Biomedical Research 2018; 29 (8): 1645-1652

ISSN 0970-938X
www.biomedres.info

Phytochemical screening of different organic crude extracts from the stem bark of *Ziziphus spina-christi* (L.).

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Abstract

Zizyphus spina-christi **(L.) (Rhamnaceae family) is a subtropical plant known as 'Nabq' or 'Sidr' which is used for various medicinal purposes. It has used in folk medicine in pain related ailments throughout these regions. Aim of our work is to phytochemically investigate the plant's bark in our laboratory with Chromatographic approach. The crude ethanol extract of dried bark of *Z. spina-christi* (L.) was fractionated by diethyl ether, chloroform, ethyl acetate and finally with n-butanol. Finally the fractions were investigated for the chemical constituents by using gas chromatography-mass spectrometry. Thirty six compounds from the four fractions of the stem bark of *Z. spina-christi* (L.) have been identified and recognized, especially betulin, quercetin, stigmasterol, sitosterol, ethyl oleate and phytol by means of GC-MS analyses.**

Keywords: *Zizyphus spina-christi* (L.), Ethanol extract, Betulin, Phytol, Stigmasterol, α-sitosterol, Quercetin.

Accepted on February 17, 2018

Introduction

Ziziphus spina-christi (L.), locally known as Sidr, is a multipurpose tree species belonging to the botanical family Rhamnaceae. It is an important cultivated tree and one of the few truly native tree species of Arabia (Saudi Arabia, Jordan, and Egypt) that is still growing along with many newly introduced exotic plants [1]. It is one of the important fruit crops in the dry parts of tropical Asia and Africa. *Ziziphus spina-christi* (L.) fruits are highly nutritious and rich in vitamin *C. Ziziphus* (Rhamnaceae) species are used in folk medicine to treat blisters, bruises, chest pains, dandruff, fractures, headache, and mouth problems [2]. *Ziziphus spina-christi* (L.) leaves are traditionally used to treat ulcers, wounds, eye diseases, bronchitis, and skin diseases as an anti-inflammatory agent. The seeds are sedative and are used to halt nausea, vomiting and abdominal pain associated with pregnancy [3]. The fresh leaves are applied on swollen eye at night [4]. The roots are used to cure and prevent skin diseases [5]. Fruits are used to promote the healing of fresh wounds, and treat dysentery, bronchitis, coughs and tuberculosis [6]. It is also used to relief digestive disorders, obesity, urinary troubles and microbial infections [7,8]. Some pharmacological screening studies indicated that the aqueous extract of *Ziziphus spina-christi* (L.) root bark has an antinociceptive activity in mice and rats [9] and a central depressant effect in mice [10]. The methanol extract of *Ziziphus spina-christi* (L.) stem bark has antidiarrheal effects in rats [11].

Previous phytochemical studies on the different species of the genus *Ziziphus* led to the isolation and characterization of peptide, cyclopeptide alkaloids, flavonoids, sterols, tannins, betulinic acid and triterpenoidal saponin glycosides [7,12-15]. *Ziziphus spina-christi* (L.) extracts were evaluated for their phytochemical content in the stem bark as well as the antimicrobial and cytotoxic activities. The phytochemical analysis indicates the presence of tannins, flavonoids, terpernoids, saponin glycosides and alkaloids in *Ziziphus spina-christi* (L.) [16]. From the butanol extract of the leaves of *Ziziphus spina-christi* (L.) growing in Egypt, four triterpenoidal saponin glycosides were isolated and named christinin-A-D, respectively. Christinin-A was the major saponin [17].

Medicinal plants were investigated for phytochemical contents by GC-MS and HPLC with ethanol and hexane extracts and their toxicity by MTT and comet assay on human Peripheral Blood Mononuclear Cells (PBMCs) [18]. Phytochemical investigations on *Ziziphus spina-christi* (L.) have shown that this plant contains many biologically important phytochemicals. From the different species of the genus *Ziziphus*, peptide and cyclopeptide alkaloids, flavonoids, sterols, tannins, betulinic acid and triterpenoidal saponin glycosides have been isolated and chemically identified [19-21]. Betulic and ceanothic acid, three cyclopeptide alkaloids as well as four saponin glycosides [22] and several flavonoids have been isolated from the leaves of *Ziziphus spina-christi* (L.) [23]. This study deals with the investigation and identification of the chemical constituents by using Gas Chromatography-Mass Spectrometry (GC-MS) in the bark of *Ziziphus spina-christi* (L.) extracted by different organic solvents depending on polarity.

Materials and Methods

Plant material

Fresh bark of the *Ziziphus spina-christi* (L.) were collected from the Hail region with GPS coordinates (270 29\ 05\\ N, 410 41\ 44\\ E), (270 30\ 51\\ N, 410 42\ 01\\ E), (270 32\ 18\\ N, 410 41\ 42\\ E), (260 0\ 21\\ N, 400 28\ 20\\ E), Saudi Arabia. The plant was identified and the specimen deposited in the Herbarium of the Department of Biology, University of Hail. The bark of plant was broken into pieces and air dried.

Preparation of the plant extract

The dried bark of *Ziziphus spina-christi* (L.) (5.2 kg) were extracted with absolute ethanol repeatedly (3 times × 20 L). The ethanol extract was filtered and concentrated under reduced pressure (119.59 g). The yield % was 2.3%. The ethanol extract was then fractionated using diethyl ether, chloroform, ethyl acetate and finally with n-butanol. The diethyl ether, chloroform, ethyl acetate and butanol fractions were filtrated and dehydrated over anhydrous sodium sulphate and concentrated under reduced pressure. The yield % of these fractions was 41.19 g, 8.45 g, 7.24 g and 8.86 g, respectively.

GC-MS analysis

The samples were subjected to GC-MS analysis (Thermo Scientific ISQ LT Trace 1310). Injector temperature was 250°C, and column description: TG-SQC GC Column 15 m x 0.25 mm x 0.25 mm, Temperature programming was maintained from 50°C to 290°C with constant rise as follows: Oven temperature was initially 50°C, increased up to 150°C at a rate of 7°C/min (held for 1 min), increased up to 250°C at a rate of 5°C/min (held for 5 min) and finally increased up to 290°C at a rate of 10°C/min (held for 2 min). The ion source and MS transfer line temperatures were 300°C. The crudes were injected with a splitless mode. Mass spectra were taken at 70 eV; fragments from 40 to 1000 Dalton. The final confirmation of constituents was made by computer matching of the mass spectra of peaks with the Wiley and National Institute Standard and Technology (NIST) libraries mass spectral database.

Phytochemical screening

Ziziphus spina-christi (L.) bark extracts were subjected to qualitative phytochemical analysis for the presence of various classes of active chemical constituents such as tannins, saponins, glycosides, flavonoids, alkaloids, terpenes, steroids, etc. using standard procedures [24,25].

Results and Discussion

GC-MS analysis was carried out in organic crude extracts (diethylether, chloroform, ethyl acetate and butanol extracts) fractionated from the ethanol extract of the stem bark of *Ziziphus spina-christi* (L.). The comparison of the mass spectrums with the data base gave more than 90% match.

Chemical composition of diethyl ether extract of Ziziphus spina-christi (L.)

Table 1. Identified compounds in diethyl ether extract of Ziziphus spina-christi (L.).

RT (min)	Identified compounds	Chromatogram % area	MW
7.34	3-dodecene, (Z)-	3.15	168
11.09	1-tetradecene	19.84	196
13.18	Butyl hydroxytoluene	7.00	220
14.50	1-hexadecanol	21.30	242
18.26	1-hexadecanol, 2-methyl	17.82	256
22.04	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	18.94	284
25.01	Ethyl linoleate	3.65	308
25.11	(9-octadecenoic acid (Z)-, ethyl ester) ethyl oleate	5.68	310
25.61	Octadecanoic acid, ethyl ester (Ethyl stearate)	2.62	312

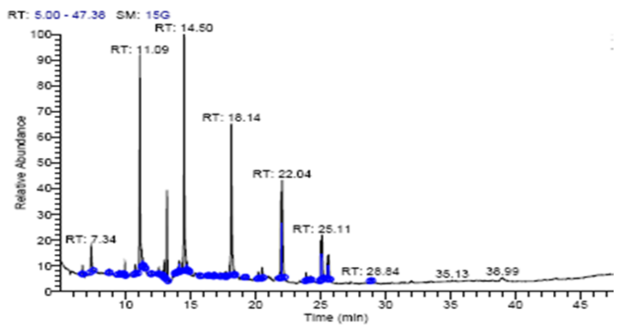

Figure 1. GC-MS chromatogram of diethyl ether extract of Ziziphus spina-christi (L.).

The diethyl ether extract was found to contain nine different compounds, representing 100% of the total extract. The identified compounds are listed in Table 1 according to their elution order on TG-SQC GC Column (Figure 1). The major compounds detected were 1-Hexadecanol (21.30%), hexadecanoic acid, ethyl ester (Ethyl palmitate) (18.94%) and 1-Hexadecanol, 2-methyl (17.82%). The volatile constituents of the leaves oil of Iranian *Ziziphus spina-christi* (L.) is also reported to have hexadecanol as a main component (9.7%) [26]. Octadecanoic acid, ethyl ester (Ethyl stearate) (2.62%) were also found to be the minor components of the diethyl ether extract.

Chemical composition of chloroform extract of Ziziphus spina-christi (L.)

Table 2. Identified compounds in chloroform extract of Ziziphus spina-christi (L.).

RT (min)	Identified compounds	Chromatogram % area	MW
9.01	Pyrrolidine, 1-(1,6-dioxooctadecyl)-pyrrolidine,	1.22	351
11.22	Dichloroacetic acid, tetradecyl ester	2.33	324
12.84	2,6,10,15-tetramethyl heptadecane,	1.94	296
12.96	Quercetin, quercetin 7,3',4'-trimethoxy 4H-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	0.46	344
13.17	Butylated hydroxytoluene	1.96	220
14.5	1-hexadecanol	2.27	242
14.61	Octadecane, 1-chloro-	4.67	288
18.26	1-hexadecanol, 2-methyl	4.95	256
20.59	7,9-di-tert-butyl-1-oxaspiro(4, 5)deca-6,9-diene-2,8-dione	8.30	276
22.08	Hexadecanoic acid, ethyl ester	8.69	284
25.11	(9-Octadecenoic acid (Z)-, ethyl ester) Ethyl oleate	3.38	310
25.64	Ethyl stearate	1.40	312
29.25	Phenol, 2,2'-methylenebis(6-(1,1-dimethyl ethyl)-4-methyl-	1.89	340
42.17	Stigmasterol	0.65	412
43.21	α-Sitosterol	0.68	414
43.64	Betulin	55.23	442

From Table 2, it's clear that the chloroform extract contain 16 different compounds, representing 100% of the total extract. The identified compounds are listed in Table 2 according to their elution order on TG-SQC GC Column (Figure 2). The major compounds detected were betulin (55.23%), hexadecanoic acid ethyl ester (8.69%), 7, 9-di-tert-butyl-1-oxaspiro (4, 5) deca-6,9-diene-2,8-dione (8.30%). As shown in the table, quercetin (0.46%), stigmasterol (0.65%) and α-Sitosterol (0.68%) were present in minor quantities in the chloroform extract. The methanol extract of ripe edible fruits of *Ziziphus spina-christi* (L.) and Z. jujuba are also reported to contain quercetin [27].

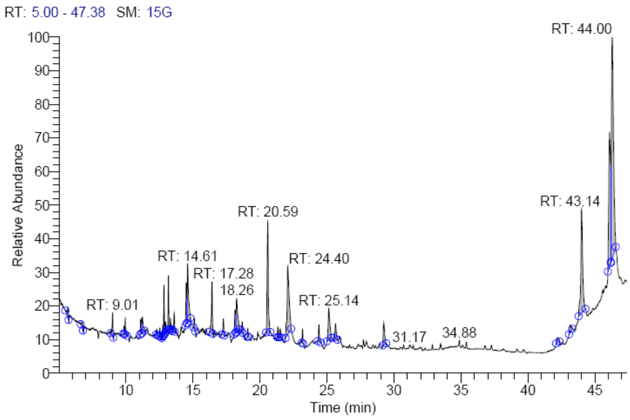

Figure 2. GC-MS chromatogram of chloroform extract of Ziziphus spina-christi (L.).

Chemical composition of ethyl acetate extract of Ziziphus spina-christi (L.)

Table 3. Identified compounds in ethyl acetate extract of Ziziphus spina-christi (L.).

RT (min)	Identified compounds	Chromatogram % area	MW
12.81	Lanceol, cis (2E)-2-(4,7-Dimethyl-3,4,4a,5,6,8a-hexahydro-1(2H)-naphthalenylidene)-1-propanol	3.87	220
20.78	Hexadecanoic acid, methyl ester (Palmetic methyl ester)	2.15	270
23.82	8,11-Octadecadienoic acid, methyl ester	1.80	294
23.92	9-Octadecenoic acid (Z)-, methyl ester (Methyl oleate)	4.65	296
24.19	Phytol (2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, (R-(R*, R*-(E)))-)	2.66	296
36.13	Heptacosane	2.44	380
41.49	Campesterol	2.92	400
42.18	Stigmasterol	15.98	412
43.21	α-Sitosterol	43.89	414
43.64	Betulin	15.45	442
44.8	Ethyl iso-allocholate	4.20	436

The ethyl acetate extract was found to contain 11 different compounds, representing 100% of the total extract. The identified compounds are listed in Table 3 according to their elution order on GC column (Figure 3). The major compounds detected were α-sitosterol (43.89%), stigmasterol (15.98%), and betulin (15.45%). The minor components of the ethyl acetate extract are found to be 8,11-octadecadienoic acid, methyl ester (1.80%), hexadecanoic acid, methyl ester (Palmetic methyl ester) (2.15%) and phytol (2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, (R-(R*, R*-(E)))) (2.66%). The oil from the aerial parts of *Z. jujuba* is also reported to contain phytol [28].

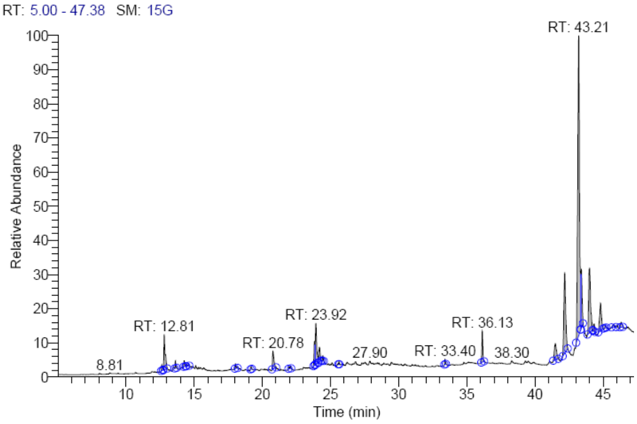

Figure 3. *GC-MS chromatogram of ethyl acetate extract of Ziziphus spina-christi (L.).*

Chemical composition of butanol extract of Z. spina-christi

Table 4. *Identified compounds in butanol extract of Z. spina-christi.*

RT (min)	Identified compounds	Chromatogram % area	MW
6.46	1,2-dihydeo-4-ethoxy-2-quinol one	4.71	189
8.72	1-butoxy-1-isobutoxy-butane	5.3	202
18.90	9-octadecenoic acid (Z)- oleic acid	1.82	282
19.62	Cholestan-3-ol, 2-methylene-, (3á, 5à)-	1.83	400
21.79	1,1-Dimethyltetradecylhydrosulfide	5.52	258
23.62	Dotriacontane	6.58	450
23.90	Phytol	21.31	296
24.38	14-a-H-pregna	11.59	288
29.84	Di-(9-octadecenoyl)-glycerol	6.78	620
32.48	Z,Z-4,16-octadecadien-1-ol acetate	3.44	308
42.17	Stigmasterol	4.26	412
43.21	á-Sitosterol (Stigmast-5-en-3-ol, (3á)-)	23.51	414
43.64	Betulin	3.45	442

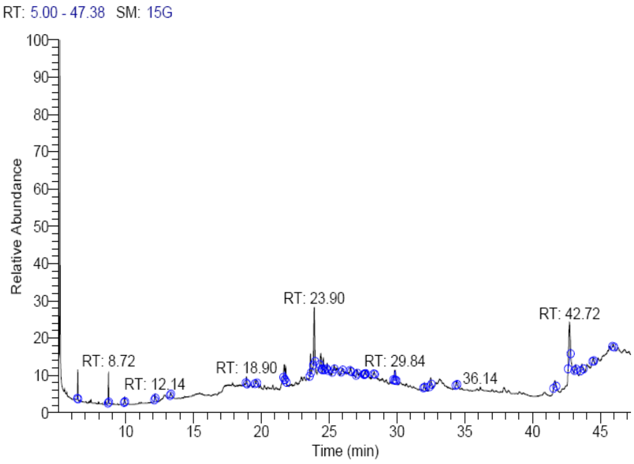

Figure 4. *GC-MS chromatogram of butanol extract of Ziziphus spina-christi (L.).*

The n-butanol extract was found to contain 13 different compounds, representing 100.1% of the total extract. The identified compounds are listed in Table 4 according to their elution order on TG-SQC GC column (Figure 4). The major compounds detected were á-sitosterol stigmast-5-en-3-ol, (3á)- (23.51%), phytol (21.31%) and 14-á-H-pregna (11.59%). Interestingly, phytol is also present as the main constituents of the oil from the aerial parts of *Z. jujuba* (29.1 %) [27]. As shown in Table 4, 9-octadecenoic acid (Z)-oleic acid (1.82%), cholestan-3-ol, 2-methylene-, (3á, 5à) (1.83%), Z,Z-4,16-Octadecadien-1-ol acetate (3.44%) and betulin (3.45%) were also found to be the minor components of the n-butanol extract.

Comparison between different extracts of Ziziphus spina-christi (L.)

Table 5. *Comparison between different extracts of Ziziphus spina-christi (L.).*

RT (min)	Identified compounds	Diethyl ether extract	Chloroform extract	Ethyl acetate extract	n-butanol Extract
6.46	1,2-Dihydeo-4-ethoxy-2-quinolone	-	-	-	+
7.34	3-Dodecene, (Z)-	+	-	-	-
8.72	1-butoxy-1-isobutoxy-butane	-	-	-	+
9.01	Pyrrolidine,1-(1,6-dioxooctadecyl)-pyrrolidine,	-	+	-	-
11.09	1-Tetradecene	+	-	-	-
11.22	Dichloroacetic acid, tetradecyl ester	-	+	-	-
12.81	Lanceol, cis(2E)-2-(4,7-dimethyl-3,4,4a,5,6,8a-hexahydro-1(2H)-naphthalenylidene)-1-propanol	-	-	+	-

12.84	2,6,10,15-tetramethyl heptadecane,	-	+	-	-
12.96	Quercetin, quercetin 7,3',4'-trimethoxy 4H-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy- (CAS)	-	+	-	-
13.17	Butyl hydroxytoluene	+	+	-	-
14.5	1-Hexadecanol	+	+	-	-
14.61	Octadecane, 1-chloro-	-	+	-	-
18.26	1-hexadecanol, 2-methyl	+	+	-	-
18.9	9-Octadecenoic acid (Z)-Oleic acid	-	-	-	+
19.62	Cholestan-3-ol, 2-methylene-, (3á,5à)-	-	-	-	+
20.59	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	-	+	-	-
20.78	Hexadecanoic acid, methyl ester (Palmetic methyl ester)	-	-	+	-
21.79	1,1-Dimethyltetradecylhydrosulfide	-	-	-	+
22.04	Hexadecanoic acid, ethyl ester(Palmitic acid, ethyl ester)	+	-	-	-
23.62	Dotriacontane	-	-	-	+
23.82	8,11-Octadecadienoic acid, methyl ester	-	-	+	-
23.92	9-Octadecenoic acid (Z)-, methyl ester (Methyl oleate)	-	-	+	-
24.19	Phytol (2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, (R-(R*,R*-(E)))-)	-	-	+	+
24.38	14-á-H-Pregna	-	-	-	+
25.01	Ethyl linoleate	+	-	-	-
25.11	(9-Octadecenoic acid (Z)-, ethyl ester) ethyl oleate	+	+	-	-
25.61	Octadecanoic acid, ethyl ester (Ethyl stearate)	+	+	-	-
29.25	Phenol,2,2'-methylenebis(6-(1,1-dimethylethyl)-4-methyl-	-	+	-	-
29.84	DI-(9-Octadecenoyl)-glycerol	-	-	-	+
32.48	Z,Z-4,16-Octadecadien-1-ol acetate	-	-	-	+
36.13	Heptacosane	-	-	+	-
41.49	Campesterol	-	-	+	-
42.17	Stigmasterol	-	+	+	+
43.21	á-Sitosterol Stigmast-5-en-3-ol, (3á)-	-	+	+	+
43.64	Betulin	-	+	+	+
44.8	Ethyl iso-allocholate	-	-	+	-

'+': Present; '-': Absent.

Comparing the chemical components of different extracts of *Ziziphus spina-christi* (L.) showed that few compounds are specifically present in certain extracts. tradecene, hexadecanoic acid, ethyl ester (ethyl palmitate), and ethyl linoleate are found only in diethyl ether extract. Seven different compounds are only found in the chloroform extract, namely pyrrolidine, 1-(1,6-dioxooctadecyl)-pyrrolidine; 2,6,10,15-tetramethyl heptadecane; quercetin, quercetin 7,3',4'-trimethoxy 4H-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy; octadecane, 1-chloro-; 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione; phenol, 2,2'-methylenebi([6-(1,1-dimethylethyl)-4-methyl-. The ethyl acetate extract alone, when compared with other extracts, had contained the following seven compounds: Lanceol, cis (2E)-2-(4,7-dimethyl-3,4,4a,5,6,8a-hexahydro-1(2H)-naphthalenylidene)-1-propanol; hexadecanoic acid, methyl ester (Palmetic methyl ester); 8,11-octadecadienoic acid, methyl ester; 9-octadecenoic acid (Z)-, methyl ester (Methyl oleate); heptacosane; campesterol; and ethyl iso-allocholate. The butanol extract alone, when compared with other extracts, had contained the following nine compounds: 1,2-dihydeo-4-ethoxy-2-quinol one; 1-butoxy-1-isobutoxy-butane; 9-octadecenoic acid (Z)-oleic acid; Cholestan-3-ol, 2-methylene-, (3á,5à)-; 1,1-dimethyltetradecylhydrosulfide; dotriacontane; 14-á-H-PREGNA; Di-(9-octadecenoyl)-glycerol; and Z,Z-4,16-octadecadien-1-ol acetate.

There are some compounds present in many extracts (Table 5 and Figure 5). Butyl hydroxytoluene (1) (BHT) also known as dibutylhydroxy toluene, is a lipophilic organic compound, chemically a derivative of phenol, that is useful for its antioxidant properties [29]. BHT is also used to prevent peroxide formation in diethyl ether and other laboratory chemicals. For example, Sigma Aldrich mentioned that 'Diethyl ether contains 1 ppm BHT as inhibitor, anhydrous, ≥ 99.7%' (Retrieved 11 September 2012). To make sure if the natural origin of BHT and not diethyl ether we inject diethyl ether as blank that gave negligible peak area compared to the main peak., 1-hexadecanol (2), 1-hexadecanol, 2-methyl (3), (9-octadecenoic acid (Z)-, ethyl ester) ethyl oleate (4) and octadecanoic acidethyl ester (Ethyl stearate) (5) were exist in both diethyl ether extract (7.00%, 21.30%,17.82%, 5.68% and 2.62%, respectively) and chloroform extract (1.96%, 1.99%, 3.03%, 3.38%, 1.23%, respectively).

Phytol (6) was present in n-butanol extract (21.31%) in higher concentration as compared with ethyl acetate extract (2.66%). Stigmasterol (7), sitosterol (Stigmast-5-en-3-ol, (8) and betulin (9) were found in three extracts (chloroform, ethyl acetate and butanol extracts). Stigmasterol (7) was present in ethyl acetate extract (15.98%) in large quantities as compared to butanol extract (4.26%) and chloroform extract (0.65%). á-Sitosterol (Stigmast-5-en-3-ol) (8) was present in ethyl acetate extract in large quantity (43.89%), more than that present in butanol extract (23.51%) and chloroform extract (0.68%). Betulin (9) was present in chloroform extract in large quantity (55.23%) as compared to ethyl acetate (15.45%) and butanol (3.45%) extracts.

Table 6. *Phytochemical constituants of extracts of Z. spina-christi stem-bark.*

Chemical constituents	Ethanol extract	Diethyl ether extract	Chloroform extract	Ethyl acetate extract	n-butanol extract
Saponin glycosides	+	+	+	++	+++
Carbohydrate	+	-	-	+	++
Flavonoids	+	+	+	+	++
Terpernoids	+	+	+	++	++
Alkaloids	+	+	+	+	++
Anthraquinone	-	-	-	-	-

+++: Strong intensity reaction; ++: Medium intensity reaction; +: Weak intensity reaction; -: Nondetected.

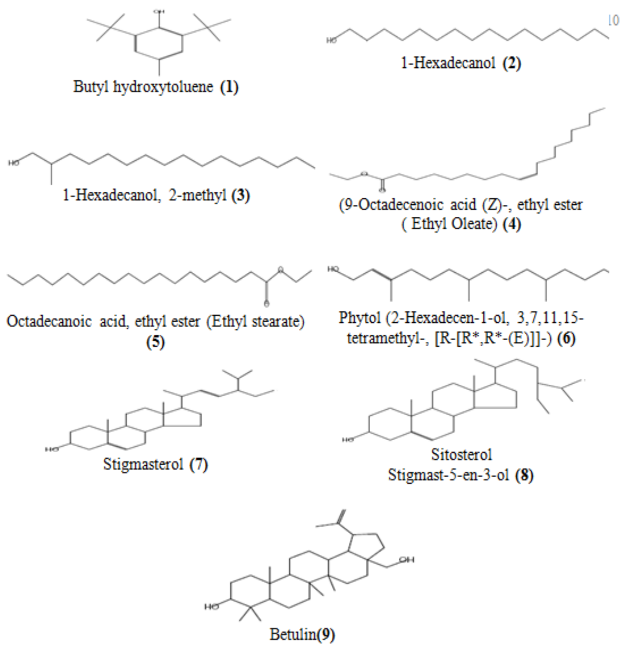

Figure 5. *Chemical structures of different chemical compounds identified in the stem bark of Z. spina-christi (Wiley and National Institute Standard and Technology (NIST) libraries mass spectral database of GC-MS).*

From the above Table 6, it's clear that the phytochemical screening for ethanolic extract, diethyl ether extract, chloroform extract and n-butanol extract showed presence of flavonoids, terpenoids and saponin glycosides and alkaloids. The butanol extract is the best one. While free and combined anthraquinones were absent, carbohydrates were also absent in both diethyl ether and chloroform extracts.

Phytochemical analysis of *Ziziphus spina-christi* (L.) leaves showed the presence of four saponin glycosides in the butanol extract [22]. As christinin-A was the major saponin glycoside, the butanol extract and chistinin-A were used to evaluate the potential antidiabetic activity and toxicity of *Ziziphus spina-christi* (L.) leaves [30]. Preliminary phytochemical analysis showed the presence of major classes of secondary metabolites such as tannins, alkaloids, flavonoids, cardiac glycosides, etc. in both of the extracts. Saponins, protein and amino acids were absent in both the extracts. Seed extract showed the absence of steroids and terpenoids while their presence was revealed in fruit extract [31].

Conclusion

In GC-MS analysis, 36 different chemical compounds were identified in the stem bark of *Z. spina-christi*. Diethylether, chloroform, ethyl acetate and butanol extracts fractionated from the ethanol extract of *Ziziphus spina-christi* (L.) contain 9, 17, 11 and 13 compounds, respectively. Diethyl ether extract is better than chloroform extract for isolation of butyl hydroxytoluene, 1-hexadecanol, 1-hexadecanol, 2-methyl, (9-octadecenoic acid (Z)-, ethyl ester) ethyl oleate and octadecanoic acid, ethyl ester (Ethyl stearate). Phytol was isolated effectively by n-butanol than ethyl acetate from the ethanolic extract of *Z. spina-christi*. Stigmasterol, á-Sitosterol Stigmast-5-en-3-ol, (3á) was isolated from ethanolic extract of *Ziziphus spina-christi* (L.) using ethyl acetate. Betulin was present in chloroform extract in large quantity as compared with ethyl acetate and butanol extracts. Butanol extract of *Ziziphus spina-christi* (L.) is rich in carbohydrates, flavonoids, terpenoids and saponin glycosides and alkaloids compared to other extracts. Further detailed investigations on the isolated compounds are needed to identify the phytoconstituents and study there antimicrobial and cytotoxic activities.

Acknowledgments

This work was supported by King Abdulaziz City for Science and Technology (Grant No. ص م88-35).

Conflicts of Interest

None

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