Original article

The GC-MS analysis and cytotoxic effect of *Ricinus communis* L. extracts using brine shrimps lethality test

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Abstract

Medicinal plants have always played a vital role in human life. The aims of this study are to screen cytotoxic effect of *Ricinuc communis* L. seeds and leaves extracts using brine shrimps lethality test, and to perform a composition analysis using gas chromatography mass spectrometery (GC-MS). Soxhlet extraction of *Ricinuc communis* L. seeds and leaves was carried out using petroleum ether chloroform and methanol. Methanol extract of leaves has been subjected to fractionation using column chromatography and the first eluded fraction was also subjected to lethality test and GC-MS analysis. The results show that the *Ricinus communis* L. seeds are more toxic than the leaves that is demonstrated with lowest lethal dose (LD50) of petroleum ether extract of seeds (LD50 is 100 µg \ ml). The leaves chloroform extract is more toxic (LD50 is 206.608 µg\ml) than the methanol (LD50 is 341.861 µg\ml) and petroleum ether (LD50 is 397.163 µg\ml). The column chromatography methanol fraction had weak toxicity (LD50 is 634.682 µg\ml). GC-MS of *Ricinus communis* L. leaves methanol extract shows that the leaves contain alkaloids, hydrocarbons, alcohols, esters, and fatty acids, the most predominant compounds are n-Hexadecanoic acid (palmitic acid) with the highest peak area (peak area is 22.02%) followed by (1,2-dihydro-4-methoxy-1-methyl-2-oxo,3-Pyridinecarbonitrile) (peak area is 17.41%) and gamonelic acid(Gamma-linolenic acid) (peak area is10%).The GC-MS analysis of column chromatography of methanolic fraction shows the predominance of pentadecanoic acid 14 methy, methyl ester (peak area is 30.68%), methyl linolenate (peak area is 24.34%) and hexadecanoic acid (peak area is 21.45%). The GC-MS results of chloroform extract of *Ricinus communis* L. seeds shows that ricinoleic acid is predominant in the extract (peak area is 20.8%, followed by 9-Tricosene (peak area is 8.77%).

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Introduction

Natural plants are considered primary source of chemical compounds which have medicinal effect. From that point of view, extraction, isolation and characterization of these compounds could lead to discovery of new drugs. Among these medicinal plants. The species *Ricinus communis Linn* belongs to family *Euphorbiaceae*, monotypic genus, *Ricinus*, and subtribe, *Ricininae.* This plant is popularly known as castor plant. Its indigenous plant of Africa.

Castor oil beans have wide range of traditional uses including purgative, wound and scarf healer, expel worm and parasites from intestine, hair growth, expectorant, tonic, organic nutrient source particularly for agricultural purposes as it

contains 10% proteins, 6.6% nitrogen, insecticidal, also its used to treat dermatitis, ringworm and wart. The leaves have also been recommended in the form of decoction or poultice and as an application to breasts of women to increase the secretion of milk (Bentley *et al*., 2007).

The *Ricinus communi L.* or castor plant has high medicinal value such as anti-oxidant, antihistamic, antinociceptive, antiasthmatic, antiulcer, immunemodulatory, antidiabetic, hepatoprotective, antifertility, anti-inflammatory, antimicrobial, central nervous system stimulant, lipolytic, wound healing, insecticidal and larvicidal and many other medicinal properties.(Jitendra and Ashsh, 2012).

Lord, *et al.*, (2003) reported that *Ricinus communis L.* seeds extract shows cytotoxic effect due to the presence of toxic material called ricin which mainly inhibits the synthesis of protein in cells and cause cell death; therapeutically it can be used to target cancer cells.

Also Darmanin, *et al*., (2009) reported that the leaves have another range of cytotoxic phytochemicals which induces apoptosis via translocation of phosphatidyl serine to the external surface of cell membrane and loss the mitochondrial potential. These compounds include three monoterpenoids, camphor, 1,8-cineole, α-pinene and sesquiterpenoid: β-caryophyllene.

Alkaloid ricinine (3-cyano-4-methoxy-Nmethyl-2-pyridone) is one of the important phytochemicals which obtained from castor plant that belongs to the alkaloid piperidine group. Its present in all parts of the plant but especially in young plants and its only cyano-subsitiuted pyridine occurred naturally (Waller *et al.,* 1961). Ricinine intake can cause vomiting and other toxic reaction such as liver and kidney damage, convulsions and hypertension (Peng, *et al*., 2014).

This study is intended to screen the cytotoxic effect of *Ricinuc communis L.* leaves and seeds extracts using the method of Brine shrimps Lethality Test and calculation of LD_{50} using *Probit* analysis program also composition analysis using GC-MS analysis of *Ricinus communis L.* extracts.

R.communis L. leaves and seeds samples were collected and identified in herbarium, Soxhlet extraction of leaves was carried out using petroleum ether, chloroform and methanol. Methanol extract was fractionated using column chromatography as it has high antioxidant potential and the first eluded column fraction was also involved in the study as it has the highest antioxidant activity among all column fractions, the seeds extracted with only petroleum ether using Soxhlet apparatus.

MATERIALS AND METHODS

Plants materials

Ricinus communis L. leaves and seeds samples were collected from their origin sources identified and authenticated in the department of Botany– Alneelain University. Specimens were deposited at the herbarium Alneelain University.

Chemicals and Reagents

Chemicals

Chloroform, Methanol, Petroleum ether, shrimps eggs, sea water.

Apparatus

Sensitive balance, Soxhlet extraction apparatus, mortar and pistle, porsaline Bitry dishes, Pasteur pipette, Vials, Incubator,

Collection of samples

The leaves and seeds of *Ricinus communis L*. were collected from the farms of faculty of agriculture university of Khartoum, *Bahri* area. The taxonomy and identification of the plant carried out at the department of botany, faculty of science, *AlNeelain University*. The leaves were washed with water and dried under the room conditions, the seeds grinded with mortar and pestle.

Soxhlet extraction method

Twenty (20) g of powdered leave, fifteen (15g) paste of seeds were accurately weighed and were separately extracted in Soxhlet apparatus with 200ml petroleum ether, chloroform and methanol respectively. The temperature of the heater adjusted according to the boiling point of each solvent, the solvents then evaporated under room temperature then dry extracts were obtained.

Toxicity test (Brine Shrimp Lethality Test)

Artemiasalina (shrimp eggs) were placed in natural sea water, and eggs hatched within 48 hrs, providing a large number of larvae (nauplii). The tested sample (20 mg) was dissolved in 2 ml of solvents. From this solution 5, 50 and 500 µl were transferred to vials (triplicate for each concentration), forming

concentrations of 10, 100 and 1000 µg/ml respectively. The solvent was allowed to evaporate overnight. Volume was made up to 5 ml with seawater. 10 larvae were placed in each vial using a Pasteur pipette. Vials were incubated at 25–27°C for 24 hrs under illumination. Etoposide (7.4625 µg/ml) was used as positive control, and number of survived larvae were counted. Data will be analyzed by Finney Probit Analysis computer program to determine LC_{50} values with 95% confidence intervals (McLaughlin. 1991).

Determination of LD_{50}

Finney Probit Analysis program was used to calculate LD_{50}.

Gas chromatography- mass spectrometry analysis

The qualitative and quantitative analysis of the sample were carried out by using GM/MS technique model (GC/MS-QP2010-Ultra) from Japan's *'Simadzu* Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm).The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60°C with rate 10°C /min to 300°C as final temperature degree with 3 minutes hold time , the injection port temperature was 300°C, the ion source temperature was 200°C and the interface temperature was 250°C.The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 26 minutes .Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library ,the National Institute of Standards and Technology (NIST). Results were recorded

RESULTS AND DISCCUSION

Extraction percentage

The extraction of *Ricinus communis L.* was performed successively using different solvents (petroleum ether, chloroform and methanol respectively) using Soxhlet apparatus and maceration with methanol.

Results in table (1) and table (2) show the extraction percentage of *Ricinus communis L.* seeds and leaves respectively.

Table (1) summarizes yield percentages of *Ricinus communis L.* seeds using different solvents.

Solvent	Weight of seeds (g)	Weight of extract (g)	Yield %	Consistency
P. ether	80.7195	35.001	43.36	Yellow oil
Chloroform	80.7195	10.225	12.66	Dark yellow
Methanol	80.7195	7.1860	8.90	Brown extract

Petroleum ether solvent has extracted castor oil from *Ricinus communis L.* seeds about 43.36% which is the highest yield compared with chloroform and methanol, the yield percentage of the present study was in accordance with (Jibrin *et al*. 2015) which carried out study on optimization percentage of castor oil yield

extracted from castor beans using response surface methodology and Box-Behnkin design the maximum yield of oil extracted was 50.9% (hexane), 49.0 %(petroleum ether).

Table (2) shows extraction percentages of *Riciuns communis L.* leaves using different solvents

Solvent	Weight of leaves (g)	Weight of extract (g)	Yield %	Consistency
P. ether	25.0028	1.223	4.89	Green paste
Chloroform	25.0028	1.0188	4.07	Green paste
Methanol	25.0028	2.9034	11.31	Green paste

In *R. communis L.* leaves, methanol has given highest yield (11.31%) compare to petroleum ether and chloroform. The present results is in accordance of a study carried by (Jennifer, *et al*, 2017), the study showed that for 100 g each in the different solvent the percentage yields were determined and were found to be 5.2, 6.0, 6.8, 7.2 and 8.3% for ethanol, water, petroleum ether, ethyl acetate and methanol respectively, thus methanol has the highest yield (8.3%).

Toxicity test (Brine Shrimp Lethality Test)

Toxicity screening for *R.communis L.* leaves and seeds extracts was studied using brine shrimp lethality test and calculation of LD_{50} was carried out using *Probit* analysis program.

Table (3) shows the results of brine shrimp lethality test for petroleum ether, chloroform and methanol extracts of *R. communis L.* leaves, petroleum ether extract for *R.communis L.* seeds and column chromatography fraction of *R.communis L.* leaf methanol extract

C (µg\ml) / Extract		10	100	1000	Extract C (µg\ml)
P. ether extract of *R.communis L.* leaves	Survive	7.00 ±1.50	7.7 ±0.88	3.3 ±0.7	6±0.87
	Dead	3.00 ±1.50	2.3 ±0.88	6.7 ±0.7	4±0.87
Chloroform extract of *R.communis L.*leaves	Survive	8.00 ±1.5	7.0 ±1.70	0.00	5±1.4
	Dead	2.00 ±1.5	3.0 ±1.70	10.00	5±1.4
Methanol extract of *R.communis L.* leaves	Survive	7.7 ±0.88	6.00 ±1.00	0.00	4.3±1.2
	Dead	2.3 ±0.88	4.00 ±1.00	10.00	5.7±1.2
P.ether extract of *R.communis L.* seeds	Survive	6.3 ±1.76	5.00 ±1.00	0.00	3.8±1.1
	Dead	3.7 ±1.76	5.00 ±1.00	10.00	2.6±1.1
Column chromatography fraction of *R. communis L* leaves	Survive	8.3 ±0.88	6.7 ±0.33	0.00	5±1.3
	Dead	1.7 ±0.88	3.3 ±0.33	10.00	5±1.3

Table (4) shows the calculation of LD_{50}

Extract	Mortality%			LD_{50} (µg\ml)
	10	100	1000	
P.ether extract of *R.communis L.* leaves	30.0	23.3	66.6	397.163
Chloroform extract of *R.communis L.* Leaves	20.0	30.0	100.0	206.608
Methanol extract of *R.communis L.* Leaves	23.3	40.0	100.0	341.861
P.ether extract of *R.communis L.* Seeds	36.6	50.0	100.0	100.000
C.chromatography fraction of *R.communis L.* Leaves	16.6	33.3	100.0	634.682

Keys; C.: column

Table (5) shows the range of LD_{50} and the toxicity (Mayer, *et al.* 1982)

Toxicity	LD_{50} (µg\ml)
Non- toxic	>1000
Weak	500-1000
Moderate	100-500
Strong	20-100
Very active	<20

For the toxicity of *R.communis L.* seeds, only petroleum ether extract was tested because it has given the highest yield percentage among the seed extracts.

The results compare the toxicity of *R.coummunis L.* leaves and seeds, the results show that the seeds petroleum ether extract which resemble around 80% (castor oil) has higher toxicity with LD_{50} 100µg\ml (strong), and that's due to presence of a toxic compounds called ricinoleic acid and ricin which cause the inhibition of protein synthesis (Lord, *et al*, 2003).

Oppositely the petroleum ether extract of the leaves is the least toxic (moderate) among leaves extracts with LD_{50} 397.163µg\ml, chloroform leaves extract is more toxic than the petroleum ether of leaves with LD_{50} 206.608 and that's could be due to the presence of a toxic compound ricinine.

The column chromatography fraction of *R.communis L.* Leaves which is separated from methanol leaves extract has weak toxicity with LD_{50} 634.682µg\ml. While methanol extract of leaves has moderate toxicity LD_{50} 341.861µg\ml) and that could be due to the higher concentration of the compound ricinine (1,2-dihydro-4-methoxy-1-methyl-2-oxo-3-Pyridinecarbonitrile) peak area is 17.1% present in methanol leaves extract AS GC-MS shows.

Similar study carryout out by (Mukram, *et al*. 2000) which tested the toxicity of different Malaysian medicinal plants using brine shrimp lethality test, among these plants *Ricinus communis L.*, the results showed that methanol (90%) extract of *Ricinus communis L.* leaves has moderate toxicity with LC_{50} 942 µg\ml.

GC-MS of *Ricinus communis L.* leaves extract shows that the leaves contains alkaloids, hydrocarbons, alcohols, esters, the most predominant compounds are n-Hexadecanoic acid (palmitic acid) with the highest peak area 22.02%,(1,2-dihydro-4-methoxy-1-methyl-2-oxo-3-Pyridinecarbonitrile,) with peak area of 17.41% (this compound is expected to be

ricinine as MS results shows) and gamonelic acid (Gamma-linolenic acid) with 10%.

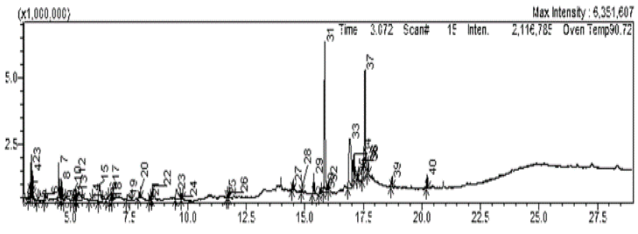


Figure (1) GC-MS Chromatogram of *R.communis L.* leaves methanol extract.

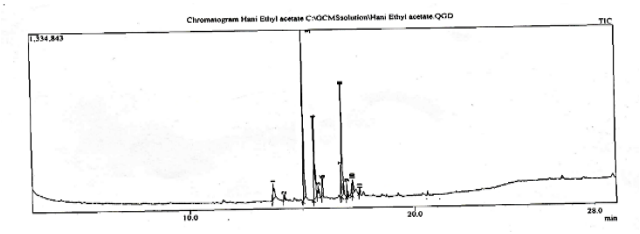


Figure (2) shows the chromatogram of C.C fraction of *R. communis L.* leaves methanol extract.

The compounds separated seem to have close range of polarities which makes the separation in GC-MS column rather difficult, and that's why they have close range of retention times (13.678 to 17.547 minutes).

Appearance of acids and their esters as in a case of linolenic acid and methyl linolenate, linolelaidic acid methyl ester, n-Hexadecanoic acid and pentadecanoic acid 14 methyl, methylester also appearance of long chain alcohols.

GC-MS of chloroform extract of *Ricinus communis L.* seeds:

GC-MS analysis of chloroform extract of *Ricinus communis L.* seeds has been carried out.

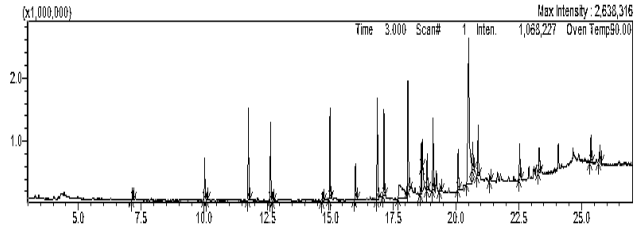


Figure (3) shows GC-MS chromatogram of chloroform *R.communis.L* seeds.

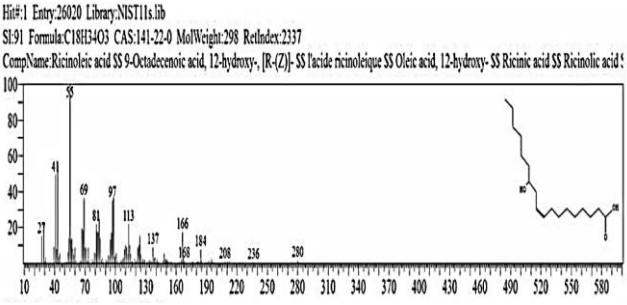


Figure (4) shows molecular weight and structure of ricinoleic acid obtained from GC-MS

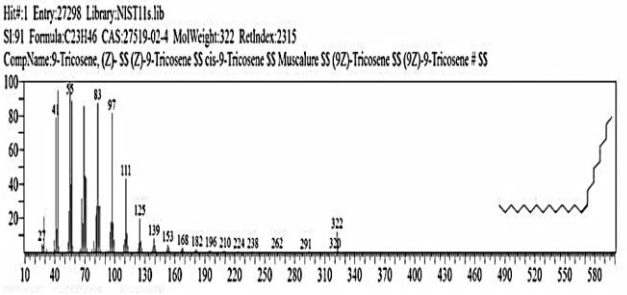


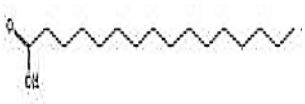
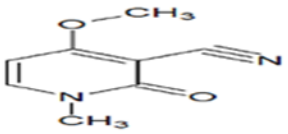
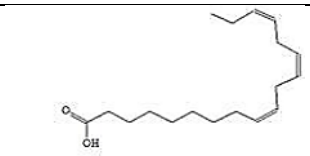
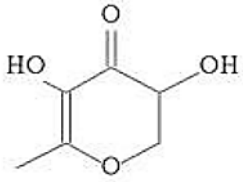
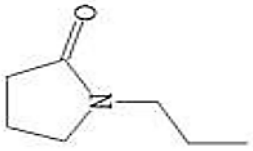
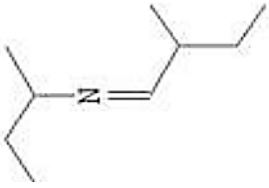
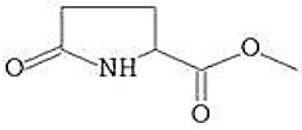
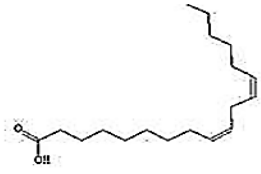
Figure (5) shows molecular weight and structure of Tricosene-Z

CONCLUSION

Extraction yield of *Ricinus communis L.* seeds using petroleum ether is 43.36%, while methanol extract of *Ricinus coomunis L.* leaves is (11.31%).

Ricinus communis L. seed has strong toxicity and more toxic than the leaves that is demonstrated with lower lethal dose (LD_{50}) of seeds petroleum ether extract (LD_{50} is 100 µg \ ml). For the leaves extracts, chloroform extract is more toxic (LD_{50} is 206.608 µg\ml) than the methanol (LD_{50} is 341.861 µg\ml) and petroleum ether (LD_{50} is 397.163 µg\ml). The column chromatography methanol fraction has weak toxicity (LD_{50} is 634.682 µg\ml). GC-MS analysis shows that n-Hexadecanoic acid (palmitic acid) with highest peak area (peak area is 22.02%) is predominant in *Ricinus communis L.* methanol leaves extract. While pentadecanoic acid 14 methy,methyl ester (peak area is 30.68%) is predominant in column fraction of methanol extract. And finally chloroform extract of *Ricinuc communis L.* seeds has predominant ricinoleic acid (peak area is 20.8%).

Table (6) shows top nine compounds; the name of separated compounds, retention time, peak area, molecular weight of MS and compound classification.

ID #	Name	Ret. Time	Area %	M.W	Molecular structure	Classification
1	n-Hexadecanoic acid	15.854	22.2	256		Saturated Fatty acid
2	1,2-dihydro-4-methoxy-1-methyl-2-oxo-3-Pyridinecarbonitrile,	16.919	17.1	164		Alkaloid
3	Gamolenic Acid	17.568	10.4	278		Unsaturated (fatty acid)
4	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one,	6.223	4.84	144		Heterocyclic compound
5	2-Pyrrolidinone	5.325	3.98	85		Alkaloid
6	2-methyl-N-(2-methylbutylidene)-1-Butanamine	3.319	3.32	155		Alkaloid
7	L-Proline, 5-oxo-, methyl ester	9.516	3.23	143		Alkaloid
8	9,12-Octadecadienoic acid (Z,Z)-	17.481	3.18	280		Unsaturated Fatty acid

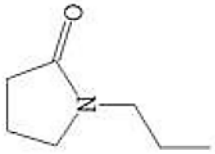
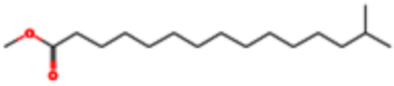
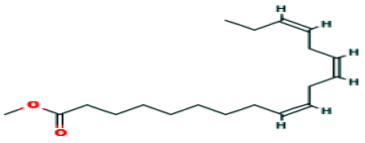
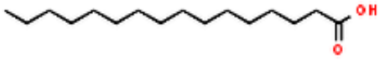
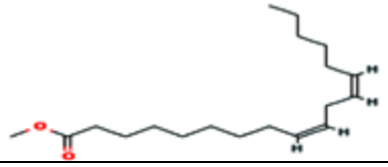
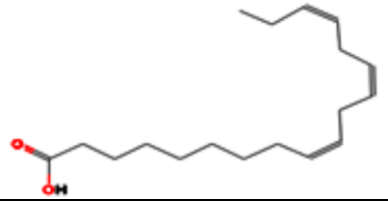
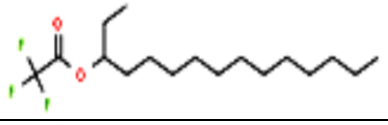
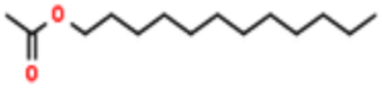
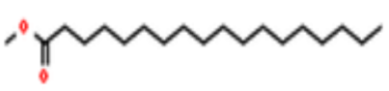
9	1-propyl-2-Pyrrolidinone	4.486	2.83	127		Alkaloid

Table 7 shows the results of compounds separated in GC-MS analysis of column chromatography fraction of *R.communis L.* leaves methanol extract and their retention time, structure and classification

NO	**R.time** Mintute	**Area %**	**Name**	**MS-Structure**	**Classification**	**M. W**
3	15.074	30.68	14 methy,methyl ester Pentadecanoic acid,		Saturated fatty acid methyl ester	270
8	16.789	24.34	9,12,15-Octadecatrienoic acid methyl ester (Methyl linoleate)		Unsaturated fatty acid methyl ester	292
4	15.534	21.45	n-Hexadecanoic acid		Saturated fatty acid	256
7	16.718	5.24	9,12- Octadecadienoic acid methyl ester (Linolelaidic acid methyl ester)		Unsaturated fatty acid methyl ester	294
10	17.235	4.46	9,12,15- Octadecatrienoic acid.(Z,Z,Z)-(linoleic acid)		Unsaturated fatty acid	278
1	13.678	4.36	3-Trifluoroacetoxy-pentadecane		Fluoro- substituted ester	324
6	15.881	3.18	Lauryl acetate		Fatty ester	228
9	16.987	2.91	Methyl stearate		Saturated fatty acid methyl ester	298

5	15.698	1.76	n-Tetracosanol-1		Saturated fatty alcohol	354
2	14.168	1.03	E-2-Tetradecen-1-ol		Unsaturated fatty alcohol	212
11	17.547	0.59	1-Eicosanol		Saturated fatty alcohol	298
		100 %				

Table 8 shows the results of GC-MS of chloroform extract of *Ricinus communis L.* seeds, 27 compounds have been separated. Ricinoleic acid is predominant in the extract with peak are 20.8%, followed by 9-Tricosene,(z)-(8.77%).

NO	R.Time	Area	Area%	Name	Classification
1	20.510	9666686	20.80	Ricinoleic acid	Unsat- fatty acid
2	18.098	4076993	8.77	9-Tricosene, (z)-	Unsat-alkene
3	16.881	3344011	7.19	n-Hexadecanoic acid	Sat-fatty acid
4	17.772	3086050	6.64	1,2-dihydro-4-meth, 3-Pyridinecarbonitrile	Alkaloid
5	11.758	2567909	5.52	2,4-bis(1,1-dimethylethyl) Phenol	Alcohol
6	17.145	2420807	5.21	9-Hexacosene	Unsat-alkene
7	15.002	2329910	5.01	1-Nonadecene	Unsat-alkene
8	19.096	2162832	4.65	Behenic alcohol	Alcohol
9	12.639	2000467	4.30	1-Heptadecene	Unsat-alkene
10	18.634	1618754	3.48	9,12-Octadecadienoic acid (z,z)-	Unsat- fatty acid
11	20.888	1473715	3.17	N-Tetracosanol-1	Alcohol
12	20.093	1334315	2.87	12- hydroxyl, methyl,9-Octadecenoic acid,	Unsat-ester
13	18.860	1234691	2.66	Octadecanoic acid	Sat-fatty acid
14	22.540	1208310	2.60	Octacosanol	Alcohol
15	16.011	1199806	2.58	1-Hexadecanol	Alcohol
16	20.672	1060439	2.28	12-hydroxy-9-Octadecenoic acid	Unsat-ester
17	10.020	1031332	2.22	3-Hexadecene,(z)-	Unsat-alkene
18	23.313	1010553	2.17	10-Undecenoyl chloride	Unsat- salt
19	25.371	839496	1.81	Decyl oleate	Ester
20	25.729	688937	1.48	17-Pentatriacontene	Unsat-alkene
21	18.666	625441	1.35	9-Octadecenoic acid,(E)-	Unsat-fatty acid
22	14.711	398426	0.86	Tridecanoic acid	Sat-fatty acid
23	7.153	268644	0.58	1-Dodecene	Unsat-alkene
24	19.385	262717	0.57	(4,4-(1-Methylethylidene) -bis-) Phenol	Alcohol
25	12.722	221764	0.48	Heptadecane	Sat-hyrocarbon
26	10.121	1866899	0.40	Tetradecane	Sat-hyrocarbon
27	21.351	159634	0.34	2,2-Methylenebis(1,1-dimethyl) Phenol	Alcohol
		46479538	100		

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CONFLECT OF INTEREST

The authors report no conflict of interes

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