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Chemical Composition and Antimicrobial Activity of *Ziziphus jujuba* Seeds Extract

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The aims of this study were to test the antibacterial activity and chemical composition of *Ziziphus jujuba* extract. The extract was obtained using 50% aqueous - ethanol extraction solution to extract *Ziziphus jujuba* seeds. The extract was prepared and evaluated for antimicrobial activity against six bacterial strains by determining minimum inhibitory concentration (MIC). The results revealed that the 50% aqueous - ethanol extract is potent in inhibiting bacterial growth of both gram-positive and gram negative bacteria. The chemical composition of fenugreek was analyzed by gas chromatography/mass spectroscopy (GC/MS). The 13-Heptadecyn-1-ol (12.95%), 7-Ethyl-4-decen-6-one (9.73%), Lineoleoyl chloride (8.54%), Linoleic acid (6.37%), 2,5-Octadecadiynoic acid, methyl ester (5.57%) and Palatinol A (4.81%) were the highest abundant compounds out of total 20 compounds were identified in the *Ziziphus jujuba* seeds extract.

Key words: *Ziziphus jujuba,* Chemical Composition, GC-MS, Antibacterial activity.

Plants are an essential part of human society since the civilization started. Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. In the last decades, various plant extracts have been the focus of great interest from researchers because they represent natural resources of new antibacterial agents with possibly novel mechanisms of action. The potential use of these products as an alternative for the treatment of several infectious diseases has been extensively screened. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[5]. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterisation of their constituents. Systematic screening of them may result in the discovery of novel active compounds.

The *Ziziphus* species (Rhamnaceae family) are considered to be multipurpose plants and have been used as foods, folklore medicines, the environmental protection plants, etc.[1]. *Ziziphus jujuba* Mill. (Rhamnaceae) mainly distribute in the tropical and subtropical regions of Asia and have

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been employed as essential oriental medicine for thousands of years. Different parts of the plant could be used as remedies in insomnia, fever, diarrhoea, wounds and ulcer, in which the fruits were claimed to be beneficial to purify the blood and aid digestion[2,3]. Also it has been widely distributed in northern China. Its fruits and seeds are usually applied in traditional medicine (TCM) for the treatment of various diseases, such as anorexia, lassitude, insomnia, anxiety, etc.[4], and many studies about their chemical constituents[5-7] as well as pharmacology activities[8, 9] have been reported.

A lot of extraction methods and analytical methods such as spectrophotometry, high performance liquid chromatography, capillary electrophoresis, gas chromatography (GC) with flame ionization detection (FID), gas chromatography–mass spectrometry (GC–MS) are developed for plant active compounds study. The combination of an ideal separation technique (GC) with the best identification technique (MS) made GC/MS an ideal technique for qualitative and quantitative for volatile and semi-volatile compounds. In addition, the use of a proper extraction method is needed.

This study aimed to evaluate the antimicrobial activity of *Z. jujuba* extracts and identify the active compounds of *Z. jujuba* seeds extract. To our knowledge this is first report on the study of antimicrobial components extracted from *Z. jujuba* seeds against the clinical Pathogens action. The assessment might provide a basis for searching the potent active compounds for the antimicrobial related search and improve the therapeutic application of Ziziphus species.

MATERIALS AND METHODS

Preparation of extracts

The Fresh Ziziphus seeds, was purchased from a local market at Riyadh, Saudi Arabia. About 100 g of fenugreek seeds were crushed in a mortar. Exactly 10 g of fenugreek seeds powder were soaked in 100ml of 50% ethanol water with agitation at 40°C. The EtOH:H_20 extract was then filtered, evaporated under steam of nitrogen using sample concentrator model Techne DB.3 (Techne, UK). The yield of the aqueous-ethanol extract was 1.8g. Aliquot of the extract was resolved in ethanol to a final concentration of 1.0 mg/mL.

Analysis and identification of compounds

The chemical composition of fenugreek extract was identified according to Priya *et al*.[10]. Chemical identification of components was assigned by matching their mass spectra with Wiley and NIST library data, standards of the main components and comparing their Kovats Retention Indices (KRI) with reference libraries[11,12] and from the literature. The component concentration was obtained by semi-quantification by peak area integration from GC peaks and by applying the correction factors.

Microorganisms

Six bacterial strains used in this study, including *Bacillus cereus, Staphylococcus aureus* as Gram positive bacterium. *Escherechia coli, Pseudomonas aeruginosa, Klebsiella peunomonia* and *Listeria monocytogenes* as Gram negative bacterium. These organisms were obtained from ATCC (American Type and Collection Center). The bacteria rejuvenated in Mueller-Hinton broth MHB (Difco, USA) at 37°C for 18 h and then stocked at 4°C in Mueller-Hinton Agar MHA (Sigma, USA).

Antibacterial assay

The method reported by Baqir *et. al*. 1985[13] has been adopted. The tests were run in triplicate. Petri plates (23x23 mm) were prepared with Trypticase soy agar and an adequate amount of inoculum was flooded onto each plate, excess inoculum was removed and the plates were dried for 30 min at 37°C. Holes (6 mm diameter) were made in the inoculated agar and filled with samples of plant extracts, plates were incubated for 24 h at 37°C. Inhibition zones when present were measured in millimeter (Table 2).

Antimicrobial activity assay

The Antimicrobial activities were determined by Kirby Bauer Disc diffusion method described by Bauer *et al*. 1966[14]. The extracts were prepared and the sterile blotting paper disc (5 mm) was soaked in the diluted extract in two different final concentrations (50 µl and 100 µl/disc). The prepared disc were dried in controlled temperature (at 37 °C overnight) to remove excess of solvent and used for study.

Determination of Minimum Inhibitory Concentrations (MIC)

The antimicrobial activity of the *Ziziphus*

jujuba extract, that shows antimicrobial activity, were determined using microdilution broth method as described by Brantner and Grein, 1994[15]. Different antibiotics [Ampicillin, amikacin, gentamicin, kanamycin, and tetracycline (10–32 ∝g/ml)] were used as reference standards (CLSI, 2011). The *Ziziphus jujuba* extract solution was prepared to obtain final concentrations of 0.25-2.0 mg/ ml for antibacterial testing. One microliter of an overnight culture of each bacterial strain, containing approximately 10^4 CFU, was applied onto a 96-well microtiter plate in the presence of MHB. The microtiter plates were incubated at 35°C for 18 h. Observations were performed at least in replicate and results were expressed as the lowest concentration of plant extracts that produced a complete suppression of colony growth, MIC.

RESULTS AND DISCUSSION

With the increase in the incidence of resistance to antibiotics, alternative natural products of plants could be of interest. Some plant extracts and phytochemicals are known to have antimicrobial properties, which could be of great importance in the therapeutic treatments. In the last years, various studies have been conducted in different countries, demonstrating the efficacy of this type of treatment[16]. The chemical composition of *Ziziphus jujuba* seeds is given in Table 1. These seeds are a rich source of fiber and protein. The fiber may be further classed as gum (gel fiber) and neutral detergent fiber. The protein fraction contains the amino acid 4-hydroxyisoleucine, which has been proven to stimulate insulin production. Whole Fenugreek seeds also contain 4.8% saponins. Fenugreek seed saponins are of steroidal nature (type furostanolsaponins) with diosgenin as the principal steroidal saponin.

The 50% aqueous-ethanol extract of *Ziziphus jujuba* seeds were screened for their antimicrobial activity at three different concentration (50,250 and 1000 µg/ml) against *Staphylococcus aureus*, *K. pneumonia* and *Listeria monocytogenes*. The results showed that the growth of *B. cereus* and *S. aureus* was inhibited at a MIC value of 72.5 and 41.25µg/ml respectively, followed by *E. coli*, *P. vulgaris* and *K. pneumonia*, while *Ps. aeruginosa* showed highest MIC value of 89.25µg/ml. The poor activity of the 50 % ethanol/water extract against most bacterial strains investigated in this study is in agreement with previous reports[17, 18]. This could be due to the insolubility of the active compounds in water or the hot water could have caused denaturation of the active compounds. It is also observed from the results that the ethanol/water extract had wide antibacterial activity (Table 2) against both gram positive and gram negative bacteria *S. aureus* and *S. typhi*, respectively, (Table 4). The activity of the

Table 1. Proximate Composition (%) of *Ziziphus jujuba* seeds extract

Component	Seeds
Moisture (%)	87.5
Total Soild	12.32
Ash	5.1
Carbohydrate (%)	15.45
Protein	3.31
lipid	1.3
Total Sugar (%)	12.9

Table 2. Mean inhibition zone diameter (mm) of 50% aqueous-ethanol of *Ziziphus jujuba* on tested microorganisms by disc diffusion method with respect to various concentrations in µg/ml

Bacteria	50 % Eth./H_2O Extract		
	50	250	1000
Bacillus cereus	7.0	6.0	13.0
Staphylococcus aureus	-	-	10.0
Escherichia coli	6.0	8.0	17.5
Pseudomonas aeruginosa	-	-	-
Klebsiella pneumonia	-	9.0	17.0
Listeria monocytogenes	-	7.0	16.5

Table 3. Minimal inhibitory concentration (MIC) of 50% ethanolic extract of *Ziziphus jujuba* seeds against different strains (µg/ml)

Bacteria	MIC
Bacillus subtilis	72.5
Staphylococcus aureus	41.25
Escherichia coli	52.5
Pseudomonas aeruginosa	89.25
Klebsiella pneumonia	42.5
Listeria monocytogenes	51.25

extracts against the Gram negative bacteria is noteworthy as these bacteria are known to exhibit high degree of resistance to conventional antibiotics[19]. The few variations in results between the disc diffusion and MIC results can be due to the different susceptibility of the bacterium to the plant extract, the rate of growth of bacteria, solvents used to extract the plant compounds and the rate of seeds extract diffusion[20].

Table 2, A side from concerns with food quality degradation, these microorganisms may be causal agents of intestinal infections in humans. According to the values of microbial growth rate in the presence of different extract concentrations, *Ziziphus jujuba* extract was presented antimicrobial capacity following the order: *E. coli ~Ps. Aeruginosa> B. cereus ~K. pneumonia> S. aureus> E. coli* and *Ps. aeruginosa* (Gram-negative) were the most sensitive microorganisms even at lower concentration. *Pseudomonas aeruginosa* was the most resistant microorganism even at higher concentration.

The MIC as low as ∝g mL^{-1} of a semi-purified fraction against gram negative and positive bacteria is suggestive of good antibacterial potential of the compounds of *Ziziphus jujuba*. Hence *Ziziphus jujuba* may yield potential molecules in the treatment of infections caused by pathogenic bacteria which have developed resistance against the known antibiotics, Singleton, 1999[21].

Chemical composition of *Ziziphus jujuba* seeds extract

Figure 1 presented the typical GC/MS chromatogram of a total of 20 compounds were recorded in solvent extracts as indicated in Table 4. Most of these identified compounds are playing a role in the biological activity of natural extracts. Some of these compounds are reported for the first time in *Ziziphus jujuba* seeds. The major compounds characterized were 13-Heptadecyn-1-ol (12.95%), 7-Ethyl-4-decen-6-one (9.73%), Lineoleoyl chloride (8.54%), Linoleic acid (6.37%),

Table 4. Identified compounds of 50% aqueous-ethanol extract of *Ziziphus jujuba*

#	Retention Time (min.)	Compound name	Mol. wt.	Molecular Formula	% of Total
1	4.475	Linoleic acid	280	$C_{18}H_{32}O_2$	6.37
2	5.765	AC1LC4P9	556	$C_{34}H_{36}O_7$	3.13
3	6.657	7-Ethyl-4-decen-6-one	182	$C_{12}H_{22}O$	9.73
4	7.346	1-Oxaspiro[2.5]octane, 5,5-dimethyl-4-(3-methyl-1,3-butadienyl)-	206	$C_{14}H_{22}O$	5.57
5	8.1	13-Heptadecyn-1-ol	252	$C_{17}H_{32}O$	12.95
6	8.549	3,5-Heptadienal, 2-ethylidene-6-methyl	150	$C_{10}H_{14}O$	3.45
7	8.641	2,5-Octadecadiynoic acid, methyl ester	290	$C_{19}H_{30}O_2$	4.81
8	9.502	AC1LCD4M	334	$C_{20}H_{30}O_4$	3.28
9	10.222	Icosapentaenoic acid	302	$C_{20}H_{30}O_2$	1.64
10	10.945	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	268	$C_{16}H_{28}O_3$	4.40
11	11.804	Androstan-17-one, 3-ethyl-3-hydroxy-, (5±)-	318	$C_{21}H_{34}O_2$	1.29
12	12.067	[5,9-Dimethyl-1-(3-phenyl-oxiran-2-yl)-deca-4,8-dienylidene]-(2-phenyl-aziridin-1-yl)-amine	414	$C_{28}H_{34}N_2O$	0.98
13	12.352	Doconexent	328	$C_{22}H_{32}O_2$	2.42
14	12.904	Columbin	358	$C_{20}H_{22}O_6$	1.75
15	13.465	Ethyl para-ethoxybenzoate	194	$C_{11}H_{14}O_3$	3.75
16	15.106	Palatinol A	222	$C_{12}H_{14}O_4$	3.84
17	24.108	Ethyl palmitate	284	$C_{18}H_{36}O_2$	1.40
18	26.813	Linolenin, 1-mono-	352	$C_{21}H_{36}O_4$	3.83
19	27.37	Lineoleoyl chloride	298	$C_{18}H_{31}Cl_O$	8.54
20	27.912	Cervonic acid	328	$C_{22}H_{32}O_2$	0.65
-		NI			16.25

seeds NI: Not identified

2,5-Octadecadiynoic acid, methyl ester (5.57%), Palatinol A (4.81%).

The structure of the highest compounds were identified in *Ziziphus jujuba* seeds extract given in Table 4 and figure 2.13-Heptadecyn-1-ol (12.95%), is phenolic compound and one of the major flavour compounds. phenolic compounds were found to inhibit the cell growth and fermentation and used as antioxidant[12,21]. Furthermore, its derivatives have also been used for therapeutic purposes. For instance, Hydroxymethylfurfural is a potential candidate for treating sickle cell anemia[11]. Lineoleoyl chloride, has been found to possess many interesting pharmacological and physiological activities, such as anti-inflammatory effects. Lineoleoyl chloride results from the hydrolysis degradation of Linoleic acid during extraction[22]. 7-Ethyl-4-decen-6-one (9.73%), Lineoleoyl chloride (8.54%), Linoleic acid (6.37%), 2,5-Octadecadiynoic acid, methyl ester (5.57%) also play a role in the activity of *Ziziphus* seeds extracts. While for the first time we identified Palatinol A (4.81%) in *Ziziphus* seeds extracts. *Ziziphus jujuba* seeds extract were found to contain small amounts of other compounds, this in line with other investigators[23,24].

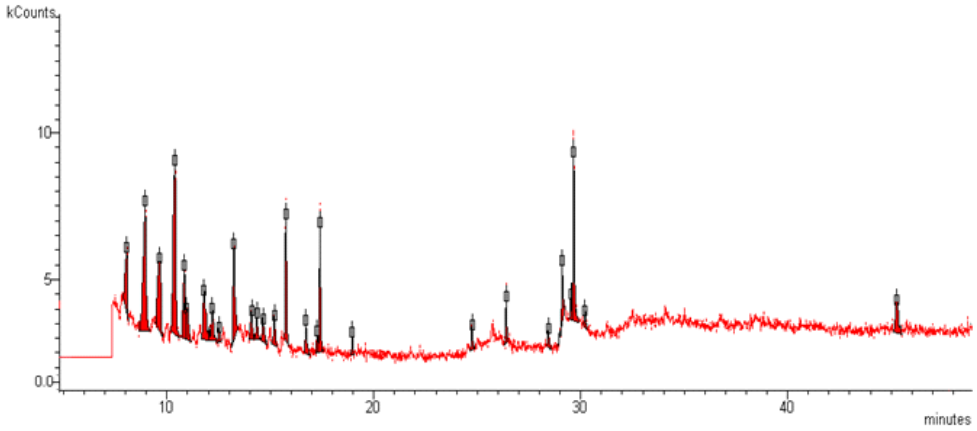

Fig. 1.Typical GC/MS chromatogram of *Ziziphus jujuba* seeds extract

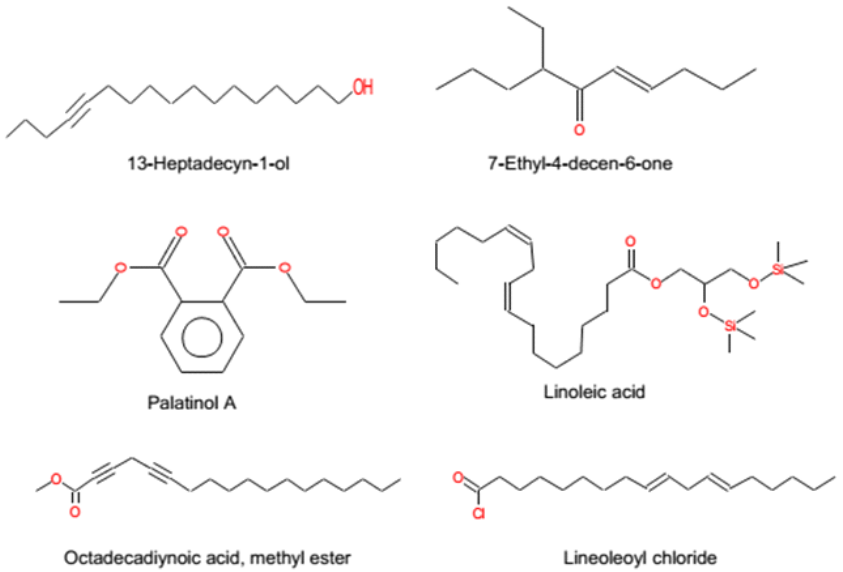

Fig. 2. Chemical structure of the highest abundant compounds were identified in the *Ziziphus jujuba* seeds extract. 13-Heptadecyn-1-ol (12.95), 7-Ethyl-4-decen-6-one (9.73), Lineoleoyl chloride (8.54), Linoleic acid (6.37), 2,5-Octadecadiynoic acid, methyl ester (5.57), Palatinol A (4.81)

CONCLUSION

In conclusion, our study was one of very few studies have confirmed that the antimicrobial activity of *Ziziphus jujuba* seeds extract against certain microorganisms. Results of this study showed that the have found for the first time that *Ziziphus jujuba* extracts are effective in inhibiting the growth of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Listeria monocytogenes*. Remarkably, they elicited no effects on immortalized normal human foreskin fibroblasts cells and nonmalignant epithelial breast cells. Triterpenic acids resulted the bioactive compounds present in the most effective extracts (ZE2 and ZE4). Our data provide a strong rational base for the use in Traditional Chinese Medicine of *Ziziphus* extracts in the treatment of cancers. Moreover, our results highlight that *Ziziphus jujuba* are valuable fruits rich in bioactive compounds with potential human health benefits. More experiments are in progress to understand the molecular targets and pathways affected by *Ziziphus jujuba*.

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