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Phytochemical screening by using GC-MS and FTIR spectrum analysis of fixed oil from Sudanese *Ziziphus spina Christi* seeds

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The current trend of using natural products from the plants, either as pure constituents or as standardized extracts, provides numerous opportunities for novel drugs, precisely the wild edible plants. *Ziziphus spina Christi* plant is considered as one of the most important therapeutic and nutritional wild edible plants because of containing bioactive compounds. Therefore, this study was carried out to investigate the petroleum ether *Ziziphus spina Christi* seed oil via using two analytical methods: GC-MS, and FTIR spectrometer. The seed oil was extracted by using Soxhlet apparatus. The GC-MS analysis indicated the presence of seventeen fatty acids, eight of them are majors constituents, namely 7-Octadecenoic acid, methyl ester [28.11%], Hexadecanoic acid, methyl ester [19.63%], Methyl stearate [16.97%], Eicosanoic acid, methyl ester [10.76%], Docosanoic acid, methyl ester [8.60%], cis-11-Eicosenoic acid, methyl ester [4.79%], Tetracosanoic acid, methyl ester [2.62%], and Squalene [2.20%]. On the other hand, the FTIR spectrum showed the presence of alcohols, phenols, alkanes, alkenes, carbonyls and Carboxylic acids and aromatic compounds in the extracts with different peak types and correspondences. The results of GC-MS and FTIR analysis showed the availability of bioactive compounds in the plant extracts, which could be responsible of the source of pharmaceutical value of *Ziziphus spina Christi* plant.

KEYWORDS

Wild edible plants; *Ziziphus spina Christi*; seed fixed oil; nutritional; novel drugs; therapeutic.

Introduction

The *Ziziphus spina Christi* shrub or tree has been considered as the most important species of Genus Ziziphus, belonging to the family of Rhamnaceae, known as Sidder in Sudan and the Middle East, and in English Christ's thorn Jujube [1]. Morphologically, it is a spiny shrub with the height of 5-10 meters, and remains green throughout the year. Geographically point of view, it is spread throughout tropical and subtropical countries precisely in North Africa, Middle East, and North west India, and in Sudan it is found over large parts of the country [2]. Most parts of Ziziphus spina Christi plant are used in folk medicine [3], *Ziziphus spina Christi* root bark extract is used in many African regions as curative source digestive system disease [4], and the stem bark is used traditionally as an antidiarrheal medicine [5]. In addition, the stem bark also has shown significant cytotoxic to cervical and breast cancers [6]. Currently, several studies have reported that *Ziziphus spina Christi* aerial parts such as leaves, flowers, fruits, and seeds possess a various

nutritional and therapeutic lineament, which have been used in folk medicine due to anti-oxidants, and anti-microbial activities. Leaves, fruits and seeds have a potent source of numerous phytochemical compounds precisely polyphenol, flavonoids, Tannins, Alkaloids, Terpenoids and Saponins [7].

The leaves of *Ziziphus spina Christi* have been considered as a treatment for several diseases such ulcers, wounds, eye diseases, bronchitis, and skin diseases as an anti-inflammatory agent [8]. The Z. spina Christi flowers are significant for producing sider honey, which is used by local citizens as nutritional and therapeutic source [9]. *Ziziphus spina Christi* fruit is edible and wealthy with phytoconstituents [10]. Morphologically the fruit is semi globe shape, yellow or reddish in colour, with the sweaty pulp, and eaten as fresh. The fruits revealed bio-activity in promote the healing of wounds and also used for dysentery [11]. The seeds are anodyne and are taken sometimes with buttermilk to stoppage nausea, vomiting, and abdominal pains related to pregnancy [12]. Despite the existence of many reports that indicated the widespread of this wild edible plant, especially in some Sudanese regions like North Kordufan State, there is still the paucity of research in this regard. Further, it should be noted that there is no internationally known documentation for Sudanese collected plant germplasm, thus many plants lack identification and documentation [13]. Previously the screening of chemical constituents also known as (phytoconstituents) was conducted by high costly and often exhausting techniques. Gas Chromatography (GC) and liquid Chromatography (LC) techniques have been unified with specific detection schemes, so recently phytochemical screening has become more plain than before via using FTIR, GC-MS and other efficient techniques [14]. The aim of this study was to investigate the chemical constituents of the Fixed Oil obtained from Sudanese *Ziziphus spina Christi* Seeds and to apply GC-MS and Mid-IR techniques to analyse the phytoconstituents in the plant.

Materials and experiments

Materials

Chemicals and reagents

Petroleum Ether, and Potassium bromide were used. All the chemicals and solvents were commercial grade and used after further purification.

Plant material

The seeds *of Ziziphus spina christi* (L) were separated manually from the fruits pulp then washed, cleaned, dried by ambient temperature and crushed into a powder using blender and kept for further analysis.

Experiments

Oil extraction

100 g of the dried powder seed of *Z. spina Christi* was extracted with 500mL 0f petroleum ether by using Soxhlet extractor for (6h). The extracted oil was concentrated under pressure and stored in 4°C, for further analysis.

Process of making GC test samples

The methods and conditions of preparation were set up according to the literature [15] with slight modification 400 µL of *Z. spina Christi* oil was added into 10 mL centrifugal tubes to which 3 mL of potassium methoxide (0.5 M) was added. The mixture was heated on water bath at 60 °C for 20 min. After cooling, 3 mL of n-hexane and 2 mL of distilled water were added and mixed completely, then the extracts were prepared for GC analysis.

The GC-MS procedure

The sample of oil extracted was subjected to (GC-MS HP6890/5973, Hewlett-Packard Company, USA) for analysis. Injector temperature was 300 °C, and column

description was Rtx 5MS -Length 30 meter - Diameter 0.25 mm- thickness 0.25 mL. Temperature programming was maintained from 60 °C to 300 °C, pressure of 100 Kpa, total flow of 50 mL/min, column flow of 1.61 mL/min, and linear velocity of 46.3 cm/sec. The ion source ionizing energy was 70 eV; scan range was 50-650 amu; MS transfer line temperatures were 250 °C; interface temperature was 280 °C; acetone solvent time was 2.50 minute. The seeds oil is injected with a split less mode. The final confirmation of constituents was accomplished by subjecting their retention times and mass fragmentation patent with those obtainable of the library, the National Institute of Standards and Technology (NIST). Then the results were recorded.

Process of making FTIR oil sample

To prepare the liquid sample of *Ziziphus spina Christi* fruit oil for FTIR analysis, a good thin and transparent of pellet Potassium bromide was prepared, and a small drop of the oil was dropped via micropipette [16].

Results and discussion

GC-MS analysis

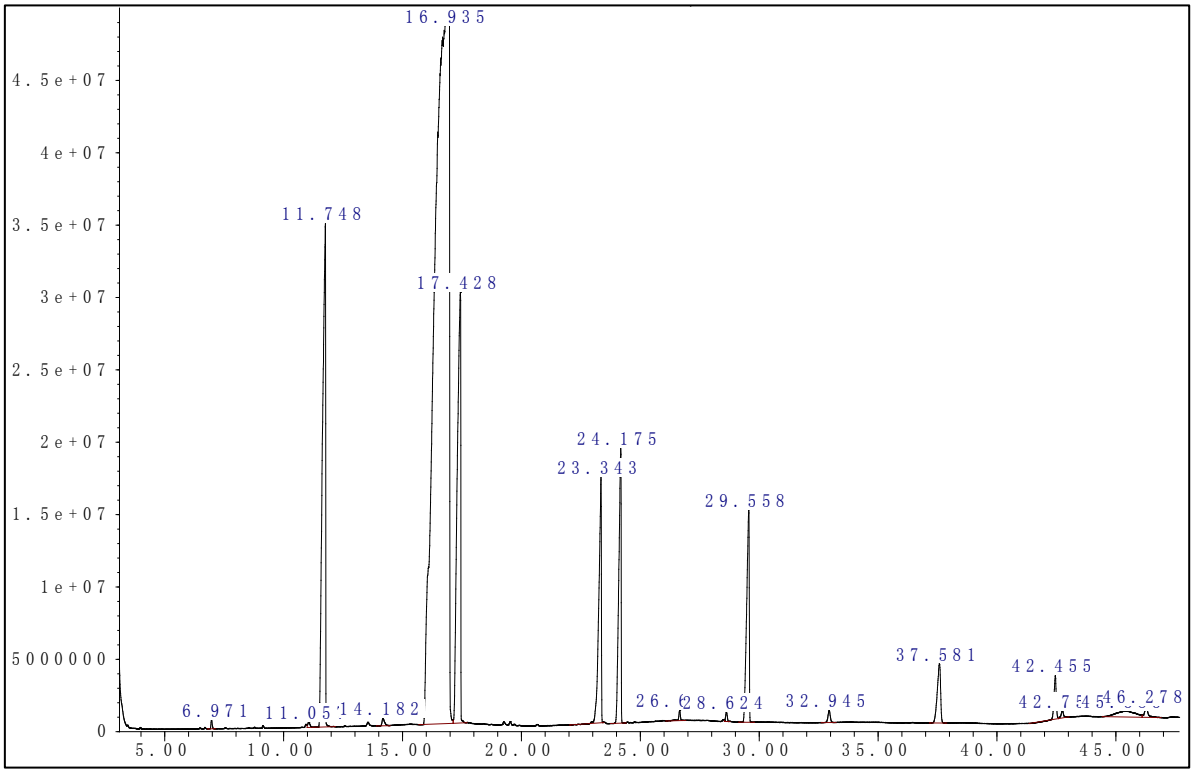

FIGURE 1 The GC Chromatogram of *Z. Spina Christi* Seed oil petroleum ether extract

TABLE 1 The chemical constituents of petroleum ether extract of ZSC Seed Oil

Peak	RT	Area	Area%	M.W	M.F	Identification Compounds
1	6.970	763651	0.429745	242	$C_{15}H_{30}O_2$	Methyl tetradecanoate
2	11.057	649323	0.365407	268	$C_{17}H_{32}O_2$	9-Hexadecenoic acid, methyl ester, (Z)-
3	11.749	34884428	19.63123	270	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
4	14.180	896311	0.5044	284	$C_{18}H_{36}O_2$	Hexadecanoic acid, 14-methyl-, methyl ester

5	16.936	49957712	28.11373	296	$C_{19}H_{36}O_2$	7-Octadecenoic acid, methyl ester
6	17.424	30167360	16.9767	298	$C_{19}H_{38}O_2$	Methyl stearate
7	23.344	8525969	4.797994	324	$C_{21}H_{40}O_2$	Cis-11-Eicosenoic acid, methyl ester
8	24.172	19131828	10.76645	326	$C_{21}H_{42}O_2$	Eicosanoic acid, methyl ester
9	26.657	1465575	0.824753	340	$C_{22}H_{44}O_2$	Heneicosanoic acid, methyl ester
10	28.626	1330517	0.748749	352	$C_{23}H_{44}O_2$	13-Docosenoic acid, methyl ester, (Z)-
11	29.556	15288997	8.603891	354	$C_{23}H_{46}O_2$	Docosanoic acid, methyl ester
12	32.943	1467448	0.825807	368	$C_{24}H_{48}O_2$	Tricosanoic acid, methyl ester
13	37.580	4656412	2.620398	382	$C_{25}H_{50}O_2$	Tetracosanoic acid, methyl ester
14	42.454	3921108	2.206606	410	$C_{30}H_{50}$	Squalene
15	42.753	1430507	0.805019	396	$C_{26}H_{52}O_2$	Pentacosanoic acid, methyl ester
16	45.380	1429511	0.804458	356	$C_{21}H_{40}O_4$	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester
17	46.276	1731971	0.974668	410	$C_{27}H_{54}O_2$	Hexacosanoic acid, methyl ester
		177698628	100			

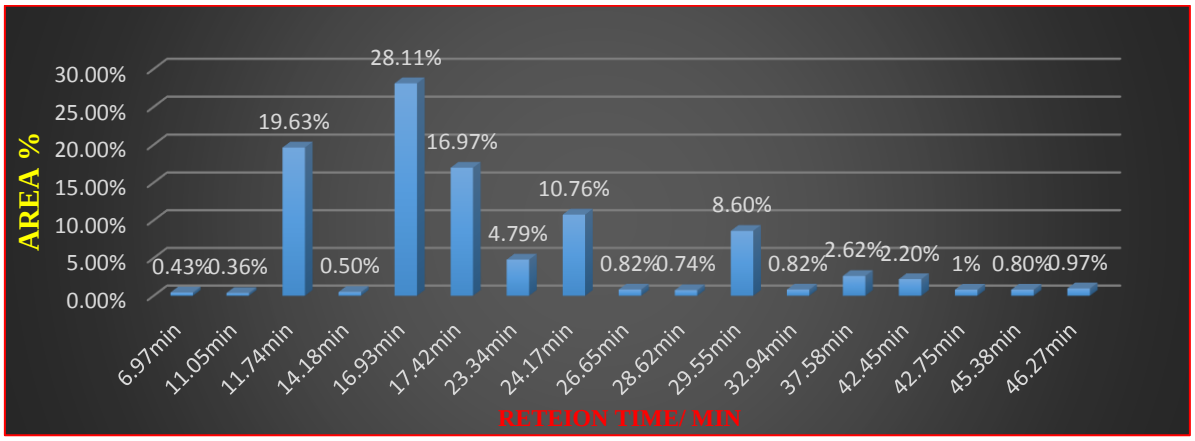

FIGURE 2 Identified compounds in the *Z. spina Christi* Petroleum ether Seed oil by using Area%, and retention time (RT)

The petroleum ether Seed oil extract of the *Z. spina Christi* was investigated by using GC-MS, and its corresponding chromatogram is presented in Figures 1 and 2, and Table 1. The identification of the compounds was confirmed *via* interpretation of their mass spectra fragment ions data and comparing their data with those available in the Wiley 9.0, NIST libraries, and with those published in literature. The GC chromatogram revealed about seventeen components, the major components were eight, namely compound **5** is 7-Octadecenoic acid, methyl ester appeared in the GC chromatogram at [16.936] minute, with MS ions m/z 296[M+], and molecular formula ($C_{19}H_{36}O_2$) produced the flowing fragment ion 69, 74, 83, 87, 97, 111, 180, 222, 264 and 55 base peaks as showed in Figure 3. The compound 7-Octadecenoic acid, methyl ester showed high peak area [28.11%], in line with [17], [18], classify like Fatty acid ester, and reported as antibacterial in [19].

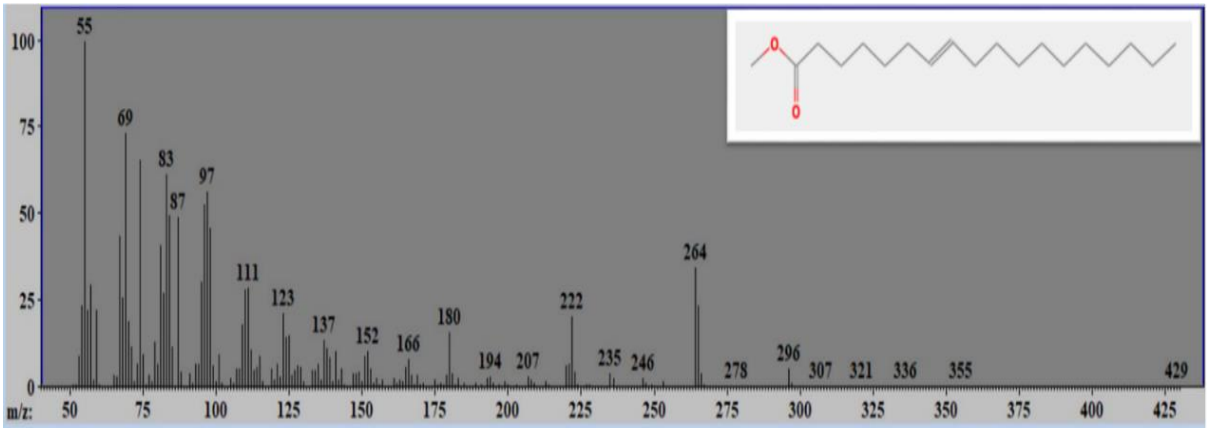

FIGURE 3 The Mass spectrum of 7-Octadecenoic acid, methyl ester

The peak at [11.749] minute with Area [19.63%] on the GC chromatogram generated fragment ion 87, 97, 129, 143, 227, 270, and 74 base peak and MS ions m/z270[M] + , with molecular formula ($C_{17}H_{34}O_2$), corresponding to Compound **3** Hexadecanoic acid, methyl ester, also known as Palmitic acid, methyl ester showed in Figure 4 and reported in [20, 21] . The compound has been considered as an anti-bacterial agent [22], also acts as anti-oxidant, reducing blood cholesterol, and anti-inflammatory agent [23].

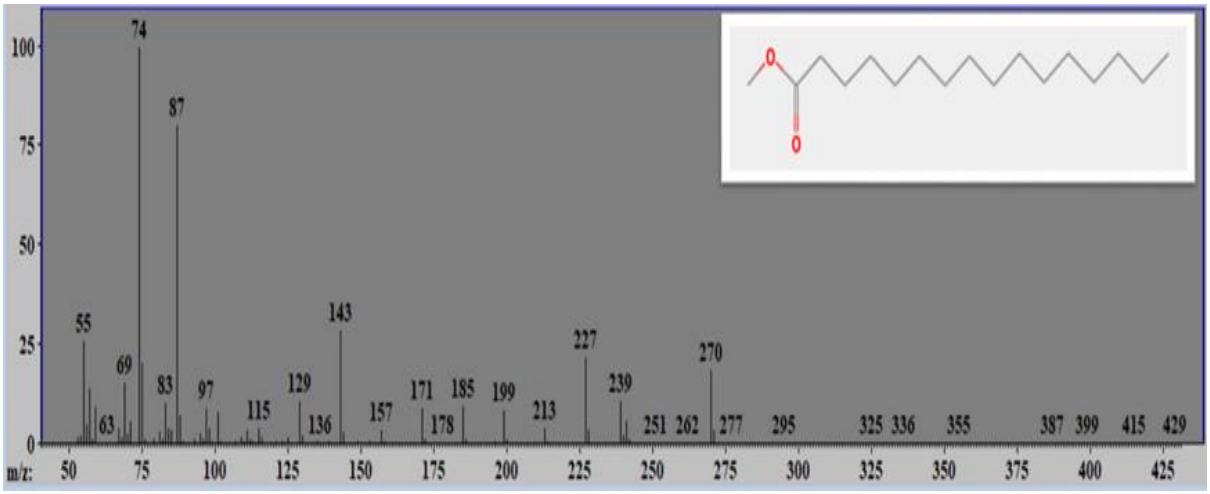

FIGURE 4 The Mass spectrum of Hexadecanoic acid, methyl ester

The peak area (16.97%) on the GC Chromatogram appeared at (17.424) minute, by producing molecular ion m/z 270[M] $^+$, fragment ion 87, 129, 143, 199, 255, 298 and 74 as base peaks, and molecular formula ($C_{17}H_{34}O_2$). It was corresponding to the compound **6** Methyl stearate as presented in Figure 5 and reported in [24]. The compound was reported as (*Meloidogyne incognita)* inhibitor [25].

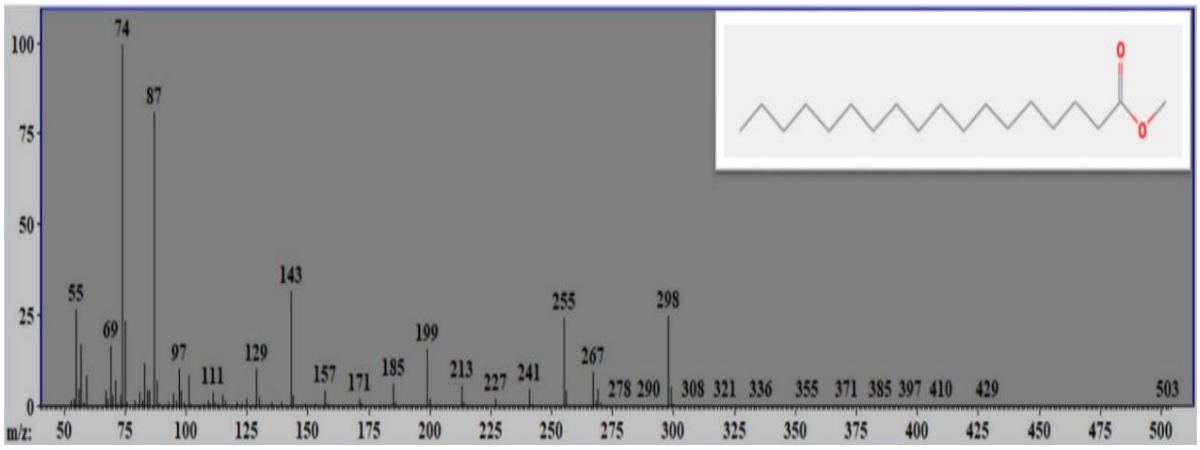

FIGURE 5 The Mass spectrum of Methyl stearate

Compound **8** appeared at (24.172) minute, by producing MS ions m/z 326[M] + with fragment ion 87, 129, 143, 227, 283, 326, and 74 as base peak as shown in Figure 6. The peak Area was 10.76%, corresponding to molecular formula ($C_{21}H_{42}O_2$). This compound was identified to be Eicosanoic acid, methyl ester [23].

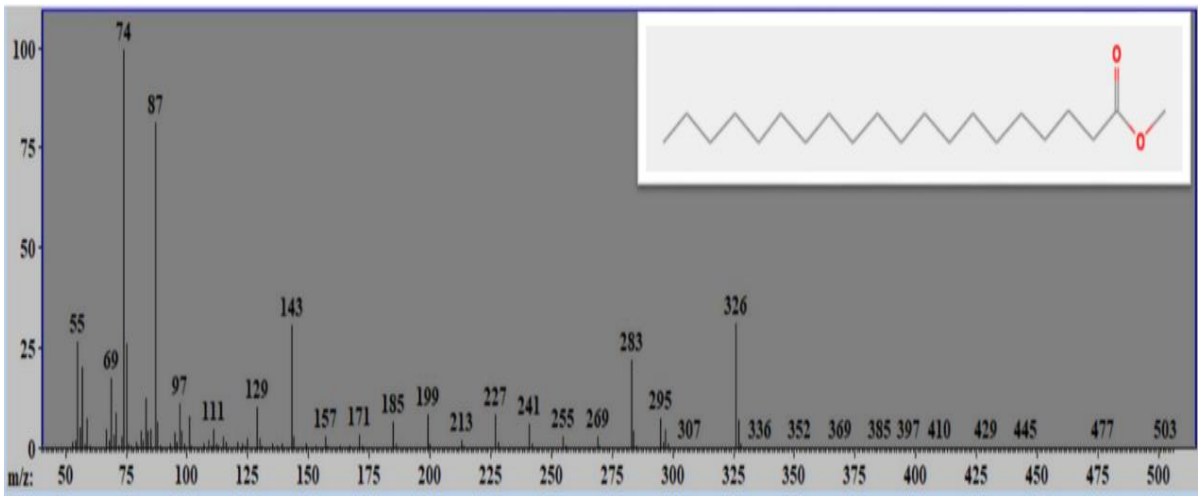

FIGURE 6 The mass spectrum of Eicosanoic acid, methyl ester

The fragment ion 87, 129, 143, 255, 311, 354, and 74 base peak are shown in Figure 7, which were appeared at [29.55] minute on the GC chromatogram with molecular ion m/z 354[M] +, Area [8.60%], and formula ($C_{23}H_{46}O_2$), corresponding to compound **11** Docosanoic acid, methyl ester, which was in agreement with the report in [26].

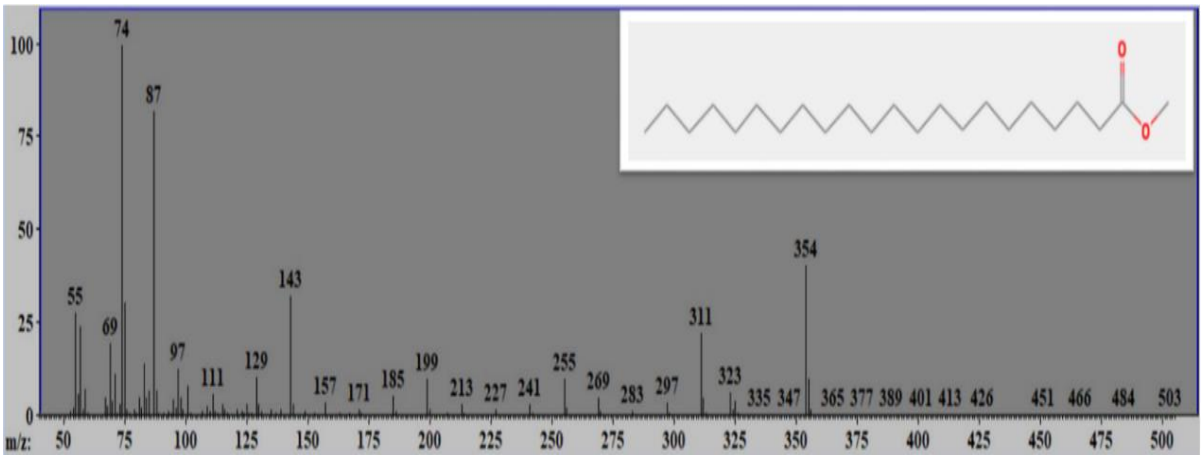

FIGURE 7 the Mass spectrum of Docosanoic acid, methyl ester

The Docosanoic acid and methyl ester were found therapeutic, and effective in diagnostic activities [26]. The GC chromatogram at (23.34) minute was observed peak area (4.79%), and fragment ion 69, 74, 83, 79, 111, 123, 208, 250, 292, base peak 55 as shown in Figure 8, which were matched with molecular formula ($C_{21}H_{40}O_2$), and MS ion m/z 324 [M]+, corresponding to the compound **7** Cis-11-Eicosenoic acid, methyl ester identified in[27].

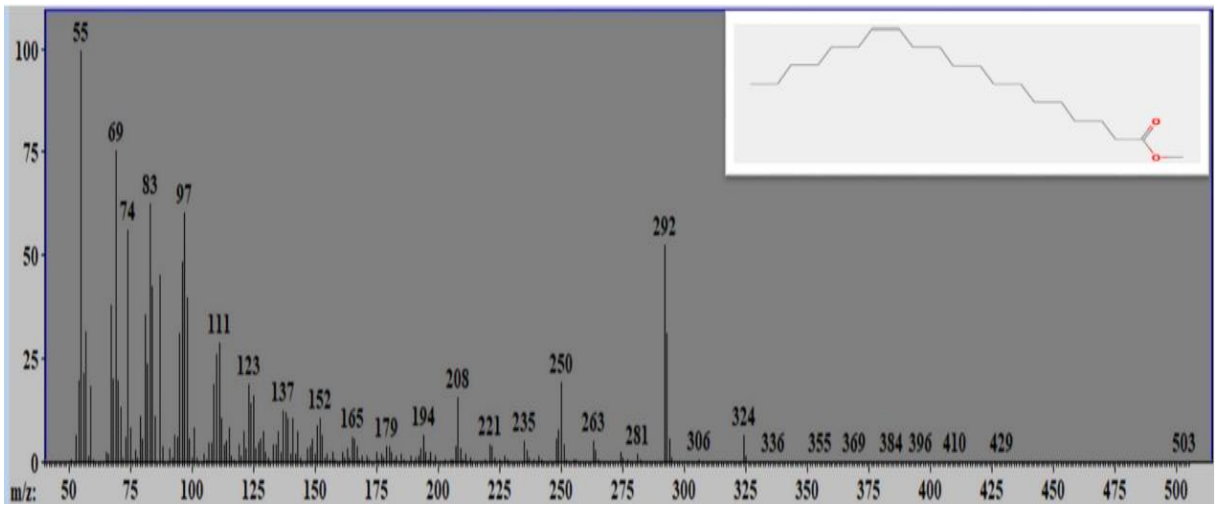

FIGURE 8 The Mass spectrum of Cis-11-Eicosenoic acid, methyl ester

There were two compounds showing a couple of different GC chromatogram peaks at (37.58, and 42.45) minutes, with molecular formula ($C_{25}H_{50}O_2$, and $C_{30}H_{50}$). They produced molecular ion m/z 382[M] $^+$, and m/z 410[M]$^+$, with base peaks 74, and 69, which agreed with compound **13** Tetracosanoic acid, methyl ester, and compound **14** Squalene, respectively. The fragment ion of the two compounds is shown in Figures 9 and 10.

The tetracosanoic acid, methyl ester, and squalene have been reported to possess antioxidants and anti-bacterial activity [28,29].

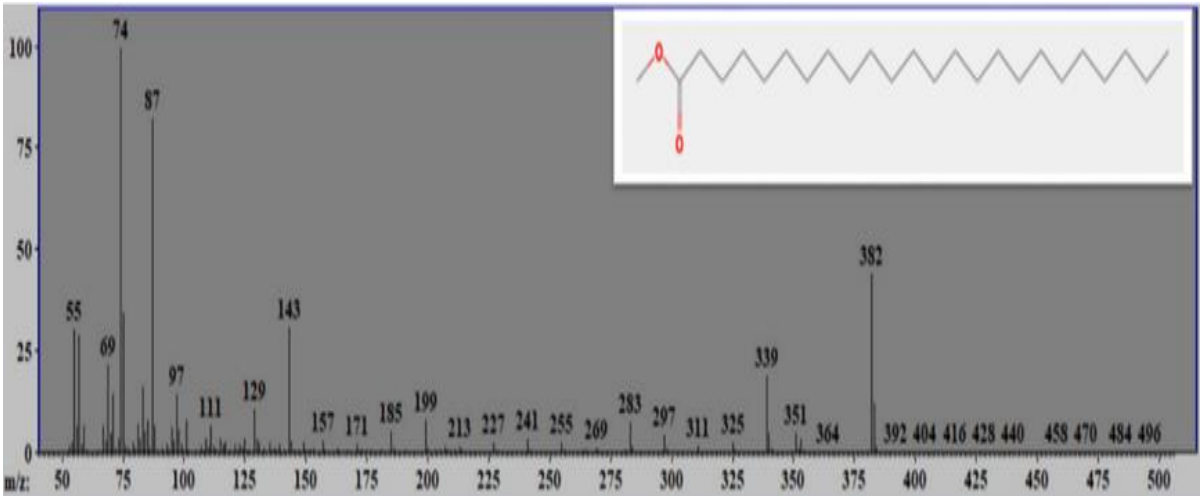

FIGURE 9 The Mass spectrum of Tetracosanoic acid, methyl ester

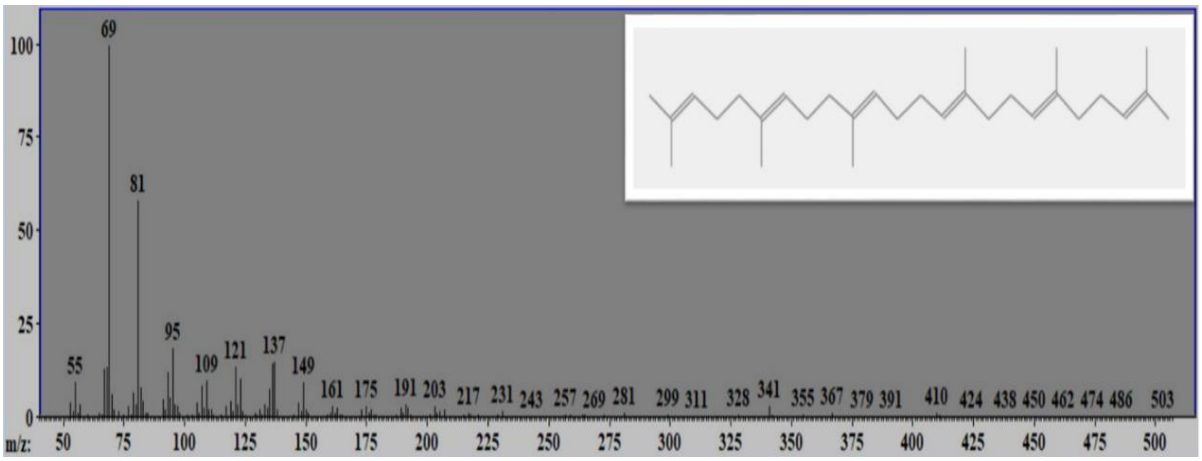

FIGURE 10 The Mass spectrum of Squalene

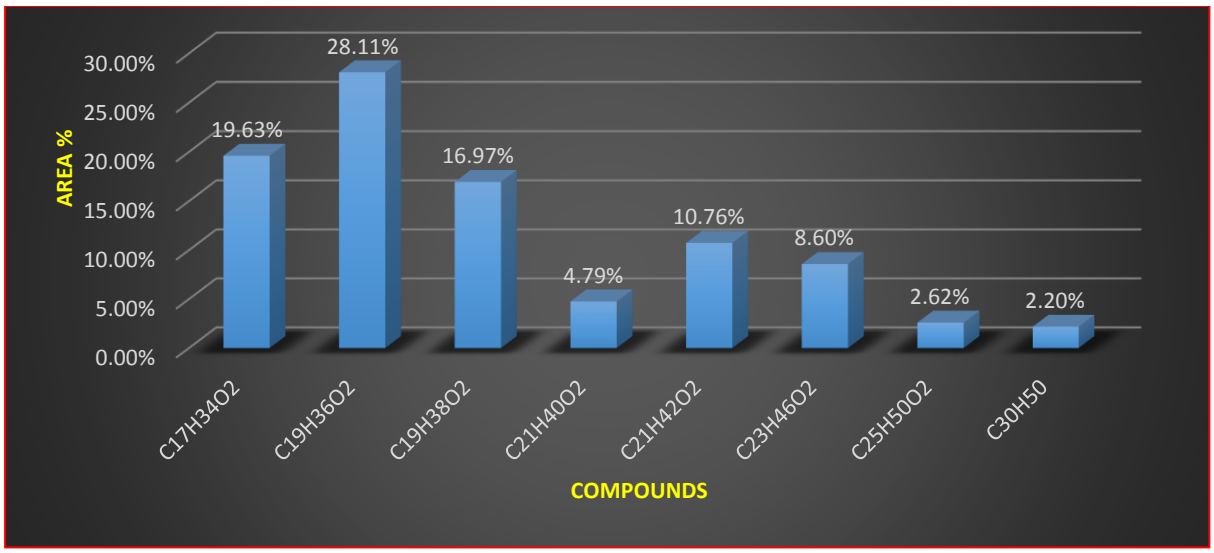

FIGURE 11 The eight major compounds in petroleum ether Ziziphus spina seed oil using Area% and Molecular Formula

The eight major compounds of petroleum ether fixed seed oil were showed in Figure 11 by their molecular formula and peak area%, the minor constituents of the extracted seed oil those are showed area% ≥1, were reported by their peak area and molecular formula, namely compound **17** was identified as Hexacosanoic acid, methyl ester, with peak area 0.97% appeared at 46.2 minute in the GC chromatogram. The compound **11** was identified as Heneicosanoic acid, methyl ester, ($C_{22}H_{44}O_2$), showing peak area of .82%. The compound **12** was identified as Tricosanoic acid, methyl ester, $C_{24}H_{48}O_2$, with peak area of .82%. The compound **15** was identified as Pentacosanoic acid, methyl ester, ($C_{26}H_{52}O_2$), with peak area of .80%. The compound **16** is 9-Octadecenoic acid (Z)-, 2, 3-dihydroxypropyl ester, ($C_{21}H_{40}O_4$) showed peak area of .80%. The remaining compounds had peak area less than .80%. The retention time started at 6.97 minute and ended at 46.27 minute. The molecular weights of the compounds ranged from 242 to 410.

FTIR spectroscopy

By using first and second derivative IR, the petroleum ether *Ziziphus spina Christi* seed oil extract was exposed to FTIR spectroscopy to produce more resolution for the probably compounds in the extract shown as absorbance band with Mid-IR region (4000-500) cm^{-1} in Figures 12 and13.

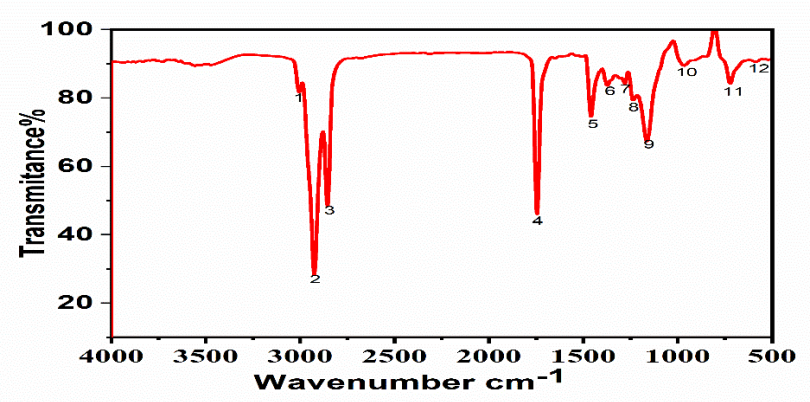

FIGURE 12 First IR spectrum of petroleum ether oil extract from *Z. spin Christi*

TABLE 2 Functional groups in petroleum ether extract of seed oil first IR

Peak No	Wavenumber cm^{-1}	Functional Groups	Identification
1	3005.36 cm^{-1}	C-H	aliphatic
2	2925.41 cm^{-1}	C-H	aliphatic
3	2855.51 cm^{-1}	C-H	aliphatic
4	1745.47 cm^{-1}	C=O	carbonyl
5	1459.27 cm^{-1}	C=C	aromatic
6	1372.92 cm^{-1}	C-O	alcohol
7	1280.74 cm^{-1}	C=C	trans
8	1235.92 cm^{-1}	C-O	alcohol
9	1164.20 cm^{-1}	C-O	ether
10	966.60 cm^{-1}	C-H	aromatic
11	721.72 cm^{-1}	C-H	aromatic
12	587.01 cm^{-1}	C-H	aromatic

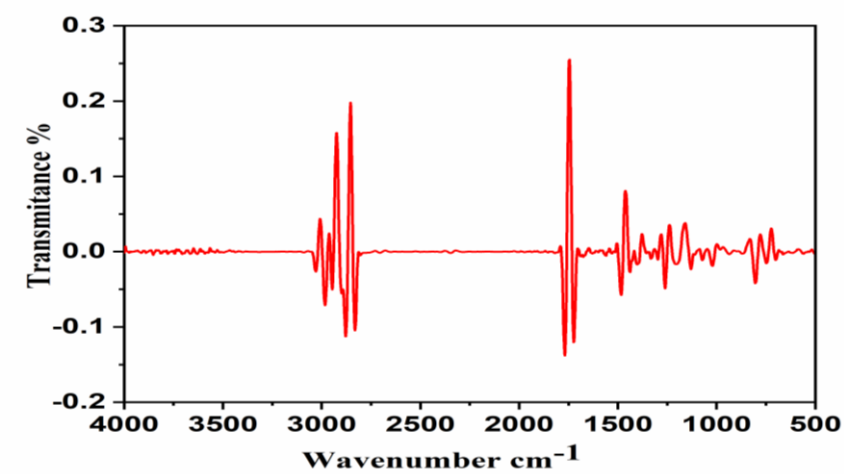

FIGURE 13 Second derivative IR of petroleum ether *Z. spina Christi* seed oil

TABLE 3 Functional groups in Petroleum ether extract of seed oil second derivative IR:

No	Regions	Wavenumber cm^{-1}	Functional Groups	Identification
		2982.55 cm^{-1}	C-H	aliphatic
		2944.25 cm^{-1}	C-H	aliphatic
1	**[3000-2000] cm^{-1}**	2872.43 cm^{-1}	C-H	aliphatic
		2829.34 cm^{-1}	C-H	aliphatic
		2791.03 cm^{-1}	C-H	aliphatic
		1764.81 cm^{-1}	C=O,	carboxylic acid
2	**[2000-1500] cm^{-1}**	1716.94 cm^{-1}	C=O,	carbonyl
		1669.06 cm^{-1}	C=O,	carbonyl
		1467.96 cm^{-1}	C-H	bending, alkane
		1424.87 cm^{-1}	O-H	bending, alcohol
		1391.55 cm^{-1}	O-H	bending, phenol
		1329.11 cm^{-1}	C-N,	aromatic amine
		1257.29 cm^{-1}	C-O	stretching, Ether
3	**[1500-600] cm^{-1}**	1190.26 cm^{-1}	C-O,	secondary alcohol
		1123.23 cm^{-1}	C-O,	secondary alcohol
		1017.09 cm^{-1}	Unidentified	Unidentified
		806.42 cm^{-1}	C=C	bending, alkene
		734.61 cm^{-1}	C=C	bending, alkene
		691.51 cm^{-1}	Unidentified	Unidentified

As for functional group and its quantified frequencies, the data was reported according to the reference [30]. The first IR spectrum petroleum ether oil extract showed a clear absence of a broad band peak at (3000-3500 cm^{-1}) as shown in Table 2, which indicated the existence of a hydroxyl group (- OH); the first three stretching bands appeared at [3005.36 cm^{-1}, 2925.41 cm^{-1}, and 2855.51 cm^{-1}], indicating the presence of C-H, aliphatic [31]. The peak number **4** observed at (1745.47cm^{-1}) was assigned to the strong stretching band of the C=O, carbonyl [32]. In the area of IR fingerprint region, (1500-600cm^{-1}) appeared seven peaks (1459.27 cm^{-1}, 1372.92 cm^{-1}, 1280.74 cm^{-1}, 1235.92 cm^{-1}, 1164.20 cm^{-1}, 966.60 cm^{-1}, 721.72 cm^{-1}, 587.01 cm^{-1}), were 1459.27 cm^{-1} due to C=C, aromatic, medium stretching band, 1372.92 cm^{-1}, and 1235.92 cm^{-1} appeared, due to weak band of alcohol. The weak band at 1280.74 cm^{-1} agreed with the existence of C=C, Trans, and stretching band at 1164.20 cm^{-1} was reported to identify medium band of C-O, ether. The three weak bands were agreed with the presence of C-H aromatic [33].

The second derivative IR spectrum of fixed seed oil showed in Figure 13, and Table 3 revealed five bands in the region (3000-2500 cm^{-1}) (2982.55 cm^{-1}, 2944.25 cm^{-1}, 2872.43 cm^{-1}, 2829.34 cm^{-1}, and 2791.03 cm^{-1}) due to C-H , aliphatic, which might indicate methyl ($-CH_3$), methylene ($>CH_2$), methyne ($>CH-$), and Special methyl ($-CH_3$) frequencies) [33]. The region at [2000-1500 cm^{-1}] showed three bands (1764.81 cm^{-1}, 1716.94 cm^{-1}, and 1669.06 cm^{-1}), implying the existence of C=O, carboxylic acid, and C=O, carbonyl [34]. We can confirm the origin of different extracts accurately and effectively, identify the medicinally important plant, and even assess the qualities of medicinal materials by investigating the finger print region [35]. Third region or finger print [1500-500] cm^{-1} of the second derivative IR spectrum corresponded to the fingerprint region, considered as high characteristic region for the compounds in the extract, and eleven bands (1467.96 cm^{-1}, 1424.87 cm^{-1}, 1391.55 cm^{-1}, 1329.11 cm^{-1}, 1257.29 cm^{-1}, 1190.26 cm^{-1}, 1123.23 cm^{-1}, 1017.09 cm^{-1}, 806.42 cm^{-1}, 734.61 cm^{-1}, and 691.51 cm^{-1}) appeared, which indicated the presence of C-H bending, alkane, O-H bending, alcohol, O-H bending, phenol, C-N, aromatic amine, C-O stretching, ether, C-O, secondary alcohol, C-O, secondary alcohol, unidentified band, C=C bending, alkene, C=C bending, alkene and unidentified band, respectively. These functional groups proved that this plant possesses a several of bioactive compounds such phenolic and flavonoids.

Conclusion

The results of Gas Chromatography Mass Spectrum (GC-MS) analysis showed that the fixed oil extracted from *Ziziphus spina Christi* seeds rich with various bioactive compounds such as fatty acids, which showed different bioactivity such as anti- bacterial, -anti-inflammatory, and anti-tumour. Besides, the Fourier-transform infrared spectroscopy (FTIR) analysis showed several functional groups such as alcohols, phenols, alkanes, alkenes, carbonyls, carboxylic acids, and aromatic compounds, where most of these compounds have been reported to possess significant bioactivity.

Future research may address the pharmaceutical and nutritional value of ingredients, and novel drugs in the *Ziziphus spina Christi* plant.

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