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Gas Chromatography-Mass Spectrum andFourier-transform infrared spectroscopy analysis of Fixed Oil from Sudanese *Ziziphus spina Christi* Fruits Pulp

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ABSTRACT

The study was carried out in reason of investigating the phytochemical constituents that could be present in the Ziziphus spina Christi Sudanese plant, by using two different analytical methods such as the Gas Chromatography-Mass spectrum (GC-MS) and the Fourier-transform infrared spectroscopy (FTIR). The *Z. spina Christi* Fruit pulp oil was extracted with four solvents methanol, petroleum ether, acetone, and isopropyl alcohol, using Soxhlet apparatus, The Gas Chromatography-Mass Spectrum (GC-MS) was carried out to analyze the methanolic fruit pulp oil extract, the results indicated to the presence of thirty-two phytochemical compounds. Ten of them are majors, namely9-Octadecenoic acid compounds, methyl ester, (E) - (17.07%), 7-Oxabicyclo[4.1.0]heptane,1-methyl-4-(2-methyloxiranyl)- (16.43%), 9-Octadecenoic acid (Z) -, methyl ester (10.6%), Hexadecanoic acid, methyl ester (8.78%), methyl stearate (6.71%), Docosanoic acid, Methyl ester (4.22%), cis-13-Eicosenoic acid, methyl ester (3.43%)%), methyl 18-methylnonadecanoate (2.98%), squalene (2.38%), 9-tricosine, (Z) - (2.4%).While the Fourier-transform infrared spectroscopy (FTIR) analysis was carried out for (methanol, petroleum ether, acetone, and isopropyl alcohol) fruit pulp oil extract the result showed the presence of many active functional groups such as alcohols, phenols, alkanes, alkenes, carbonyls, and Carboxylic acids and aromatic compounds in the extracts with different peak types and correspondences. The GC-MS and FTIR analysis showed the availability of bioactive compounds in the Ziziphus spina fruits pulp oil extracts, and these ingredients may be responsible for pharmaceutical value and could lead to the discovery a novel drugs

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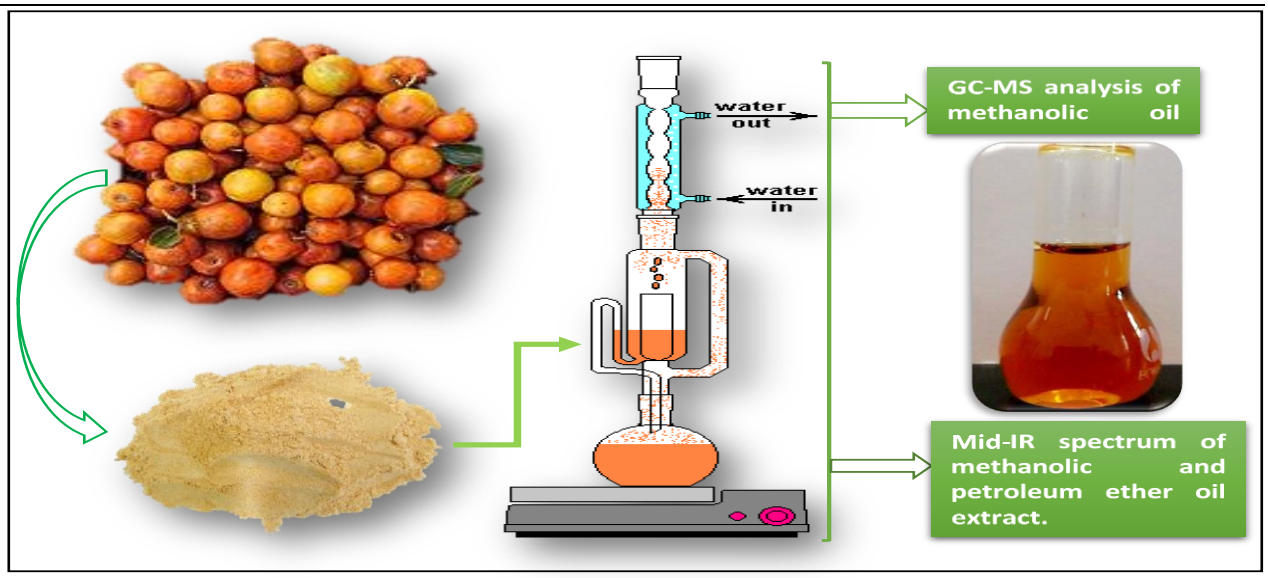
GRAPHICAL ABSTRACT



Introduction

Rhamnaceae plant family consider as one of the important plant family consisting of several genera, one of them Ziziphus genus include *Ziziphus spina Christi* species, the *Ziziphus spina Christi* species known in African countries and the Middle East as Sidder, in English: Christ's thorn Jujube [1]. The *Ziziphus spina Christi* was mentioned many times in holy books such Quran [2]The morphological structure of *Ziziphus spina Christi* classified as a shrub or either a tree with a length of 5-10 meters, and remains green throughout the year, the geographical distribution of *Z. spina Christi* is spread throughout tropical and subtropical countries precisely in North Africa, Middle East, and Northwest India, and in Sudan has been distributed over large parts of the country.[3]. Recently several studies reported that the *Z. Spina Christi*parts such as Fruits, Seeds, Leaf, stem bark, and roots possesses various nutritional and therapeutic lineaments, where have been used in folk medicinedue to antioxidants, and antimicrobial activities behaviors for the above-mentioned parts, where fruits and seed have a potent source of numerous phytochemical compounds precisely polyphenol, flavonoids, Tannin,Alkaloids, Terpenoids, and saponins [4] *Z. Spina Christi* fruit is edible and wealthy with phytoconstituents [5], The Ziziphus fruit shape semi globe, with a yellow or reddish color, the pulp usually sweaty ,and eaten as fresh, The *Z. Spina Christi* fruits used in folk medicine has a long history, they used to relief of digestive, disorder, and treat many microbial infections[6]the reported that the fruits consist of several important minerals such as potassium, sodium, calcium, and magnesium [7]. Despite the existence of many reports that indicated the widespread of this wild edible plant, especially in some Sudanese regions like North Kordufan State, it still lacking to research and documentation. It should be noted that there is no international documentation of Sudanese collected plant germ plasm., thus many plants lack identification and documentation[8]. Previouslythe screening of chemical constituents also known as (phytoconstituents) conducted by high costly and often exhausting as Gas Chromatography (GC) and Liquid Chromatography (LC) techniques unify with specific detection schemes, so recently phytochemical screening has become plain more than before by using Gas Chromatography-Mass

spectrum (GC-MS) and Fourier-transform infrared spectroscopy (FTIR), and other efficient techniques [9] Nowadays due to continuous and high demand develop a novel medicine from alternative sources by using less amount of extracted sample and in a short time, thus, the using of FTIR and GC-MS technique to analysis phytoconstituents functional groups of *Z. spina*fruits and seeds oil from Sudanese flora to the first time could help lead to discovering novel drugs for treating several recent diseases[10]. Moreover, the FTIR spectrum reflecting equitably the panorama of phytoconstituents in a complex system is the most reliable technique to screening and identify the functional groups [11]. Besides that, the GC-MS analysis was an efficient technique to identify the chemical constituents such as fatty acids, lipids, alkaloids, and volatile essential oil in medicinal plants [12].

The aim of this study to investigate the phytochemical constituents of the *Ziziphus spina Christi* fruits pulpfixed oil, obtained from Sudanese plant flora, extracted for the first time by (methanol, petroleum ether, acetone, isopropyl alcohol) solventsvia using two analytical methods GC-MS and Mid-IR techniques, and this will lead to discovering novel drugs in this plant.

Materials

Chemicals and reagents

Aqueous Methanol 20%, Petroleum Ether, acetone, isopropyl alcohol, KBr, Sodium Hydroxide, and Distilled Water. All the chemicals and solvents were commercial grade and used after further purification.

Plant material

Fruits of *Z.spina Christi* were purchased from the market in Khartoum, Om-Dorman, Sudan. The plant materials were washed thoroughly with distilled water to remove the dirt and other contaminations, after dried under ambient temperature, the pulp was removed carefully from seeds, and dried by using the technique of freezing and drying (Lyophilization) in **Figure 1.**

Experiments

Oil extraction

The dried pulp of *Z. spina* fruitgroundedinto powder, thereafter (100g) of the powder was extracted with chosen solvents, at a suitable temperature, by using Soxhlet extractor for (6 h), the extracted oil was concentrated under pressure and stored in 4 ºC, for further analysis in **Figure 2.**

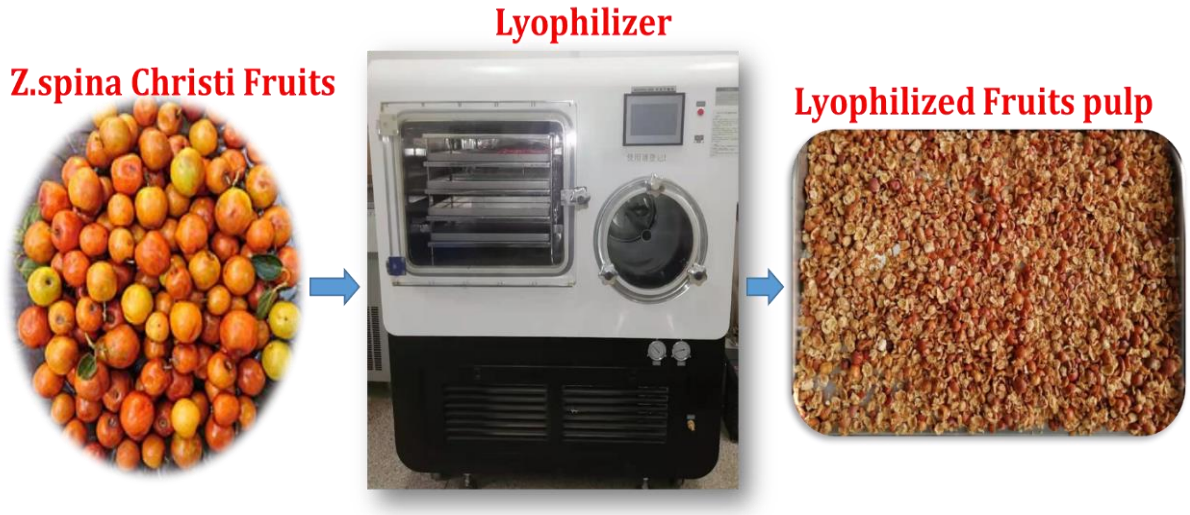


Figure 1. Preparation of Ziziphus spina Christi for extraction

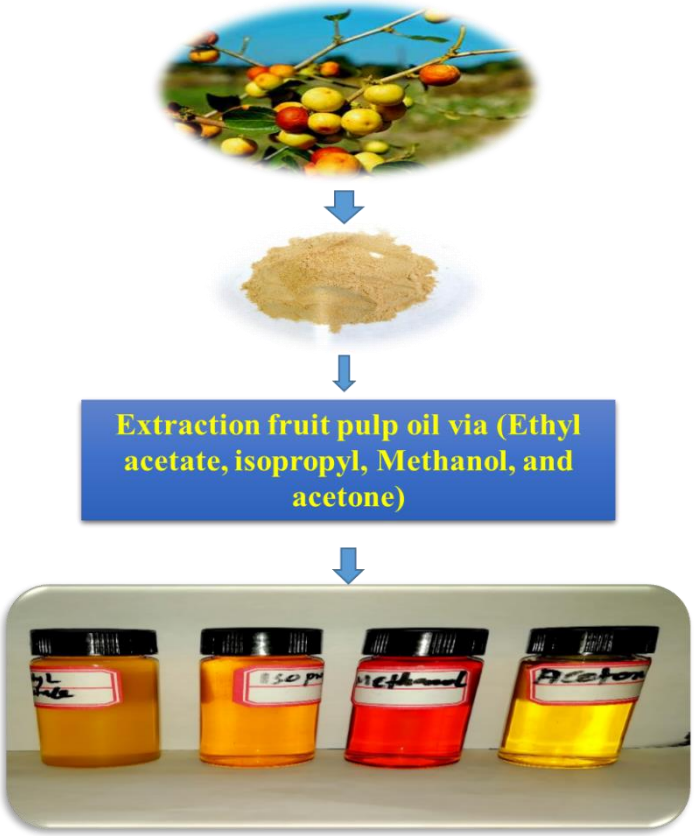


Figure 2. Different *Ziziphus spina Christi* fruits pulp oilextractions

Preparation oil for GC-MS test

The methods and conditions of preparation were set up according to the literature [13] with slight modification 500 µL of Adansonia digitata seed oil was added into 10 mL centrifugal tubes to which 3 mL of H_2SO_4–MeOH solution (1 %) was added. The mixture was heated on a water bath at 70 °C for 20 min. After cooling, 3 mL of n-hexane and 2 mL of distilled water were added and mixed completely. The extracts were prepared for GC analysis.

Preparation of FTIR Liquid sample (Fruit Oil)

To prepare the liquid sample of *Ziziphus spina Christi* fruits pulp oil for Fourier-transform infrared spectroscopy (FTIR) analysis, a good thin and transparent pellet KBr (Potassium bromide) was prepared, and a small drop of the oil was dropped via micropipette.

GC-MS analysis

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: Rtx 5MS -Length 30 meter -Diameter 0.25 mm- thickness 0.25 ml., Temperature programming was maintained from 60°C to 300°C, pressure 100 Kpa, Total flow 50 ml/min, Column Flow 1.61 ml/min, Linear Velocity 46.3 cm/sec. The ion source ionizing energy was 70 eV; Scan range: 50-650 amu; injector at 160 °C, oven initially at 250 °C, Acetone solvent Time 2.50 min., the fruits pulp oil were injected with a split less mode. The final confirmation of constituents was accomplished via comparing their retention times and mass fragmentation patent with those available in the library, the National Institute of Standards and Technology (NIST). Results were recorded.

Results and Discussion

Methanolic extract of the *Z. spina Christi*Fruit pulp oil was investigated by GC-MS, and its corresponding chromatogram was presented in **Figure 2**.

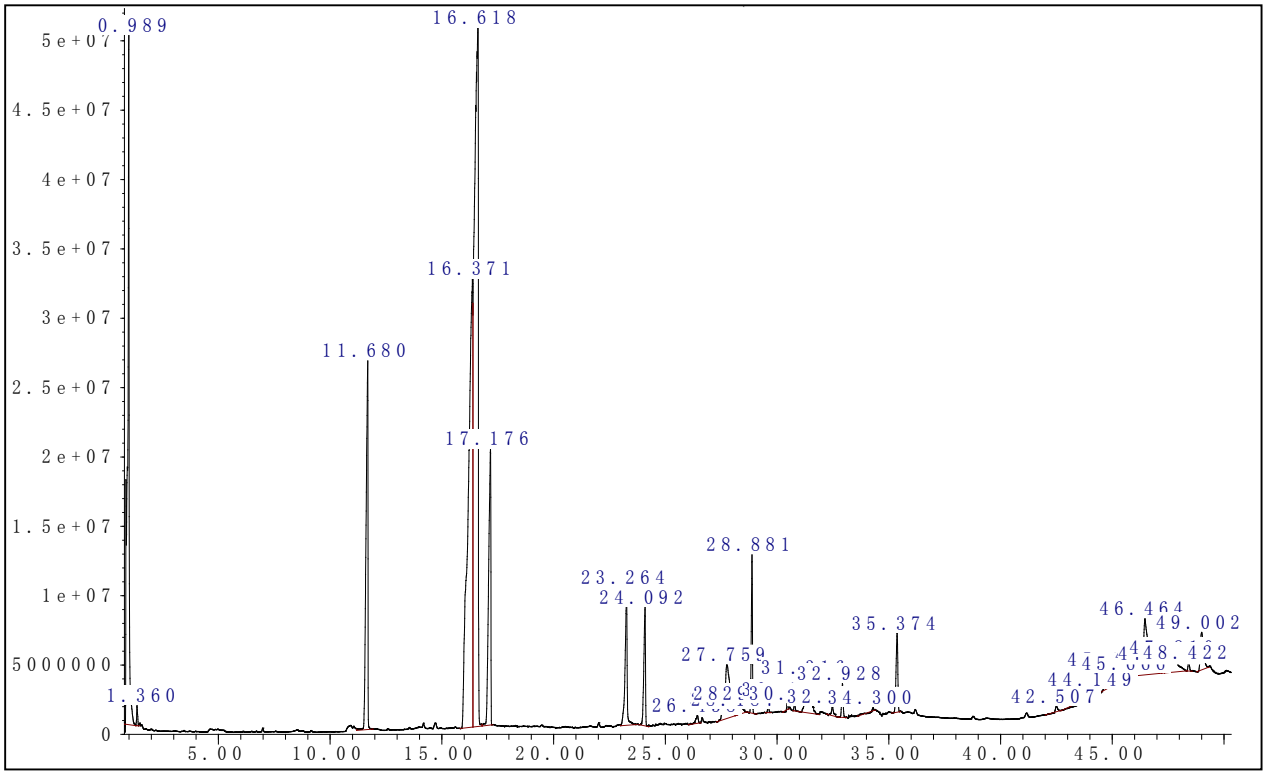


Figure 3. GC Chromatogram of methanolic extract of *Z. Spina Christi* Fruit's pulp oil

Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patent **Table1** with those available in the Wiley 9.0, NIST libraries and with those published in the literature. The GC chromatogram revealed the presence of **thirty-two** components which **ten** of themare major showed in **Table 2**, namely compound **5** is 9-Octadecenoic acid, methyl ester, (E)-,appearedon the GC chromatogram at (16.62) minutes with peak area (17.07%), it was generated molecular ion m/z at 296 $[M]^+$ corresponded to an elemental composition ($C_{19}H_{36}O_2$), in itsmass spectra, as well as the following fragment ions:59, 83, 97, 98, 264,and 55as base peak (BP), same to that available in literature [14], compound 9-Octadecenoic acid, methyl ester, (E) classified as unsaturated Fatty acid ester revealed antimicrobial, anti-inflammatory, anticancer, and diuretic activity [15], compound**1**is 7-Oxabicyclo[4.1.0]heptane,1-methyl-4-(2-methyloxiranyl)- with area (16.43%) correspond to the peak at (0.99)minutes on the GC, showed molecular ion$[M^+]^+$ m/z 112, and formula ($C_{10}H_{16}O_2$), with fragment ions: 71, 83, 101, 112, and 55 as base peak. compound **4** appear at (16.3734) minute on the GC, correspond to 9-Octadecenoic acid (Z)-, methyl ester with peak area (10.60%), which produced molecular ion m/z at 296 $[M^+]$ with molecular formula ($C_{19}H_{36}O_2$) in it MS spectra, beside to fragment ions: 69, 81, 96, 111, 180, 222, 264, 296 and 55 as base peak, it is good matched with reference [14]. The bioactivity of 9-Octadecenoic acid (Z)-, methyl esteris already has been reported as flavor, anti-cancer, and anti-inflammatory [16].

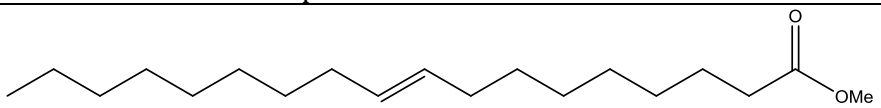
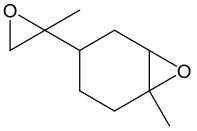
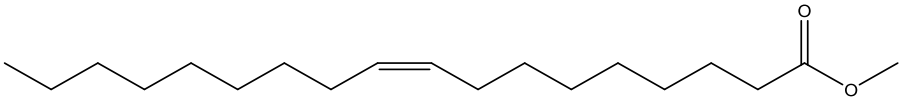
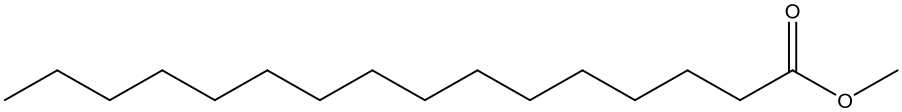
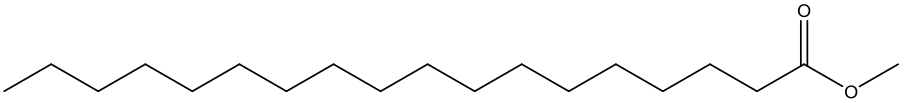
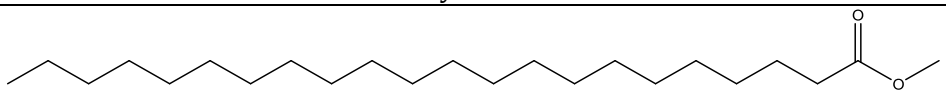
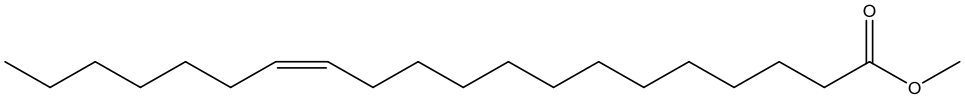
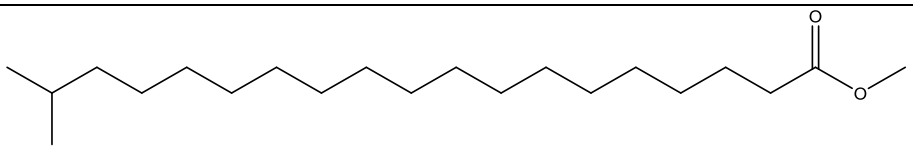
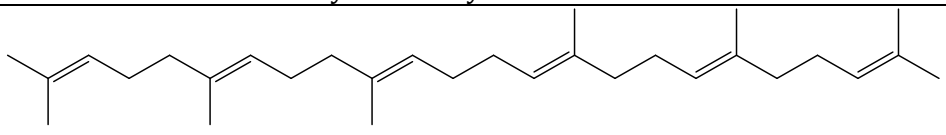
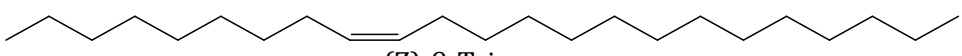
Table 1. The chemical constituents of methanolic extract of fruit's pulp oil MS

Peak	RT	M+	BP	Key fragment ions	Name of compound
1	0.990	168	55	71, 83, 101, 112	7-Oxabicyclo[4.1.0]heptane,1-methyl-4-(2-methyloxiranyl)-
2	1.350	136	95	51, 107, 121, 136, 148	4,7-Methano-1H-indene, octahydro-
3	11.682	270	74	87, 143, 227, 270	Hexadecanoic acid, methyl ester
4	16.373	296	55	69, 81, 96, 111, 180,222, 264, 296	9-Octadecenoic acid (Z)-, methyl ester
5	16.625	296	55	59, 83, 97, 98, 264	9-Octadecenoic acid, methyl ester,(E)-
6	17.174	298	74	87, 143, 199, 225, 298	Methyl stearate
7	23.264	324	55	69, 97, 111, 292	cis-13-Eicosenoic acid, methyl ester
8	24.092	326	74	87, 143, 283, 326	Methyl 18-methylnonadecanoate
9	26.441	667	73	147, 221, 281, 355, 429, 503	Cyclononasiloxane, octadecamethyl-
10	27.758	266	129	55, 59, 95, 115, 185	9-Octadecenal, (Z)-
11	28.138	280	129	55, 158, 71, 95, 116, 185	Cyclopropaneoctanal, 2-octyl-
12	28.186	264	55	57, 70, 95, 129, 130, 185, 217	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide
13	28.294	282	55	55, 57, 70, 95, 117, 129, 130, 185,267, 320	9-Octadecenoic acid, (E)-
14	28.878	354	74	87, 143, 311, 354	Docosanoic acid, methyl ester
15	29.604	563	73	130, 147, 209, 221, 281, 355, 429	Benzeneethanamine, N-[(pentafluorophenyl)methylene]-.beta.,3,4-tris[(trimethylsilyl)oxy]-
16	30.460	432	55	57, 69, 95, 133, 191, 205, 387	5-Fluoro-3-trifluoromethylbenzoic acid, hexadecyl ester
17	30.786	368	75	55, 57, 97, 129, 143, 207, 325, 368	Tricosanoic acid, methyl ester
18	31.315	294	131	73, 75, 132, 189, 279	beta.-Eudesmol, trimethylsilyl ether
19	32.483	370	73	54, 68, 107, 132, 207, 221, 281, 355, 429	Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester
20	32.931	382	74	55, 57, 75, 97, 143, 231, 382	Tetracosanoic acid, methyl ester
21	34.302	234	55	69, 117, 132, 175, 218, 264, 413	Butanedioic acid, 2,3-dimethoxy-, diethyl ester
22	35.375	410	69	82, 95, 121, 137, 191	Squalene
23	42.510	416	151	55, 69, 95, 191, 207, 281, 416, 417	beta.-Tocopherol
24	44.146	359	207	55, 72, 97, 147, 221, 281, 355, 429	(E)-2-bromobutyloxychalcone
25	43.035	464	55	57, 67, 109, 161, 281, 375	Oleyl alcohol, heptafluorobutyrate
26	45.666	207	207	55, 68, 97, 147, 281, 355	Benzo[h]quinolone, 2,4-dimethyl-
27	46.461	265	55	57, 68, 109, 161, 207, 265, 281, 329, 377	1,2-Benzisothiazol-3-amine tbdms
28	47.167	207	207	55, 69, 97, 147, 207, 281, 331	Benzo[h]quinolone, 2,4-dimethyl-
29	47.302	420	207	55, 57, 97, 147, 281, 323, 377	5,14,23-Octadecatrien-14,15-diol
30	47.812	207	207	55, 57, 69, 197, 147, 281, 355, 429	Benzo[h]quinolone, 2,4-dimethyl-
31	48.242	264	207	57, 68, 101, 147, 264, 281, 355, 441	Benzene, 2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-

Compound **3** is Hexadecanoic acid, methyl ester, classified as Fatty acid methyl ester [12], was appeared at (11.68) minute, with peak area (8.78%), with m/z at 270 $[M]^+$, and formula $C_{17}H_{34}O_2$, this compound has fragment ions: 87, 143, 227, 270, and 74 as base peak good agreement with those reported in [17], which has anti-oxidant, decrease the cholesterol in the blood, and anti-inflammatory effect [18]. Compound **6** is methyl stearate was showed molecular ion m/z at 298 $[M],^+$ which corresponded to formula ($C_{19}H_{38}O_2$), appeared at (17.17) minutes on the GC chromatogram, with the area (6.71%), the data of this compound was matched with reference [19], also was reported as Gamma-Aminobutyric acid (GABA) aminotransferase inhibitor, anti-inflammatory, intestinal Lipid metabolism regulator gastrin inhibitor anthelmintic, and ant nociceptive [19]. There are two peaks in GC chromatogram appeared at 23.26, and 28.87 minutes with area 3.43%, and 4.22% respectively, which they produced two molecular ion m/z at 324 [M+], and m/z at 354 [M]and two base peak (55, and 74), corresponding to molecular formula ($C_{21}H_{40}O_2$, and $C_{23}H_{46}O_2$), respectively. These two compounds were identified as cis-13-Eicosenoic acid, methyl ester **7**, and Docosanoic acid, methyl ester **14**, Where compound **14**was reported to possess therapeutic, and diagnostic activities [20]. The GC-Ms chromatogram showed three peaks at (24.09, 35.37, and 449.00) minutes, with molecular formula$C_{21}H_{42}O_2$, $C_{30}H_{50}$, and $C_{23}H_{46,}$ and their molecular ions m/z at 326 $[M^+]$, m/z at 410 $[M^+]$, and m/z at 322 $[M^+]$, and three base peaks: 74, 69, and 55, corresponded to compounds Methyl 18-methylnonadecanoate**8** (2.98%), Squalene **22** (2.38), and 9-Tricosene, (Z)- (2.40) **32**, there is data were agree with references [16,21].The compound **22** and **32** were shown several bioactivities as antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemo-preventive, lipoxygenase- inhibitor, pesticide, and anti-fungi [15, 22].

The compound **28** appeared at two different retention time (47.167, and 47.812) minutes by showing the same name of compound Benzo[*h*]quinolone, 2,4-dimethyl-, the compound shoed same base peak in fragment ion and molecular ions m/z at 207 $[M^+$, The remaining compounds had peak area less than 2%. The retention time started at 0.990 minutes and ended at 48.242 minute. The molecular weights of the compounds ranged from 136 to 667.

Table 2 Theten major compounds of methanolic fruits pulp oil extract

No	Compound name and structure	M.F	Area%
1	 9-Octadecenoic acid, methyl ester, (E)	$C_{19}H_{36}O_2$	17.07
2	 7-Oxabicyclo[4.1.0]heptane,1-methyl-4-(2-methyloxiranyl)-	$C_{10}H_{16}O_2$	16.43
3	 9-Octadecenoic acid (Z) -, methyl ester	$C_{19}H_{36}O_2$	10.60
4	 Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	8.78
5	 methyl stearate	$C_{19}H_{38}O_2$	6.71
6	 Docosanoic acid, Methyl ester	$C_{23}H_{46}O_2$	4.22
7	 cis-13-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	3.43
8	 methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	2.98
9	 squalene	$C_{30}H_{50}$	2.38
10	 (Z)-9-Tricosene	$C_{23}H_{46}$	2.40

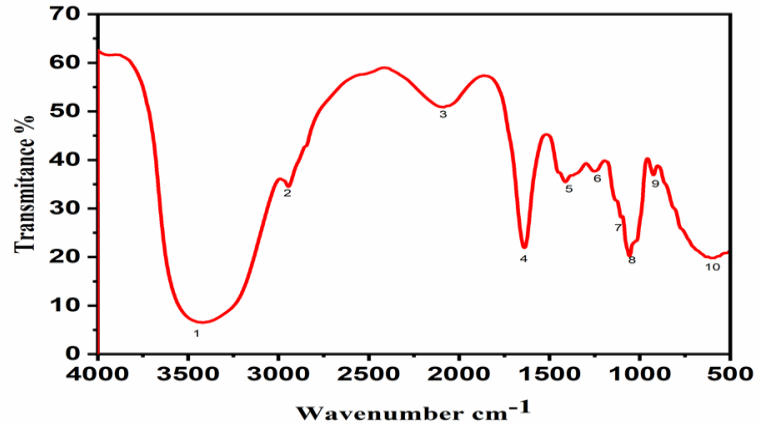


Figure 4 FTIR peaks of methanol fruits pulp oil extract

Table 3 the functional groups in methanol extract of *Z. spina* Christi fruits oil

Peak. No	Wave length cm^{-1}	Functional Group	Identification
1	3420.36 cm^{-1}	OH, (v.br.)	Alcohol
2	2944.9 cm^{-1}	C-H, (w)	Aliphatic
3	2086.44 cm^{-1}	C≡C, (br.)	Aliphatic
4	1638.99 cm^{-1}	C=O, (s)	Carbonyl
5	1412.08 cm^{-1}	C=C, (w)	Cis
6	1252.71 cm^{-1}	C=C, (w)	Trans
7	1103.34 cm^{-1}	C-O, (w)	Ether
8	1057.35 cm^{-1}	C-O, (s)	Phenolic
9	924.54 cm^{-1}	C-H, (w)	Aromatic
10	591.1 cm^{-1}	C-H, (br.)	Aromatic

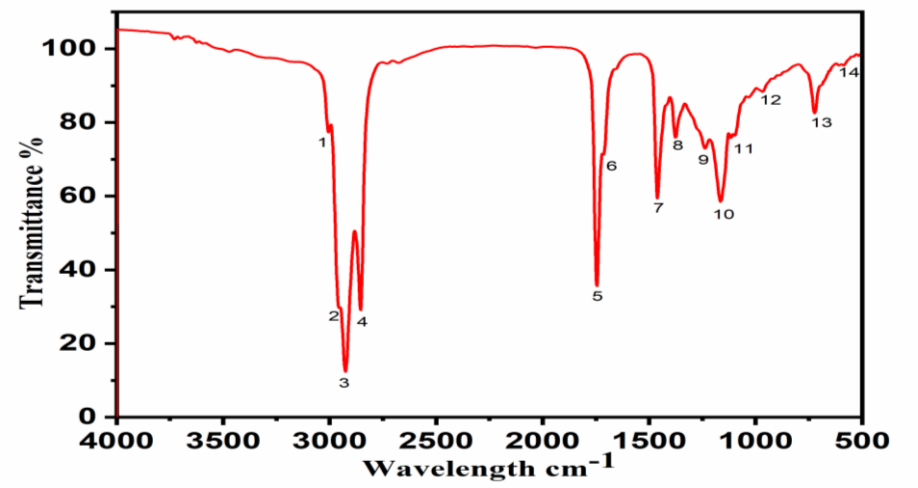


Fig 5. FTIR peaks of petroleum other fruits pulp oil extract

Table 4 the functional groups in petroleum ether extract of fruits pulp oil extract

Peak. No	Wave length cm^{-1}	Functional Group	Identification
1	3005.95 cm^{-1}	C-H,(w)	Aliph
2	2956.1cm^{-1}	C-H,(w)	Aliph
3	2925.55cm^{-1}	C-H,(s)	Aliph
4	2854.4 cm^{-1}	C-H,(m)	Aliph
5	1746.5 cm^{-1}	C=O,(s)	Carbonyl
6	1712.22 cm^{-1}	C=O,(w)	Carbonyl
7	1465.07 cm^{-1}	C=C,(m)	Aromatic
8	1377.84 cm^{-1}	C-O,(w)	Alcohol
9	1238.93 cm^{-1}	C-O,(w)	Alcohol
10	1164.17 cm^{-1}	C-O,(s)	Ether
11	1098.15 cm^{-1}	C-O,(w)	Phenolic
12	965.74 cm^{-1}	C-H,(w)	Aromatic
13	722.20 cm^{-1}	C-H,(m)	Aromatic
14	608.04 cm^{-1}	C-H,(w)	Aromatic

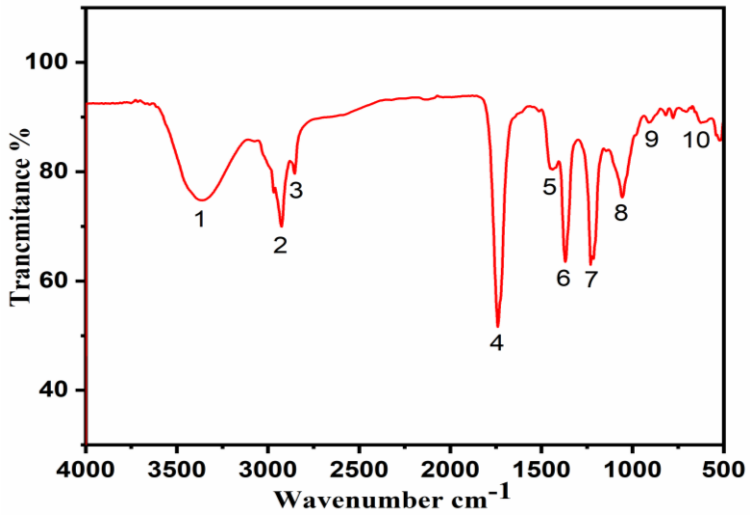


Figure 6 FTIR peaks of acetone oil extract from *Z. spina Christi* Fruit's pulp oil

Table 5. The functional groups in acetone extract of fruits pulp oil extract

Peak No	Wavenumber cm^{-1}	Functional Group	Identification
1	3361.65	OH, (br)	Alcohol
2	2926.17	C-H, (s)	aliphatic
3	2854.42	C-H, (w)	aliphatic
4	1739.74	C=O, (s)	carbonyl
5	1438.01	C=C, (w)	aromatic
6	1369.14	C-H, (s)	-C(CH3)2
7	1230.17	C-O-C, (s)	ether
8	1056.73	OH, (m)	C-OH
9	911.13	CH2,(w)	rocking
10	523.30	C-H, (w)	aromatic

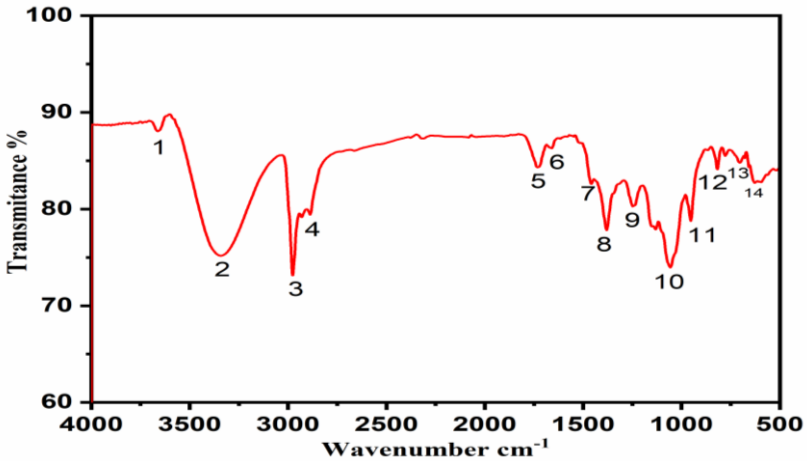


Figure 7 FTIR peaks of isopropyl alcohol oil extract of pulp oil

Table 6 the functional groups in an isopropyl alcohol extract of fruits pulp oil extract

Peak No	Wavenumber cm^{-1}	Functional Group	Identification
1	3661.62	OH, (w)	free
2	3341.48	OH, (br)	H-bonded
3	2976.55	C-H, (s)	aliphatic
4	2887.67	C-H, (w)	aliphatic
5	1731.30	C=O, (m)	carbonyl
6	1659.68	C=O, (w)	carbonyl
7	1458.38	C=C, (w)	aromatic
8	1380.73	C-H, (s)	-C(CH3)2
9	1274.21	C-O-C, (m)	ether
10	1056.51 cm^{-1}	OH, (s)	C-OH
11	952.98	C-H, (m)	aromatic
12	817.71	C-H, (m)	aromatic
13	702.53	C-H, (w)	aromatic
14	626.95	C-H, (m)	aromatic

Key: m = medium, v = very, s = strong or sharp, w = weak, br. = Broad

Functional group and its quantified frequencies. Data was reported according to reference [23]. When the analysis is done with the use of FTIR, the samples to be tested are either solid or liquid exposed to contact with infrared (IR). This is because infrared radiation has effects on the

atomic vibrations of a molecule in the sample, which leads to absorption and/or specific energy transfer [24]. In summary, the infrared spectrum consists of three wavelength regions: the far-infrared spectrum <400 cm^{-1}, the mid-infrared spectrum 400-4000 cm^{-1}, and the near-infrared spectrum 4000-13000 cm^{-1}, respectively. The mid-infrared spectrum is the most common and over-used in sample analysis in Figure 7 In addition, the far-infrared and near-infrared spectrum also contributes to providing information about the samples analyzed [24].

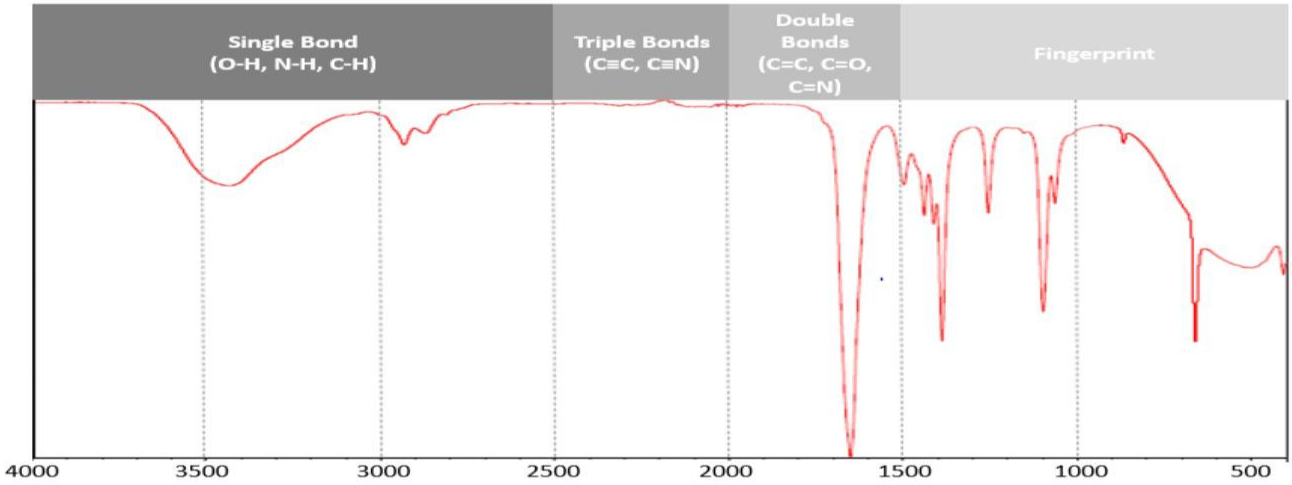


Figure 8. Mid-IR spectrum regions

The spectrum of the methanolic extract showed in Figure 4 and Table 3 the main characteristic absorption very broadband at 3420.36 cm^{-1} which appearsin the range between 3650- 3250 cm^{-1}, indicatesthe presence of-OH group [25]. The presence of the aliphatic saturated (C-H, aliphatic/w) week symmetric stretching band are observed at 2944.9 cm^{-1} also known as a narrow band observedat below 3000 cm-1, showing aliphatic compounds [26]. This is due toalkyneconstituents. The (C=O) strong stretching band was observed at 1638.99; it is due to the presence of carbonyl [27], the two peak sat 1412.08 cm^{-1} and 1252.71 cm^{-1} were shown the presence of the (C=C) week stretching band; it due to observing *cis* that possesses between two alkyl (R) groups on the same side, and trans possesses two alkyl (R) groups on the opposite side. In the fingerprint, the area was observed four stretching bands at1103.34 cm^{-1}, 1057.35 cm^{-1}, 924.54 cm^{-1}, and 591.1 cm^{-1}, and their functional groups respectively, as C-O for alkyl-substituted ether (C-O) for phenolic (s), (C-H) for, aromatic (w) and C-H for aromatic (br)., two a weak and one strong band were observed between 3005.95–2925.55cm^{-1},which indicate the presence of aliphatic saturated (C-H). Another two bands for C=O, carbonyl groups (1746.5 cm^{-1} strong bands and 1712.22 cm^{-1} weak band) [26]. C=C, aromatic (1465.07 cm^{-1}mediumbandand C-O, alcohol (1377.84 cm^{-1}, and 1238.93 cm^{-1}, both of them are weak bands), C-O, ether (1164.17 cm^{-1}, strong band), C-O, Phenolic (1098.15 cm^{-1}, weak peak), and three of C-H aromatic, (965.74 cm^{-1}weak, 722.20 cm^{-1} medium, and 608.04 cm^{-1} weak peak)[28],. The IR spectrum of petroleum ether extract in Figure 5 and Table 4 is varying from aqueous methanolic extract Figure 2, by showing some differences between the IR analysis of petroleum ether and Methanolic extract, wherein petroleum ether there was a clear absence of a broad band peak at (3000-3500 cm^{-1}), which indicated to exist of a hydroxyl group (-OH), Furthermore, the IR of petroleum ether showed stretching band at (2956.1 cm^{-1}, 2925.55 cm^{-1} and 2854.4 cm^{-1}), due

to the C-H were stronger than in methanolic extract. [29] in addition to that, the peak at 1164.17 cm^{-1} due to Ether (C-O) in petroleum ether also was stronger than that appeared inmethanolic extract, on the other hand, the week band at 1098.15 cm^{-1} due to the phenolic compound was strong in methanolic extract in compared with petroleum ether [30]. On another side, were using the macroscopic fingerprint characters of the FT-IR spectrum, we can confirm the origin of different extracts accurately and effectively, identify the medicinally important plant, and even assess the qualities of medicinal materials [31]. therefore, the area of fingerprint in region 600-1500 cm^{-1} was shown, where the methanolic oil extract showed about (5) peaks were corresponded to C=C, trans (w), C-O, ether (w),C-O, phenolic (s), and C-H, aromatic (w), respectively, and the petroleum ether oil extract fingerprint area showed about (8) functional groups C=C, aromatic(m), C-O, alcohol (w), C-O, alcohol (w), C-O, ether (s), C-O, Phenolic (w), C-H, aromatic (w), C-H, aromatic (m), C-H, aromatic (w).

IR spectrum of acetone fruits pulp extract showed in Figure 6 and Table 5 the first one was broad absorbance band is 3361.65cm^{-1}appread at region 4000-3500 cm^{-1}, indicated to OH alcohol, strong broad bands peak 2926.17 cm^{-1} showed at region 3000-3500 cm^{-1}, modified as C-H aliphatic [32], the stretching at 2854.42$cm^{-1}$is showed in the region 3000-2500 cm^{-1} as weak peak and identified as C-H aliphatic in literature [33], the peak 1739.74 cm^{-1}, showed in the region 2000-1500 cm^{-1}, identified as C=O carbonyl reported in [34].

The region of fingerprint 1500-500 cm^{-1} in acetone fruits pulp oil extract showed about five peaks with different types of stretching as flow (1438.01 cm^{-1}, 1369.14 cm^{-1}, 1230.17 cm^{-1}, 1056.73 cm^{-1}, 911.13 cm^{-1}, 523.30 cm^{-1}) corresponded to C=C aromatic [35], C-H,-$C(CH_3)_2$, C-O-C ether, OHC-OH, CH_2, rocking [36], C-H aromatic) respectively.

Isopropyl alcohol fruit pulp oil extract IR analysis result in Figure 7 and Table 6 showed about fourteens peaks in different regions, peak one was free weak presence at 3661.62 cm^{-1} assigned to OH, broad peak at 3341.48 cm^{-1}, identified as OH (H-bonded) phenolic compounds [37], the strong absorption at 2976.55 cm^{-1} because of C-H aliphatic, the weak peak at 2887.67 cm^{-1}, due to C-H aliphatic [38], the absorption at 1731.30 cm^{-1} and 1659.68 cm^{-1} were indicated to C=O carbonyl functional group [39], the finger print region in 1500-500 cm^{-1} showed about eight absorbance bands 1458.38 cm^{-1}, 1380.73 cm^{-1}, 1274.21 cm^{-1}, 1056.51 cm^{-1}, 952.98 cm^{-1}, 817.71 cm^{-1}, 702.53 cm^{-1}, 626.95 cm^{-1} assigned as (C=C aromatic, C-H-C($CH_3)_2$, C-O-C ether, OH (C-OH), C-H- aromatic, C-H- aromatic, C-H- aromatic, C-H- aromatic) respectively.

Conclusion

The results of Gas Chromatography-Mass Spectrum of methanolic fruits pulp oil extract showed about thirty-two compounds, ten of them were the major compounds showed peak area (2.38-17.07)%, most of them are reported possesses biological activity. the Fourier-transform infrared spectroscopy (FTIR) of methanolic, petroleum ether, and isopropyl alcohol fruit pulp oil extract showed that several active functional groups, which corresponded to, alcohols, phenols, alkanes, alkenes, carbonyls, and Carboxylic acids, and aromatic compounds. Where a major of these compounds have been reported could possess significant bioactivity. Therefore, this study could lead to more seeking in this plant via Scholars to investigate the pharmaceutical and Nutritional value of ingredients in the Z. spina Christi plant.

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