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GC-MS Analysis and Antimicrobial Activity of Solvent Extracts of *Zizyphus lotus* (L.) Desf.

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ABSTRACT

Dichloromethane profile of the fruits of *Zizyphus lotus* (Rhamnaceae) growing in Algeria was studied by capillary gas chromatography-mass spectrometry (GC-MS). 21 compounds were identified among which: Heptadecene-(8)-Carbonicacid(40.22%),9-Octadecenoicacid(z)-ethylester(16.94%), hexadecanoic acid (9.14%), Phenol, 2-(2-aminoethyl)(6.19%), Ethyl linoleate (3.94%) and Hexadecanoic acid, ethyl ester (3.23%). Moreover, the antibacterial activities of the ethyl acetate extract of *Zizyphus lotus* were tested against standard bacterial strains: *Serratia marcescens* ATCC 13880 , *Pseudomonas aeruginosa* ATCC 10145 , *Bacillus subtilis* ATCC 6051 , *Escherichia coli* ATCC 25922 , *Enterococcus faecalis* ATCC 29212 , *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 11778 using both disc diffusion and dilution methods .

Key words: *Zizyphus lotus*, Dichloromethane Extract, GC-MS, Antimicrobial Activity

INTRODUCTION

Most of the plant drugs which have become famous in modern therapy had a folklore origin. Fruit trees in the *Zizyphus* genus are examples of multipurpose plants with great ethnomedicinal potential all over the world. In the northern part of Africa, especially in Algeria, *Zizyphus lotus*, a large shrub sometimes arboruscent, is named "sedra". The fruit, called "nbeq" is described as demulcent and is involved in the treatment of throat and broncho-pulmonic irritations. In addition, the dried powder leaves and fruits are applied topically in the treatment of boils [1]. *Z. lotus* is used in Algerian traditional medicine for its antidiabetic properties [2,3]. Aqueous and organic extracts of *Z. lotus* are characterized by the presence of flavonoids and tannins [4].

Several species of the genus *Zizyphus* contain cyclopeptide alkaloids, which are particularly common in the Rhamnaceae. An alkaloid mixture of this species showed significant antifungal properties [5]. Several cyclopeptide alkaloids have been already isolated from the genus *Zizyphus*. Pharmacological investigations carried out on some of these species have shown sedative, analgesic, anti-inflammatory and hypoglycemic activities [6].

The present work deals with a GC / MS identification of Dichloromethane extract , then an antibacterial study of Ethyl Acetate extract .

MATERIALS AND METHODS

Plant material

The fruits of *Zizyphus lotus* were collected in June 2015, from Ouargla Algeria.

Extraction

Fruits of *Zizyphus lotus* (300 g) were macerated four times with 70% Et-OH solution by replacing the solvent every time. The hydro-alcoholic solutions were concentrated under reduced pressure and the residue was dissolved in water and kept in cold overnight. After filtration, the aqueous solution was successively extracted with CH₂Cl₂, EtOAc and *n*-BuOH three times for each. Then the extracts were concentrated to dryness. The residue obtained by CH₂Cl₂ was dissolved in Hexane and subjected to GC/MS analysis[7].

Gas Chromatography-Mass Spectrometry:

The CH₂Cl₂ extract was dissolved in hexane and injected into a GC-MS apparatus (Hewlett Packard Model 5890 series) equipped with a mass selective detector (mass HP 5972). Capillary GC-MS analysis was developed under the following conditions: Capillary column HP5-MS, 30 m x 0.32 mm (i.d.), detector temperature 300°C, injector temperature 280°C.

Antimicrobial Activity:

Ethyl acetate extract was tested against standard strains of Nine bacteria, namely *Serratia marcescens* ATCC13880, *Pseudomonas aeruginosa* ATCC10145, *Bacillus subtilis* ATCC 6051, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC11778, *Methicillin-resistant Staphylococcus aureus* (MRSA)ATCC013300 and *Staphylococcus aureus* (ATCC 25923).

Microorganisms Test:

3.7% of Mueller Hinton agar was mixed with hot distilled water and autoclaved at (121°C, 15 Psi (2 atm), 15 min). After autoclaving, it was allowed to cool to 45-50 °C in a water bath. Then the medium was poured into sterilized petri dishes with a uniform depth of approximately 5 mm [8-10].

Whatman 1 filter paper was used to prepare discs of 5 mm in diameter [9,10]. They were sterilized by autoclaving and then dried during the autoclaving cycle. The discs were then impregnated with the Ethyl acetate extract dissolved in DMSO [11].

Disc diffusion method for antimicrobial susceptibility test was carried out according to the standard method by Kirby-Bauer to assess the presence of antimicrobial activities of plant extracts. A bacterial suspension adjusted to 0.5 McFarland standard (1.5×10^8 CFU/ml) was used to inoculate Mueller Hinton agar plates evenly using a sterile swab. The discs impregnated with the plant extracts were placed individually on the Mueller Hinton agar surface with flamed forceps and gently pressed down to ensure contact with the agar surface. The discs were spaced far enough to avoid both reflection waves from the edges of the petri dishes and overlapping rings of inhibition. The plate was then incubated at 37°C for 18 hours in inverted position to look for zones of inhibition. Zones of inhibitions produced by the sensitive organisms were demarcated by a circular area of clearing around the plant extract impregnated discs.

The diameter of the zone of inhibition through the center of the disc was measured to the nearest millimeter [12,13].

RESULTS AND DISCUSSION

21 compounds were identified from Dichloromethane extract of *Zizyphus lotus* (Table 1), Terpenes, Butanoic acid, 3-methyl (1), Butanoic acid, 2-methyl (2), Hexanoic acid (3), Benzoic acid (4), 1-Amino-pyrrolidine(5), Benzaldehyde, 4-hydroxy-3-methoxy(6), Benzaldehyde, 4-hydroxy-3,5-dimethoxy(7), Phenol, 2-(2-aminoethyl)(8), 15-Tetracosenoic acid, methyl ester (9), Hexadecanoic acid (10), Thiosulfuric acid (11), Hexadecanoic acid, ethyl ester (12), 2,3-Dihydrocyclohepta (DE) Naphthale (13), Heptadecene-(8)-Carbonic acid(14), Ethyl linoleate(15), 9-Octadecenoic acid(z)-, ethylester(16), 9-Octadecenoic acid (z) (17), Nonadecanoic acid, ethyl ester (18), 1-Hexadecanol(19), 1,2-Benzenedicarboxylic acid(20), 9-Octadecenoic acid (z)-, 2,3-dihydroxy (21). The antibacterial activities of the ethyl acetate extract of *Zizyphus lotus* were tested against standard bacterial strains: *Serratia marcescens* ATCC 13880, *Pseudomonas aeruginosa* ATCC 10145, *Bacillus*

subtilis ATCC 6051 , *Escherichia coli* ATCC 25922 , *Enterococcus faecalis* ATCC 29212 , *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 11778 using both disc diffusion and dilution methods. The results showed moderate activities.

Table 1: Terpenes of *Zizyphus lotus*

N°	compound	T _R	Area%	Ratio %
1	Butanoic acid, 3-methyl	4.38	2.51	6.244
2	Butanoic acid, 2-methyl	4.53	1.62	4.018
3	Hexanoic acid	5.77	0.56	1.382
4	Benzoic acid	7.75	0.93	2.311
5	1-Amino-pyrrolidine	8.13	0.38	0.939
6	Benzaldehyde,4-hydroxy-3-methoxy	10.65	2.16	5.379
7	Benzaldehyde,4-hydroxy-3,5-dimethoxy	14.96	0.81	2.011
8	Phenol,2-(2-aminoethyl)	16.50	6.19	15.385
9	15-Tetracosenoic acid, methyl ester	20.17	0.66	1.636
10	Hexadecanoic acid	20.63	9.14	22.730
11	Thiosulfuric acid	20.71	0.55	1.363
12	Hexadecanoic acid, ethyl ester	21.10	3.23	8.041
13	2,3-Dihydrocyclohepta (DE) Naphthale	21.34	1.34	3.324
14	Heptadecene-(8)-Carbonic acid-(1)	24.18	40.22	100.000
15	Ethyl linoleate	24.29	3.94	9.797
16	9-Octadecenoic acid (z)-, ethyl ester	24.43	16.94	42.106
17	9-Octadecenoic acid (z)	24.51	2.69	6.683
18	Nonadecanoic acid, ethyl ester	24.84	1.80	4.481
19	1-Hexadecanol	27.90	0.68	1.686
20	1,2-Benzenedicarboxylic acid	31.52	1.96	4.871
21	9-Octadecenoic acid (z)-, 2,3-dihydroxy	34.64	1.71	4.247

Table 2: Diameter of inhibition zone at different concentrations of Acetate ethyl extract (µg/µL)

Name of cultured Bacteria	Diameter of Inhibition zone (Cm)	
	500 µg /µL	1000 µg /µL
<i>Serratia marcescens</i> ATCC 13880	0	1
<i>Pseudomonas aeruginosa</i> ATCC 10145	0	1
<i>Bacillus subtilis</i> ATCC 6051	1.2	0.7
<i>Escherichia coli</i> ATCC 25922	0	0.6
<i>Enterococcus faecalis</i> ATCC 29212	0	0
<i>Staphylococcus aureus</i> ATCC 25923	1.1	0.7
<i>Bacillus cereus</i> ATCC 11778	1	1.1

CONCLUSION

The GC-MS analysis of Dichloromethane extract of *Zizyphus lotus* showed 21 different compounds with Heptadecene-(8)-Carbonic acid-(1) as the major compound. Ethyl acetate extract was also tested against some bacteria strains. The results revealed significant activity against the Gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778) and Gram-negative (*Escherichia coli* ATCC25922) at a concentration of 1000 µg/µL.

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