis, palpitation, conjunctivitis, earache, stomatitis[e]	Mahmoodreza et al. (2010), Javidnia et al. (2008), Akpuaka et al. (2013), Ombito et al. (2014), Prathapa et al. (2015)

Table 6 (continued)

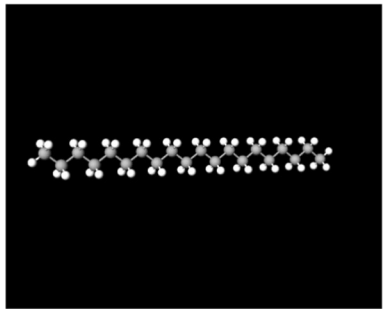
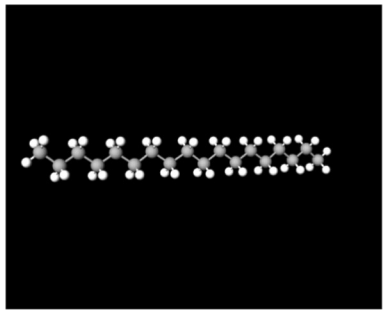
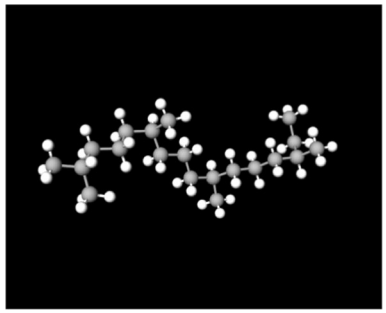
Sl. No	Compound name	Structure	Uses/biological activity	References
7	Eicosane		Anti-bacterial, anti-tumor, anti-fungal, cytotoxic	Hsouna et al. (2011)
8	Octadecane		Anti-oxidant, anti-inflammatory[a], cough, lung diseases, cold and fever detoxification[b], anti-corrosion agent[c], anti-sepsis[d]	Marzoqi et al. (2015), Zhao et al. (2017), Jyothirmayi et al. (2014), Sowmya et al. (2015)
9	Hexadecane, 2,6,10,14-tetramethyl-		Unreported	Unreported

Table 6 (continued)

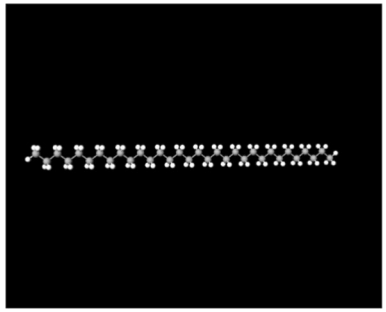
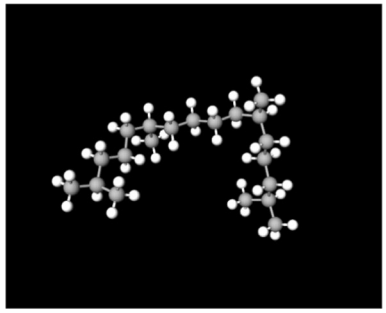
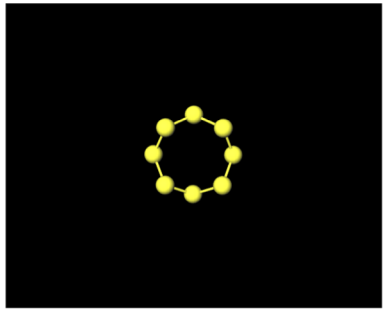
Sl. No	Compound name	Structure	Uses/biological activity	References
10	Dotriacontane		In development of organogels	Juan et al. (2009)
11	Hexadecane, 2,6,11,15-tetramethyl-		Unreported	Unreported
12	Octathiocane		Anti-oxidant, anti-cancer and anti-microbial agent	Smolecule

Table 6 (continued)

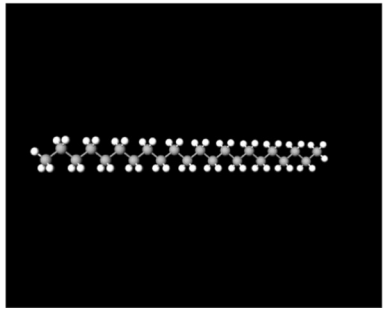
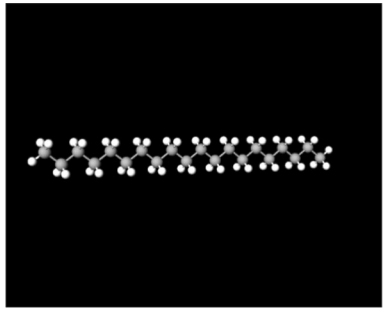
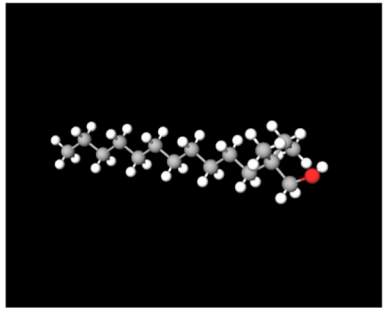
Sl. No	Compound name	Structure	Uses/biological activity	References
13	Docosane		Anti-bacterial and enhances host egg parasitization	Gumgumjee and Hajar (2015), Paul et al. (2002)
14	Eicosane		Anti-bacterial, anti-tumor, anti-fungal, cytotoxic	Hsouna et al. (2011)
15	Tridecanol, 2-ethyl-2-methyl-		Unreported	Unreported

Table 6 (continued)

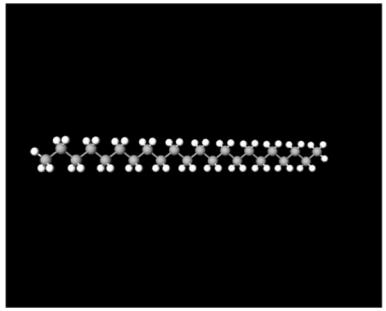
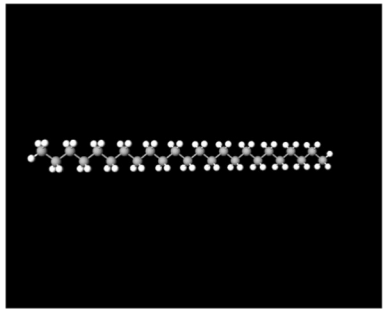
Sl. No	Compound name	Structure	Uses/biological activity	References
16	Nonadecane		Anti-HIV, anti-oxidant, anti-bacterial[a], anti-microbial[b], cytotoxic effect, anti-microbial[c,b], anti-malarial[d], unini uses like weakness of the principal organs like heart, brain, liver, general weakness, hemoptysis, palpitation, conjunctivitis, earache, stomatitis[e]	Mahmoodreza et al. (2010), Javidnia et al. (2008), Akpuaka et al. (2013), Ombito et al. (2014), Prathapa et al. (2015)
17	Docosane		Anti-bacterial[a] and enhances host egg parasitization[b]	Gumgumjee and Hajar (2015), Paul et al. (2002)
18	Tetracosane		Cytotoxic against cancerous cells[a], anti-diarrheal[b], anti-bacterial[c], anti-helmenthic, cardiotonic, laxative, anti-inflammatory, peptic ulcer treatment[d], anti-corrosive, anti-oxidant[e], anti-trichomonas[f]	Shaikh et al. (2012), Alqasim (2013), Thirunavukkarasu et al. (2016), Geeta et al. (2009), Dandekar et al. (2015), Naemi et al. (2014)

Table 6 (continued)

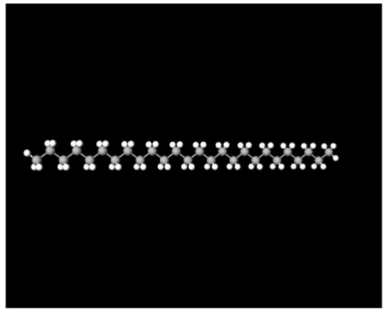
Sl. No	Compound name	Structure	Uses/biological activity	References
19	Pentacosane		Anti-bacterial	Mihailovi et al. (2011)
20	Hexacosane		Anti-microbial	Kawuri and Darmayasa (2019)
21	Celidoniol, deoxy-		Anti-bacterial[a], anti-inflammatory[b], chemical communication especially in *Anopheles stephensi* mosquito[c], pheromone of *Orgyia leucostigma*[d]	Köse et al. (2016), Zakariaa et al. (2014), Brei et al. (2004), Grant et al. (1987)

Table 7 Details of bioactive compounds in distilled water extract of the epigeic earthworm, *Eisenia fetida*, through GC–MS

Peak No	R. Time	Compound name	Base m/z	Compound nature	Molecular formula	Molecular weight (g/mol)
1	8.199	Undecane	57.00	Alkane	$C_{11}H_{24}$	156.30
2	10.218	2-Piperidinone	99.10	Lactum	C_5H_9NO	99.13
3	13.277	Undecane, 2,8-dimethyl-	57.10	Alkane	$C_{13}H_{28}$	184.36
4	14.579	Hexane, 3,3-dimethyl-	57.10	Acyclic alkane	C_8H_{18}	114.22
5	14.690	Hydrocinnamic acid	91.10	Carboxylic acid	$C_9H_{10}O_2$	150.17
6	16.480	1-Heptadecanol	57.10	Long chain fatty alcohol	$C_{17}H_{36}O$	256.46
7	17.715	(1-Benzyl-3-phenyl-prop-2-ynyl)-dimethyl-amine	158.10		$C_{18}H_{19}N$	249.3
8	18.961	Hexane, 2,4-dimethyl-	57.10	Alkane	C_8H_{18}	114.22
9	19.361	2-Tert-butyl-4-(1,1,3,3-tetramethylbutyl)phenol	191.20	Phenol	$C_{18}H_{30}O$	262.4
10	20.090	Hexane, 3,3-dimethyl-	71.10	Alkane	C_8H_{18}	114.22
11	21.488	2-Tridecene, 1-chloro-1,1,2-trifluoro-, (Z)-	56.10	alkene	$C_{13}H_{22}ClF_3$	270.13
12	23.951	Octane	57.10	Alkane	C_8H_{18}	114.22
13	24.907	Octane, 6-ethyl-2-methyl-	71.10	Alkane	$C_{11}H_{24}$	156.30
14	25.969	Cyclopentane, 1,1,3-trimethyl-	56.00	Cycloalkane	C_8H_{16}	112.21
15	28.795	Benzothiazole, 2-(2-hydroxyethylthio)-	167.00	Heterobicyclic compound	$C_9H_9NOS_2$	211.3
16	29.229	Undecane, 3-methyl-	71.15	Alkane	C12H26	170.33
17	31.108	Octathiocane	64.00	Thiocane heterocyclic series	S8	256.52
18	33.932	Heptadecyl acetate	83.15	Ester	C19H38O2	298.50

bioactive compounds in treating helminthiasis were identified. GC–MS-based approach to measure the response of earthworm to the exposure of carbofuran in the soil was reported by Mohana et al. (2013).

Thus, various chemical compounds obtained from the epigeic earthworm, *Eisenia fetida*, in different solvent extracts exhibit a wide range of medicinal/biological properties, which can be life savior and can be used in preparation of new drugs to treat a wide variety of diseases in pharmacological industries.

Conclusion

GC–MS analysis of the powdered extracts of the epigeic earthworm, *Eisenia fetida*, in four different solvent extracts revealed the presence of various biologically active compounds, which are having important medicinal value. In total, 78 compounds were obtained from four different solvent extracts, out of which 17, 22, 21 and 18 bioactive compounds were analyzed in chloroform, ethyl acetate, methanol and distilled water, respectively.

Maximum compounds were obtained from ethyl acetate (22) followed by methanol (21), distilled water (18) and chloroform (17) extracts. As per the literature, the compounds have shown anti-fungal, anti-bacterial, anti-cancer, anti-pyretic, anti-helminthic, anti-inflammatory, anti-viral, anti-allergic, anti-HIV, anti-malarial, anti-tuberculosis, anti-diabetic, anti-convulsant, anti-chistomal, anti-nociceptive, anti-oxidant properties.

Hence, based on the results of the present study, it can be concluded that the epigeic earthworm, *Eisenia fetida*, has a potent source of bioactive compounds that can be used in pharmacological activities. Further studies are also essential to isolate, characterize and purify particular active compounds responsible for particular therapeutic activities.

Online repositories/websites used

- National Institute Standard and Technology (NIST)
- PubChem
- CheBI
- MedChem Express
- Chemspider
- Smolecule
- Chemical Book
- Cymitquimic.com

Table 8 Structure and bioactivity of the compounds in the distilled water extract of the epigeic earthworm, *Eisenia fetida*, through GC–MS

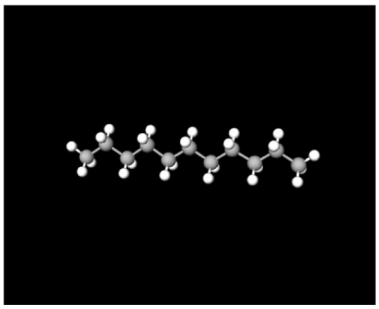
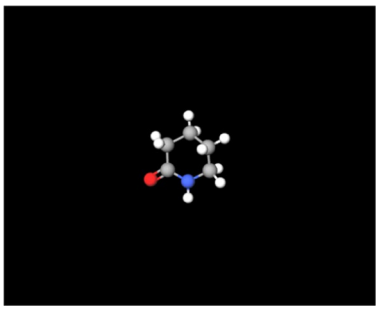
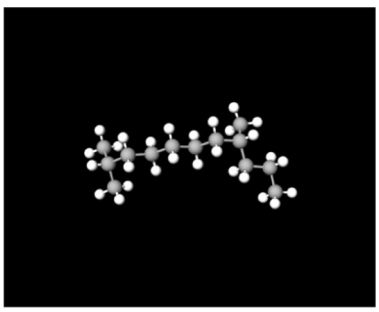
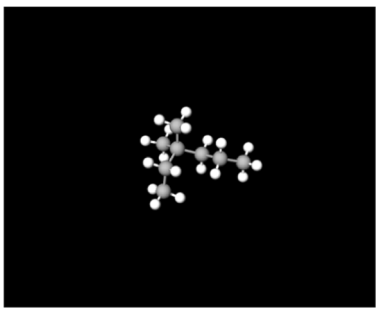
Sl. No	Compound name	Structure	Uses/biological activity	References
1	Undecane		Anti-allergic and anti-inflammatory	MCE
2	2-Piperidinone		Anti-microbial	Dawood et al. (2019)
3	Undecane, 2,8-dimethyl-		Anti-allergic and anti-inflammatory	Dabin et al. (2020)
4	Hexane, 3,3-dimethyl-		Can be used in production of phytochemical compound effective in removal of heavy metals	Ghorbani et al. (2022)

Table 8 (continued)

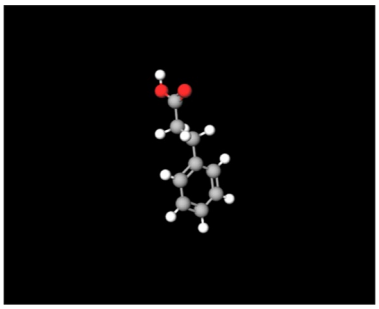
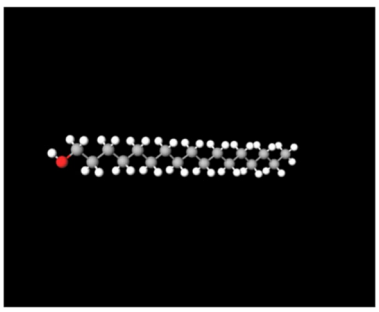
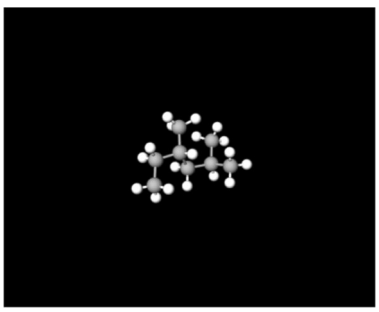
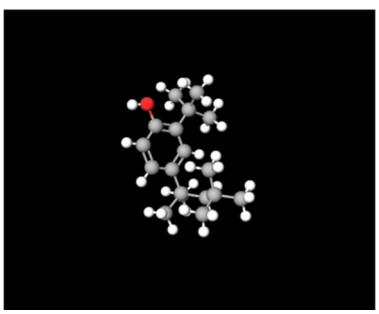
Sl. No	Compound name	Structure	Uses/biological activity	References
5	Hydrocinnamic acid		Anti-oxidant[a], anti-inflammatory[b] and anti-microbial[c]	Razzaghi-Asl et al. (2013), Nagasaka et al. (2007), Taofiq et al. (2017)
6	1-Heptadecanol		Anti-bacterial	MCE
7	(1-Benzyl-3-phenyl-prop-2-ynyl)-dimethyl-amine		Unreported	Unreported
8	Hexane, 2,4-dimethyl-		Human metabolite	ChEBI
9	2-Tert-butyl-4-(1,1,3,3-tetramethylbutyl)phenol		Unreported	Unreported

Table 8 (continued)

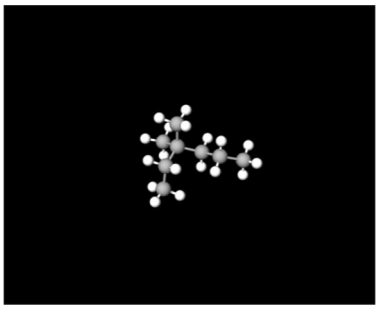
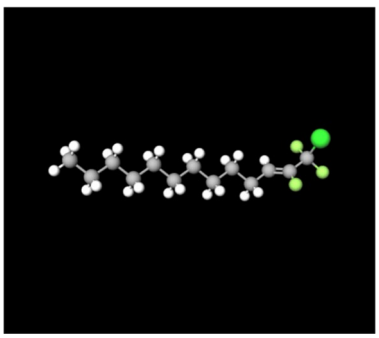
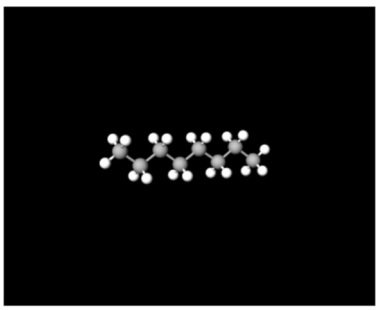
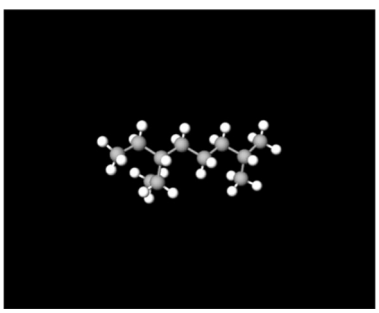
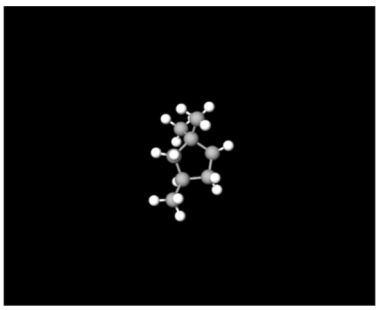
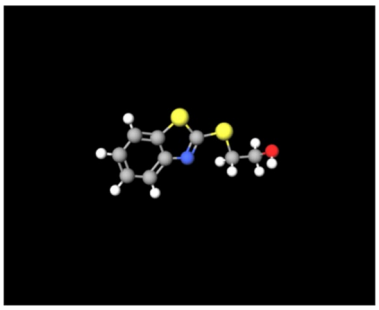
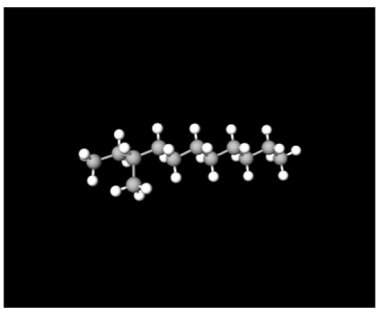
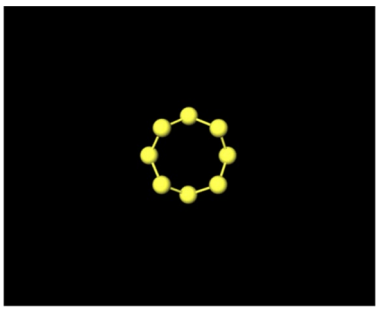
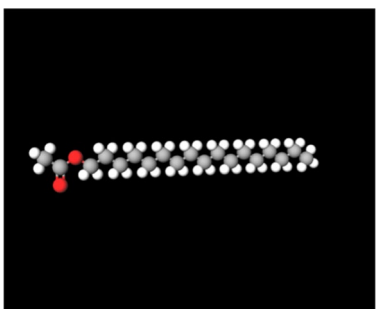
Sl. No	Compound name	Structure	Uses/biological activity	References
10	Hexane, 3,3-dimethyl-		Metabolite	Chemspider
11	2-Tridecene, 1-chloro-1,1,2-trifluoro-, (Z)-		Unreported	Unreported
12	Octane		Plays role in xenobiotics	NIH PubChem
13	Octane, 6-ethyl-2-methyl-		Unreported	Unreported
14	Cyclopentane, 1,1,3-trimethyl-		Anti-cancer	Alaqeela et al. (2022)

Table 8 (continued)

Sl. No	Compound name	Structure	Uses/biological activity	References
15	Benzothiazole, 2-(2-hydroxyethylthio)-		Anti-cancer, anti-microbial, anti-diabetic, anti-convulsant, anti-inflammatory, anti-viral and anti-tubercular	Ruhi and Nadeem (2013)
16	Undecane, 3-methyl-		Used as mild sex attractant for various types of moths and cockroaches and as an alert signal for variety of ants	Holldobler and Wilson (1990)
17	Octathiocane		Anti-bacterial, anti-fungal, anti-inflammatory and anti-oxidant	Smolecule
18	Heptadecyl acetate		Anti-inflammatory and pheromone activity	PubChem

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Author contributions
AS carried out the experiment, analyzed the data and prepared the manuscript, and PMB corrected and finalized the manuscript.

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All data analyzed during the present study are included in this article. Please contact authors for data.

Declarations

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Consent of publication
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Competing interest
There is no potential conflict of interest.

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