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FULL PAPER

Evaluating the Total Antioxidant Activity and Free Radicles Scavenger of Ziziphus Leaves, Bark Ethanoic Extracts, and the Aqueous Extract Of Ficus Leaves In Basrah

Prepared by

Fatimah Abbood Jasim
Southern Technology University
Basra Technical Institute
Basra, Iraq .
fatimah.a.jassim@stu.edu.iq

Nuha Mearad Neamah
Medical College
University of Basrah

Zahraa Hussain Jabar
Medical College
University of Basrah

Abstract

Ziziphus spina christi and Ficus carica are known for their activities and use in traditional medicine in treating many diseases besides being nutritious and healthy for eating. This study evaluated antioxidant activity in the bark and leaves, ethanoic extracts of Ziziphus spina Christi (L.) and the aqueous extract of Ficus carica. The extract's antioxidant activity was measured based on the scavenging activities of the established 1,1-diphenyl-2-picrylhydrazyl free radical scavenging method. The total antioxidant activity was measured using Total Antioxidant Capacity Reagent (TCA), using a UV-spectrophotometer against a blank, At the same time, the standard was a series of Ascorbic Acid solutions. The results revealed that the leaves extract of sider and f. carica has total antioxidant activity less than the sidr bark extract which was 300, 40 and 700 respectively. IC50 of fig leaves extract was 260.228 µg/ml and of sider leaves and bark extract were 124.433 & 1.7924 µg/ml respectively while it was 50.558 µg/ml for ascorbic acid. The low concentration of sider park has more antioxidants activity comparing with leaves from both sides and fig.

Keywords

leaves extract; free radical scavenger; Antioxidant Activity; Sider Bark properties; Ficus carica extract

1. Introduction

Plants are considered a supply of unique chemical compounds, that have capacity effects in medicine and different applications. Glycosides, steroids, alkaloids, tannins, volatile oils, fixed oils, resins, flavonoids, and phenols were active composites in plants, which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc. Different parts of the plant are utilized traditionally to treat numerous types of ailments. The fig fruits are used to cure headache, chewed for toothache and cold, whereas powdered root and leaves of the plant has been applied externally to wounds and sores[1].

Ziziphus spina-christi (L.), or Sidr (common name) is a deciduous shrub several purpose tree that belongs to the Rhamnaceae [2]. These species natural to the warmer and subtropical regions, including North Africa, South Europe, the Mediterranean, Australia, Tropical America, Asia (South and East) and the Middle East [3]. Fruits are usually used in old medication for curing of a variety of diseases, for example, they are applied to cuts and ulcers[4]. The bark of *Ziziphus* was reported to have antipyretic, sedative-hypnotic, and pain killing purposes [5]. While the Figs (*Ficus carica* L.) are an infructescences tree, a deciduous plant belonging to the Moraceae family, Fig fruit is an important crop consumed world-wide [6], where it is grown commercial, native in the Western Asia and Middle East, it has been cultivated since ancient times and is now commonly grown all over the world, both for its fruit and leaves rich with oil, the species has become naturalized in scat treed locations in Asia and North America.

Antioxidants are substances that play vital roles in stopping the pathogenic procedures associated with cancer, cardiovascular disease, macular degeneration, cataracts, and asthma, and they could beautify immune function, except that antioxidant defences shield the frame from the damaging outcomes of loose radicals generated as by-merchandise of everyday metabolism [7]. They are sometimes called the free-radical scavengers and the source can be naturally or artificially, certain plant-based food is thought to be rich in antioxidants and these are considered as a type of phytonutrient, or plant-based nutrients, the body also produces antioxidants known as endogenous while those come from the outside of the body are known as exogenous.Modern lifestyle conduct has caused many human beings to expand abnormally high levels of oxidative stress, which has been caused mainly by free radicals [8]. Reactive oxygen species (ROS), consist of free radicals such as hydroxyl (OH^-), superoxide (O^-_2), nitric oxide (NO), peroxyl (RO^-_2), lipid peroxyl (LOO^-) radical and non-free radical species like hydrogen peroxide (H_2O_2), singlet oxygen (O^{-1}_2), ozone (O_3) and lipid peroxide (LOOH) are different forms of activated oxygen [9].

ROS are produced by aerobic organisms and might without difficulty react with many organic molecules along with proteins, lipids, lipoproteins and DNA. This ROS can generate oxidative stress and produce many pathophysiological disorders such as arthritis, diabetes, inflammation, cancer and genotoxicity [10]. For protection against free radicals, organisms are endowed with endogenous (antioxidant enzymes) and exogenous defense systems. These systems are unable to protect tissues when the generation of free radicals is significantly increased[11].

The research aimed to detect the active components in both leaves and bark of *Ziziphus spina-christi (L.)* and leaves of *F. caica* , then estimated the antioxidant activities and calculated the concentration of sample required to scavenge 50% of free radicals (IC_{50}) of both extracts.

2. Experimental

2.1. Plant collection:

Fresh *Z. spina-christi (L.)* Leaves and bark were collected from trees growing in an Al - Zubair area (Basra, Iraq) between August and October of 2022. While the leaves of *Ficus carica* "figs leaves" were collected from two different cities (Karbala and Basra) then we dried those fig leaves and grounded them into fine powder.

2.2. Plant extraction:

First, the plant leaves and root bark were washed with distilled water and dried at room temperature (25^{o} C). The dried leaves and bark were grounded into powder using an electric blender. The ethanol extracts were done by adding Ten grams (10 g) of both leaves and bark powder, with 100 ml of ethanol-distilled water (8 : 2 w/v) prepared separately and placed on the magnetic sitter device without heat (DAIHAN LAB Tech. Co. LTD) for 24 h. Thereafter, it was filtered by filter papers (Qualitative filter papers No102). A ratio of 1:20 was used in forming the extract using fig leaves powder and distilled water placed on the magnetic sitter device without heat, then letting it dry. The filtered extracts were left to dry in sterilized Petri dishes in the hood. The dried extracts of leaves and bark were collected in separator glass containers and were kept in the refrigerator until the time of use in the biological experiment.

2.3. Antioxidant Activity Test

For the antioxidant activity test, 300 µL of the solution was dissolved in 3 ml of Total Antioxidant Capacity Reagent (TCA), which was prepared by mixing 7.5 ml of sulfuric acid, 0.99 gram of anhydrous sodium sulfate, and 1.24 gram of ammonium molybdate in 250 ml of distilled water. Using a UV-V spectrophotometer, absorbance was measured at 695 nm against a blank of distilled water and a series of Ascorbic Acid solutions of known concentrations as standard which were started with 0, 200,400, 600, 800 and 1000 mg/ml [12].

2.4. 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

Based on the scavenging activities of the established 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, MO), the antioxidant activity of all extracts was calculated. Three milliliters of a 0.004% (w/v) methanol solution of DPPH were added to 1000 ml of various concentrations (20,40,60,80 and 100 µg/ml) of the test substances. The absorbance at 517 nm was measured in comparison to a blank after 30 minutes of storage at room temperature in a dark area. Inhibition of free radical DPPH in percent (%) was counted by the formula:

Percentage inhibition of DPPH (%) = [(A blank – A sample) / A blank)] * 100

The absorbance A blank represented the control reaction (holding all reagents excluding the test extracts), and A sample represented the absorbance of each extract. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were counted from the recession equation, prepared from the concentration of the sample, and percentage inhibition of free radical formation/ percentage inhibition DPPH. Synthetic antioxidant reagent L-ascorbic acid (Sigma-Aldrich, St. Louis, MO), was used as positive control and duplicate were prepared in all tests [13].

2.5. GC-MS analysis:

The samples of the two dried ethanolic extracts of Sider leaves, bark and fig leaves were subjected to GC-MS Analysis in Agriculture College – University of Basrah to chemical compounds detected. By using the NIST Mass Spectral Library and the Retention Index Database, the identification of compounds was by their mass spectra and maintenance indices**.**

3. Results and discussion

3. Results

The results revealed that the 5 mg/ml leaves have a total antioxidant equal to 300 while the same concentration from sidr bark has 700, while the fig leaves extract has a total antioxidant of about 40 which detects that the 5 mg/ml (Figure. 1)

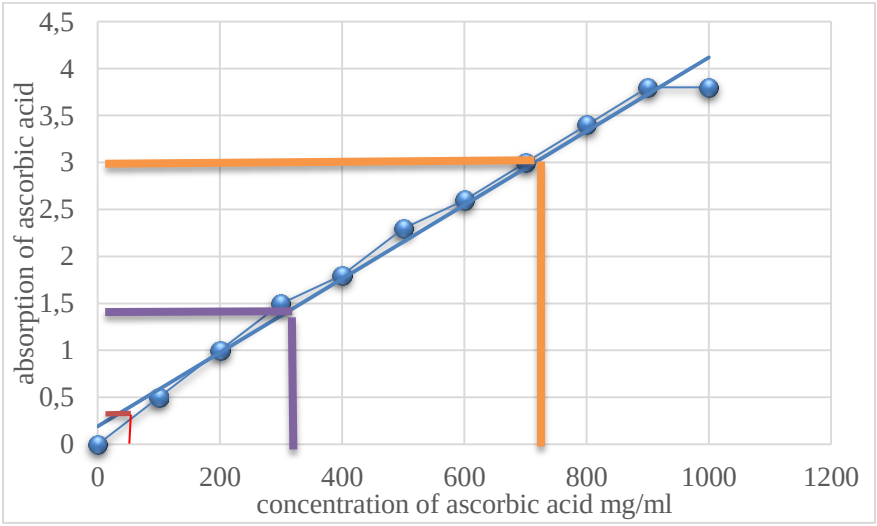

Figure 1. The total antioxidant of Fig leaves (red), Sidr leaves (orang), and bark (green) extracts.

The free radical inhibition by DPPH was high percentage sider leaves and bark extract as well as of the fig leaves extract and are shown in Figure 2. The bark extract revealed a high

activity at a low concentration. When the concentration of the fig leaf extract was rising, its activity was increased. In addition, the results revealed that IC_{50} of sider leaves and bark extract were 124.433 & 1.7924 µg/ml respectively and IC_{50} of fig leaves extract was 260.228 µg/ml while it was 50.558 µg/ml for ascorbic acid (Figure3).

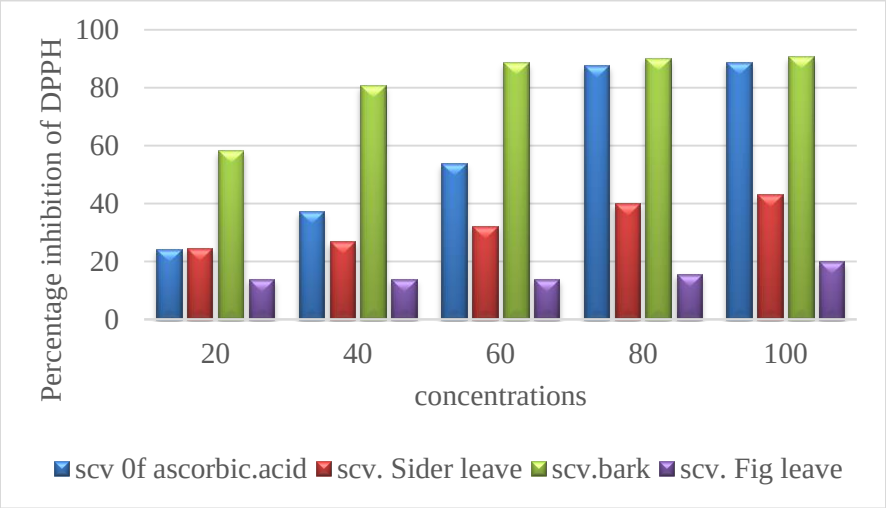

Figure 2. The inhibition percentage of free radical DPPH for fig leaves, sider leaves, and bark extracts.

The GC-MS chromatogram contained 25 peaks corresponding to 25 diverse compounds from sider bark extract while 14 different components were detected in sider leave extract (Figs. 4, 5.) Tables 1, 2 and 3 contain the concentration and identity of the detected compounds in experiment extract respectively. The GC-MS chromatogram revealed 25 peaks equivalent to 25 different compounds, as shown in Figure 6. Table 3 contains the identity and concentration of these compounds.

The concentration of identifying combinations ranged between 18.07% n-Hexadecanoic acid, 4.53 % Phenol, 2,2'-methylenebis [6-(1,1-dimethylethyl) -4- Methyl and 11.58 % Cyclopentadecanone, 2-hydroxy. Octadecanoic acid and its derivatives are communal saturated fatty acids that made up 3.40 % of the extracts.

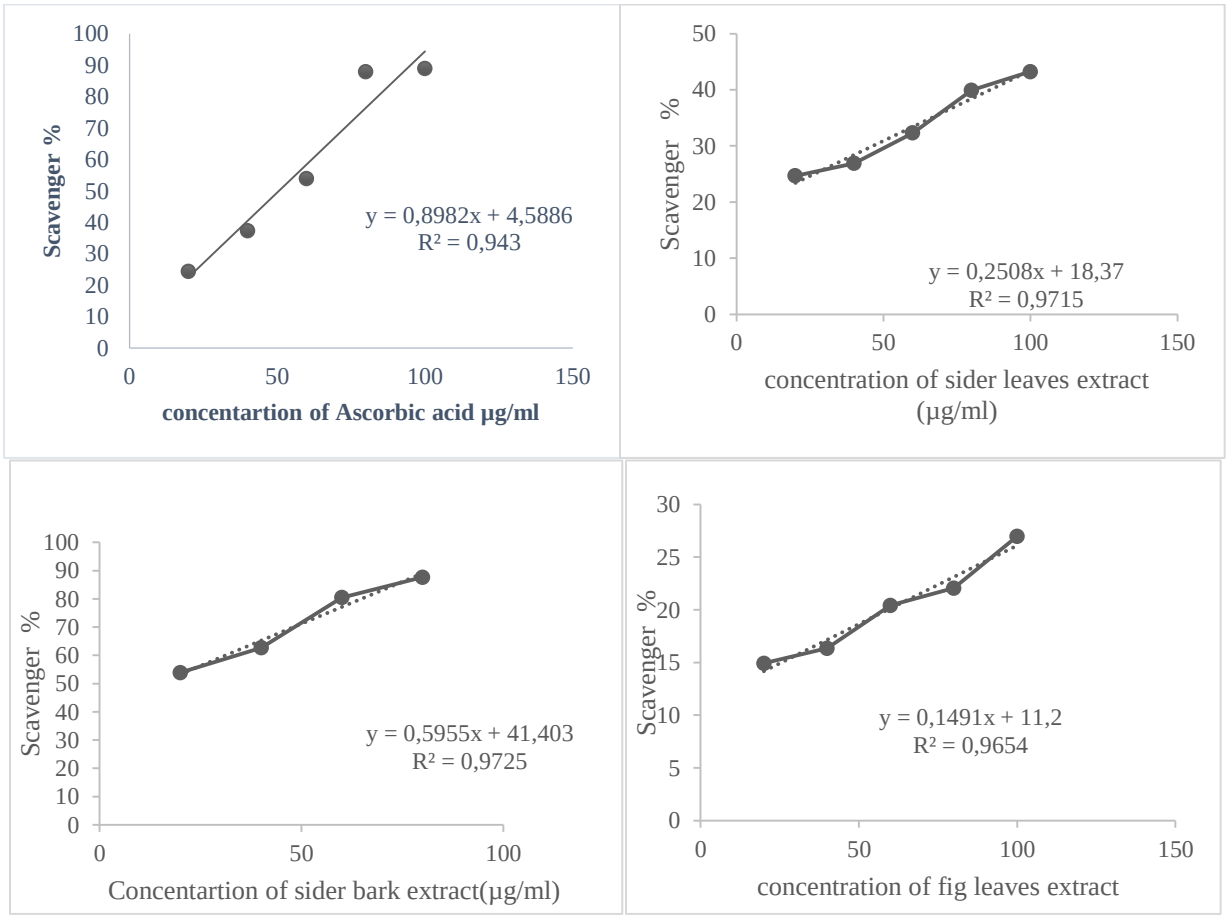

Figure (3) IC50 calculated in ascorbic acid, fig leaves, sider leaves and bark extractions.

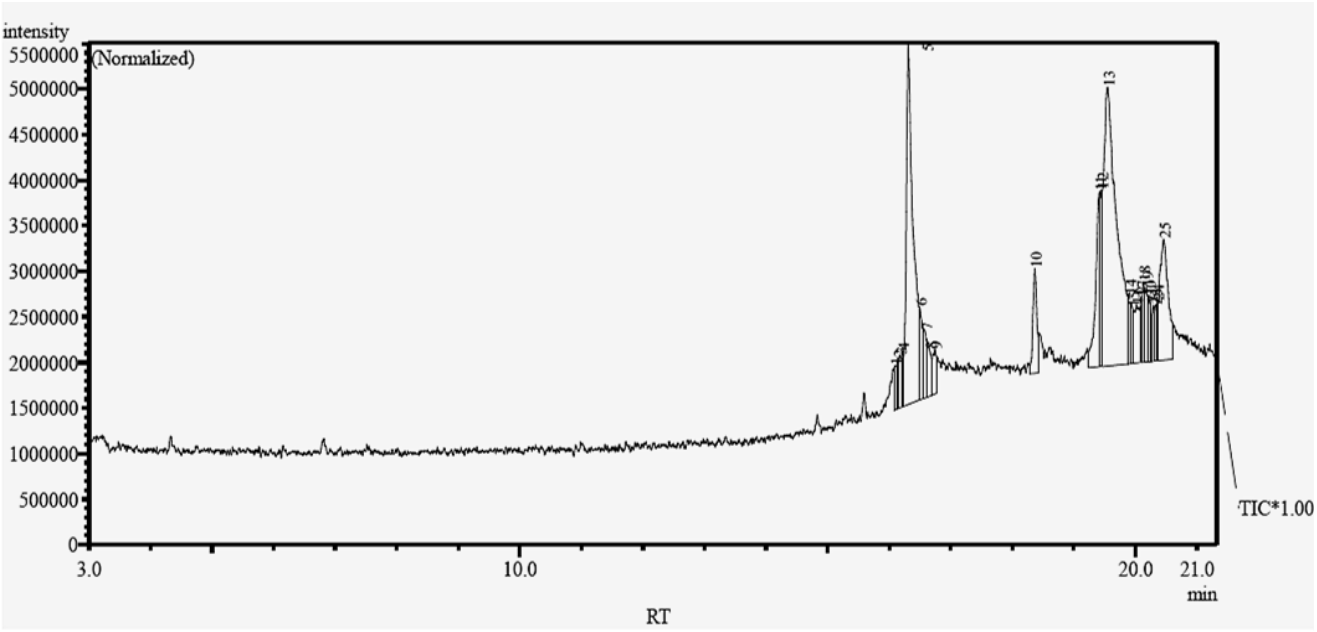

Figure 4. Chromatogram Scan by GC-MS for the sider bark extract

Table 1. Identity and retention time of compounds identified with GC-MS of sider bark extract.

Peak#	Ret. Time	Area%	Name
1	16.093	0.80	2H-9a-Methano-1benzoxepin-4,5,6,7,9,10-hexol, 5a-[(acetyloxy) methyl] octahydro-2,2,9-trimethyl-, 4,6,7,10-tetraactetate-5- benz
2	16.140	0.75	4-[3-(2,5-Dioxo-pyrrolidin-1yloxycarbonyl)-propionyl]-piperazine-1carboxylic acid, ethyl ester
3	16.173	1.09	Acetic acid, 17-(1,5-dimethyl-hexyl)-4,4,10,13,14-pentamethyl-2,3,4,5,8,10,12,13,14, 15, 16,17-dodecahydro-1H-cayclopenta [a] phenan
4	16.213	0.85	Pregnan-20-one,3,2,1,bis[(trimethylsilyl)oxy]-,O-methyloxime,(3,alpha,5,beta)
5	16.312	21.50	Pentadecanoic acid
6	16.493	2.50	Tridecanoic acid
7	16.580	1.61	Hexa-t-butylselenatrisiletane
8	16.653	1.89	Pentacosanoic acid, 2-[(trimethylsilyl)oxyl]-, methyl ester
9	16.747	1.58	Nonanoic acid
10	18.368	3.61	Heptadecyl trifluoroacetate
11	19.413	6.77	E-2-Hydroxymethylcylopentanol, bistrifluoroacetate (ester)
12	19.447	3.25	Picoolinyl 13-cyclopent-2-enyltridec-4-enoate
13	19.547	31.12	cis-Vaccenic acid
14	19.900	1.43	4-Amino-6-morpholine-5-nitropyrimidine
15	19.953	1.23	10,13,-Eicosadienoic acid, methyl ester
16	20.013	2.69	Na-(3,5-dinitrobenzoyl)tyrosine N'-[4-(dimethylamine) benzylidene) hydrazide
17	20.087	0.79	15—Methylheptatriacontane
18	20.129	1.82	Cyclododecanone, 2-pyridylcarbonylhydrazone
19	20.175	1.53	Docosyl trifluoroacetate
20	20.220	0.98	Hexacosane ,13-dodecyl-
21	20.247	0.81	Crystalline Antibiotic
22	20.293	1.13	2-Idohiistidine
23	20.327	0.83	Dichloroacetate acid, 3-tridecyl ester
24	20.360	0.73	Isopropyl stearate
25	20.461	8.71	Phenol, 2,2'-methylenebis[6-(dimethylethyl)-4-methyl-

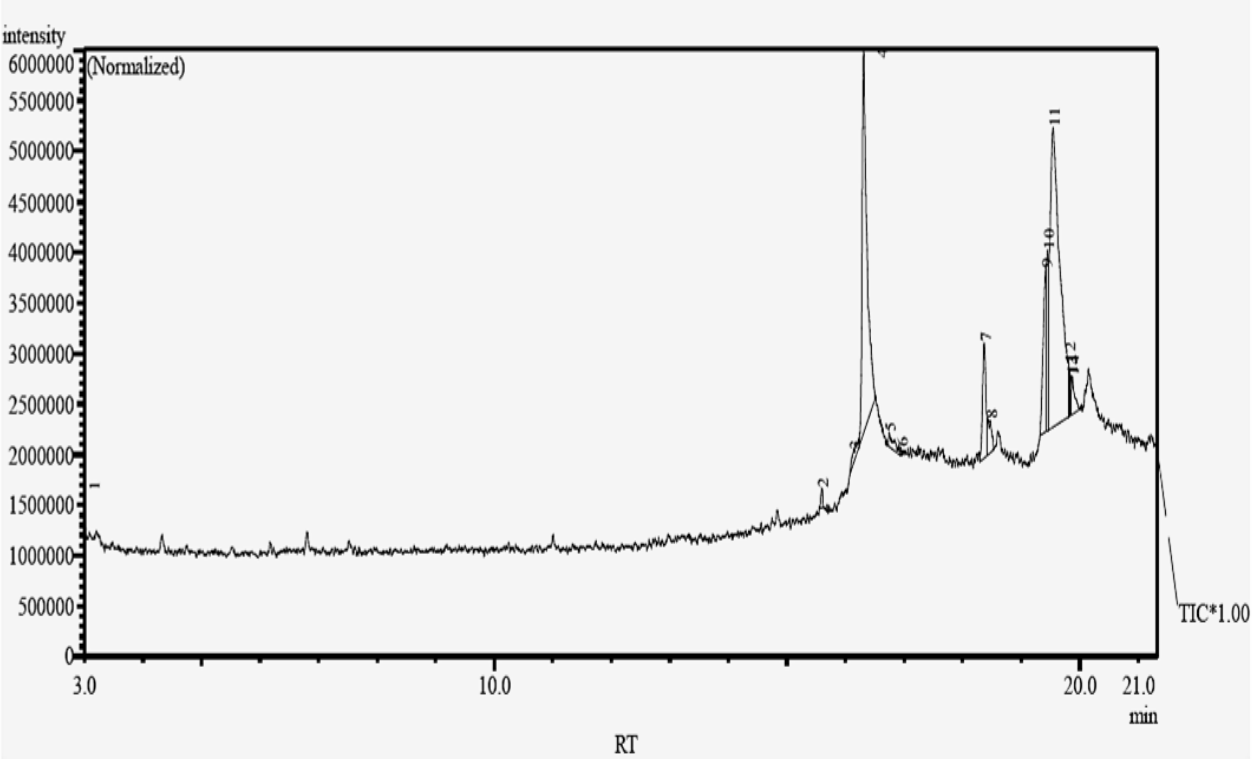

Figure 5. Chromatogram Scan by GC-MS for the sider leaves extract

Table 2. Identity and retention time of compounds identified with GC-MS of sider leaves extract.

Peak#	Ret. Time	Area%	Name
1	3.020	0.46	Serverogenin acetate
2	15.596	0.52	Hexadecanoic acid. Methyl ester
3	16.133	1.24	Methyl 23-hydroxycholate tetrakis(trimethylsiyl)-
4	16.309	27.48	Pentadecanoic acid
5	16.740	1.19	2-Hexyldecanoic acid
6	16.947	0.09	n-Octadecyl-N'-6-[N-azirdyl]hexylthiourea
7	18.336	5.87	1-Heneicosyl formate
8	18.473	1.94	13-Oxabicyclo[10,1,0]tridecane
9	19.413	8.64	9-Octadecenoic acid(Z), 2-hydroxy-1-(hydroxymethyl)ethyl ester
10	19.447	4.46	Methyl 9,12-heptadecadienoate
11	19.543	44.61	cis-9-Hexadecenal
12	19.813	1.23	Hexacosanoic acid, propyl ester
13	19.847	0.70	Eicos-9-ene-1,20-diacetate
14	19.873	1.57	Hexadecanoic acid, dodecyl ester

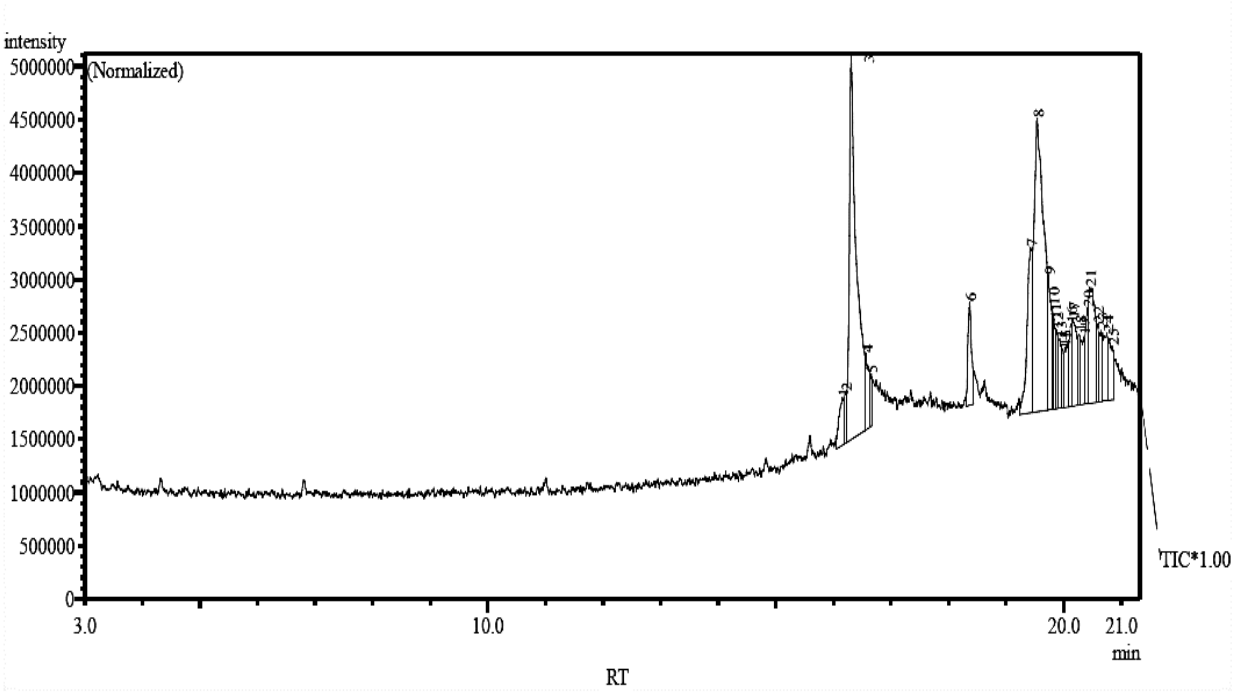

Figure 6. Chromatogram Scan by GC-MS for the fig leaves extract**.**

Table 3. Peak No., Identity, percentage and retention time of compounds identified with GC-MS for the fig leaves extract.

Peak#	Ret.Time	Area%	Name
1	16.153	2.07	Ethyl3,4-dichloro-5-[4-chloro-5-[4-(2,2,2-trichloroethoxycarbonyloxy) phenyl]oxazol
2	16.207	0.85	Propanoic acid, 2-(3-chlorobenzoylamino)-2-(5,6-dihydro-3-cyano-4H-cyclopenta[b]thien-2-ylamino)-3,3,,3-trifluoro-, ethyl ester
3	16.309	23.00	N Hexadecanoic acid
4	16.567	2.47	N Hexadecanoic acid
5	16.656	0.81	Fumaric acid, 3,3-dimethylbut-2-yl nonadecyl ester
6	18.367	3.25	n-Nonadecanol-1
7	19.420	7.49	Phosphine, 9-methyl-3-hexyn-2-yl)diphenyl-
8	19.539	23.65	Cyclopentadecanone,2-hydroxy-
9	19.733	3.17	Chloromethyl 5-chloroundecanoate
10	19.805	1.33	Thiocyanic acid, 4 alpha-methyl-5-alpha.-cholestan-3.alpha-yl ester
11	19.833	1.36	Fumaric acid, cis-non-3-enyl hexyl ester

12	19.880	0.85	1-Dimethyl(pentafluorophenyl)silyloxytridecane
13	19.933	1.69	Urea, 1,3-bis(tricycle[5,2,1,0)]dec-2-yl)-
14	19.987	1.11	E,E-10,12-hexadecadienal
15	20.013	2.34	Solasodine benzoate
16	20.120	1.51	17,21-octacosadienoic acid, pyrrolidide
17	20.155	3.07	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester
18	20.273	1.71	Phosphonochloridous acid (1-methylethyl)-, 5-methyl-2-(1-methylethyl)cyclohexyl ester
19	20.342	1.77	Pentadecanoic acid, 15-bromo
20	20.407	2.08	Hexaco1-mMethyl-1-octadecyloxy-1-silacytopentane
21	20.454	6.56	Phenol, 2,2-methylenbis[6-(1,1,dimethylethlen)-4-methyl-
22	20.587	1.01	Cyclopropane, 1 bromo-1-(3-dimethyl-1-pentenylidene)-2,2,3,3-tetramethyl-
23	20.652	1.66	1-Cyclohexano[2,3-b]Cholestan-1-‘one
24	20.753	2.69	Triacontane
25	20.840	2.48	5-Cholesten-3beta-yl isobutyl carbonate

4. Discussion .

The antioxidant activity was thought to arise from the presence of flavonoids and steroids in the extracts that were taken from fig leaves in addition to that, it is considered to be a rich source of polyphenolic compounds.

Phytochemical surveys on *Z. spina-christi* have revealed it has many biologically essential phytochemicals composed. Several studies have revealed that some alkaloids, isoquinoline, cyclopeptide, flavonoid terpenes and their glycosides have been detected in different volumes in most species of *Ziziphus* [14], [15]. The results showed that both leave and bark have similarities in their components with some differences in quantity and quality. Most plant leaves contain ceanothic and betulinic acids, numerous flavonoids, peptide, saponins, erols, and Polyphenols (as tannins and triterpenes) [16]. From the several *Ziziphus* species, the cyclopeptide alkaloids, tannins, betulinic acid flavonoids and triterpenoids saponin glycosides have been isolated and chemically recognized [17]. Steroids, β-sitosterol, β-D-glucoside, reduced tannins, and 4 saponin glycosides had been isolated from the leaves as well as glucose, fructose, sucrose, raffinose, galactose, and rhamnose had been extracted from various parts of the plants [18]. The extract of *Z. spina-christi* contained cyclopeptides ceanothic acid (a ring-A homolog

of betulinic acid), butic acid, similar as saponin, glycoside , flavonoids, protein, lipids, free sugar, and mucilage [13, 14]. Polyphenols (such as tannins) and glycosides are also extracted from the leaves.

The antioxidant activity of several flavonoids, consider scavenging because of scavenger free radicals, even higher than vitamin E, vitamin C, or glutathione [21].

The major components detected in leave and bark sider extract were Pentadecanoic acid (21.50 %), cis-Vaccenic acid, Phenol, 2,2'-methylenebis[6-(dimethyl ethyl)-4-methyl-, E-2-Hydroxymethylcylopentanol, bistrifluoroacetate (ester) and Na-(3,5-dinitrobenzoyl)tyrosine N'-[4-(dimethylamine) benzylidene)hydrazide. Comparing with the major compounds detected by Al-Rlrahman 7-methyl-6-oxo-1,2,34-tetrahydro-6hpyr, n-hexadecanoic acid, ethyl formate, gamma-sitosterol, D-liomone, the ethyl ester of hexadecanoic acid, octadecanoic acid, and ethyl ester due to the highly contents of phenol and flavonoids compounds [22]. The phytochemical analytic and GC-MS reading conducted by the ethyl acetate extract from stem bark of Ziziphus spina growing in Saudi Arabia showed both the stem bark and the roots gave positive results for more secondary metabolites, included 11 components. These variances might be due to geographical and environmental factors as well as the time of harvest and age of the plant which were recognized as key influences the chemical composition. Secondary metabolites have kindly antioxidant possessions, the resulted as well as Abdl- Rlrahman, 2018 demonstrated the possible antioxidant action of *Z. spina* roots extracts which can be used as an obtainable source of natural antioxidants to reduce oxidative stress with resulting health benefits [22] .

In the current study, *F. carica* L. leaves, sider stembark, and leaves extracts were subjected to valuation of total antioxidant contented as well as, DPPH through the IC_{50} values which was less in leave than bark, that capable with a remarkable antioxidant activity. The antioxidant activity differences observed among different species of *Ziziphus* may be attributed to phenolic compounds, which depend on the area [20 , 21], species [25], genotype [26] and/or the extraction technique [24 , 25]. These data extend to approve the presence of substantial amounts of phenolics in Tunisian *Ziziphus* extracts indicating that they are a significant source of antioxidants that may provide health-promoting advantages to consumers and they are also highly nutritious and rich in vitamin C [29] also in Oman [30]

The presence of free radicals causes some changes and damages to the structure of cells, which cause them to fail to do the desired task that they are made for or that will even start attacking the body and causing harm. Free radicals damage all components of cells, including DNA, proteins, and lipids and the destruction of cells in the normal reduced state can cause toxic effects through the production of peroxides [31]. There has been a growing interest in the use of strong antioxidants for medicinal purposes, particularly for oxidative stress-related metabolic disorders [32]. The high antioxidant activity of the different bioactive compounds in *F. carica* fruit ethanoic extract also proved it to be a potential source of free radical scavenging antioxidants

Total phenolic compounds, flavonoids, condensed tannin substances and antioxidant activity of methanolic extracts of different *Ziziphus* species parts. Plant polyphenols are a wide

group of secondary metabolites that can variety from simple molecules, like phenolic acids to highly polymerized constituents such as tannins [27, 29]. As well as the phenolic compounds founded in fig tree parts [34], [35]. There are composites with a large number of derivatives in the plant kingdom. All the phenolic compounds, but especially flavonoids, have been reported to have numerous biological properties such as antioxidant activity, which can terminate or retard the oxidation process by scavenging free radicals. These antioxidants are considered possible, protection agents for reducing oxidative damage to the human body from ROS[36]. Also, they act as anti-inflammatory mediators [37], antimicrobial activity [38] and inhibition of platelet accumulation [39].

5. Conclusions

The results conclude the leaves extract from both sidr and fig has total antioxidant less than the sidr bark extract also the capacity of free radical's activity of sider bark extract more than leave extract which detected by DPPH. These abilities resulted from the extract components, mainly unsaturated fatty acid, phenols and flavonoids.

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