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Chemical Composition And Antibacterial Activity Of The Essential Oil Isolated From Seinat (Cucumis Melo Var. Tibish) Seeds

Azhari Siddeeg, Elmuez Alsir,Yanshun-Xu, Qixing-Jiang, Wenshui-Xia

State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, 214122, China.
Department of Food Science and Technology, Faculty of Engineering and Technology, University of Gezira, Wad Medani, P.O. Box 20, Sudan.
Email: xiaws@jiangnan.edu.cn; azhari_siddeeg@yahoo.

ABSTRACT: The chemical composition of the essential oil constituent from Seinat (Cucumis melo var. tibish) seeds was analyzed by gas chromatography-mass spectrometry (GC-MS). Forty components were identified; with 9, 12-Octadecadienoic acid, methyl ester (15.27%), 2H-Pyran-2-one, tetrahydro-6-nonyl (14.60%) being the main constituents in the essential oil. Based on their functional groups, the ester compound group was highest content (31.20%) compared with the other groups. In vitro antibacterial activity against three strains of Gram-positive bacteria and three strains of Gram-negative bacteria was studied. The essential oil of seinat seeds had pronounced antibacterial activity on all the tested bacteria. Minimum inhibitory concentration (MIC) was in the range from 0.5 to 5 mg/mL of sample.

Keywords : Seinat seeds, essential oil, GC-MS, antibacterial activity.

Seinat (Cucumis melo var. tibish) is a type of melon that belongs to the Cucurbitaceae family. It is grown mostly in Sudan and is not well-known in neighboring countries and is cultivated for its edible seed. Seinat seed contains crude oil content of 31.1%, moisture content of 4.2%, 24.7% fiber, 28.5% protein, 4.3% ash and 6.9% content of total sugars. Seinat seed is an excellent source of edible oils, comprising the main fatty acids namely: linoleic acid 61.10%, oleic acid 18.75%, palmitic acid 10.37%, and stearic acid 9.18% [1]. Seinat seeds are roasted and eaten. Other well-known and more investigated oilseed proteins such as pumpkin and melon seeds are utilized directly as snacks after salting and roasting mostly in Arabian countries [2]. Recently, the essential oils and extracts of many plant species parts have become popular, and efforts to characterize their bioactive principles have gained momentum in many food processing and pharmaceutical applications [3]. Essential oils and their components have been used extensively as flavor ingredients in a lot of confectionery products, food and beverage, and also in numerous applications, including toothpaste. Many of these compounds are commonly classified as safe [4]. There are many methods have been applied for extracting volatile compounds from different foods, a few difficulties may be spotted when analyzing volatile compounds from oils, because some of these compounds are fat soluble and hence, the performance of the extraction method would be significantly reduced [5]. The toxicity of antibacterial agents to humans and other animals is commonly considered low; antibacterial agents are used to treat bacterial infections. In addition, there are many antibacterial agents, such as food preservatives and organic acids that are used to inhibit food borne bacteria and also to increase the shelf life of processed foods [6]. Many naturally occurring compounds found in edible and medicinal plants, herbs, seeds and spices have been shown to possess antibacterial functions and could avail as a source of antibacterial agent against food pathogens [7]. To the best of our knowledge, no previous reports are available on the chemical composition of the essential oil isolated from Seinat (Cucumis melo var. tibish) seeds and its antibacterial activity. Thus, the main objective of this research was to determine the chemical composition of the essential oil isolated from seinat seeds by using GC-MS. In addition, to checking the in vitro antibacterial activity of the essential oil.

2. Materials and Methods

2.1 Raw material

Dried seinat fruits were brought from a local farm in Wad Medani City, Gezira State, Sudan, in September of 2013 after harvesting, and transported to the Food Processing and Ingredients laboratory in Jiangnan University, China. The seeds were removed manually from the fruits and were kept dry at room temperature in desiccators.

2.2 Extraction of the essential oil

The essential oil of seinat was isolated according to the method of Hanbali et al. [8].The dried seinat seed flour was subjected to steam distillation in a Clevenger apparatus for 3 h. The aqueous phase was extracted with diethyl ether. The ether phase was dried over anhydrous sodium sulphate and stored in a dark glass bottle at 4ºC until analysis.

2.3 Chemical composition of the essential oil

The volatile compounds were sampled with a SPAM–fiber and separated with a GC/MS. Volatile compounds were separated on a CP-Sil-8CB (Varian, Walnut Creek, CA, USA), fused silica capillary column (30 m length, 0.25 mm, id, and 0.25 µm film thicknesses) in a Varian model 3800 gas chromatograph. The split less mode injector was maintained at 220ºC and the flame ionization detector (FID) at 250ºC, volatile compounds were separated with a capillary column DB WAX (30m×0.25µm, J and W Scientific, Folsom, CA, USA).The separation was performed as follows: the oven temperature was set at 40ºC, held for 3 min, ramped to 100ºC at the rate of 6ºC/min and then to 230ºC at 10ºC/ min. The constant column flow was 0.9ml/min. Mass spectra was obtained in the Electron Impact (EI+) mode with an energy voltage of 70eV; the mass range was 33 to 450 m/z. volatile compound identification was carried out by matching the compounds with the mass spectra of standard compounds found in the wily 130 K and national institute of standards and technology (NIST) 98 library of MS

spectra based on their retention indices.

2.4 Microbial strains and media

The antibacterial activity of the essential oil of seinat seeds was evaluated against three Gram-positive bacterial strains: Streptococcus pyogenes ATCC 12344, Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 21332, and three Gram-negative bacterial strains: Salmonella typhimurium CMCC 50013, Shigella dysenteriae CMCC 51302 and Escherichia coli ATCC 25922. The microorganisms were provided by the Microbiology Laboratory in School of Food Science and Technology, Jiangnan University, Wuxi city, China. Each culture was activated by transferring a loopful into nutrient broth (4 ml) followed by incubation at 37ºC for 16 h. The optical density of each active culture was adjusted at 615 nm by using fresh broth to give standard inoculums of 10^8 CFU/mL.

2.5 Determination of antibacterial activity

The antibacterial activity was studied by the agar well diffusion method [9]. The bacterial suspension was spread uniformly on the agar surface. Agar surface was perforated with 6 mm-diameter holes, aseptically cut and filled with 100 µL of the essential oil isolated from seinat seeds. The essential oil was used in the concentration of 0.5, 2.5, and 5 mg/mL. Dimethyl-sulphoxide (DMSO) was used as the negative control. The plates were incubated at 37ºC for 21 h and then examined to verify inhibition. A positive result was defined as inhibition zone of 9 mm or more around the holes [10]. Penicillin (20 µg/mL) was used as a reference for Gram-positive bacteria and Streptomycin (40 µg/mL) was used as a reference for Gram-negative bacteria. Experiments were performed in triplicate, and the developing inhibition zones were compared with those of reference discs. The minimum inhibitory concentration (MIC) values were determined for the bacterial strains which were sensitive to the essential oil in disc diffusion assay.

3. Results and Discussion

3.1 Chemical composition of the essential oil

As illustrated in Table 1 and Fig. 1, forty components of volatile compounds were identified in the essential oil isolated from seinat seeds. The results showed that 9,12-Octadecadienoic acid, methyl ester and 2H-Pyran-2-one, tetrahydro-6-nonyl were the highest content (15.27 and 14.60% respectively), followed by 9,12-Octadecadienoic acid, ethyl ester (10.06%), Diethyl Phthalate (9.23%), Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl (9.21%) and Ethyl 9-octadecenoate, E (4.44%). According to their functional groups, the ester compound group was found in higher proportion (31.20%) compared with the other groups. There are some compounds that were identified in the essential oil of seinat seeds which belong to unsaturated fatty acids and their derivatives such as pentadecanoic acid, octadecanoic acid, hexadecanoic acid, 9,12-Octadecadienoic acid and 9,12-Octadecadienoic acid, these same compounds were identified by Albishri, Almaghrabi and Moussa, [11] in watermelon and muskmelon cultivars in Saudi Arabia and also were identified by De melo, Narain and Bora, [12] for melon (Cucumis melo hybrid AF-522) seeds, belong the same family (Cucurbitaceae). However precise active principles have not been examined, but the essential oil of seinat contained hexenal; this compound's derivatives were suggested as possible active compounds [13].

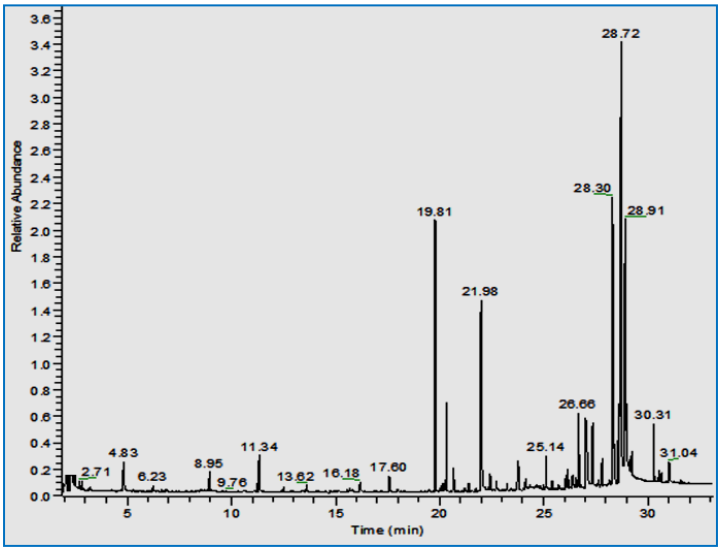

Fig 1. Gas chromatography-mass spectrometry chromatogram of volatile compounds of the essential oil isolated from Seinat (Cucumis melo var. tibish) seeds.

3. 2 Antibacterial activity

The results showed that the essential oil of seinat seed has antibacterial activity against all the bacterial strains tested (Table 2). Gram-positive bacteria were more susceptible than Gram-negative bacteria because it's considered less resistance. The results illustrated that the essential oil had the highest antibacterial inhibition against Staphylococcus aureus with a mean zone of inhibition of 22.13 mm while the lowest mean zone of inhibition (15.67 mm) was with Shigella dysenteriae. According to a research by Alves et al. [10] activity of inhibition was expressed in terms of the diameter of the inhibition zone: <9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active and >18 mm, very active, these results suggest that the volatile oil of seinat (Cucumis melo var. tibish) seeds can also be arranged based on their bacterial inhibition activity level. Since essential oil of seinat seed showed antibacterial activity against the all tested bacteria, particularly Gram-positive bacteria strains, the real extent of its inhibitory activity was evaluated by determining minimum inhibitory concentration (MIC) values, which are shown also in Table 2.

Table 1. Chemical composition of the essential oil isolated from seinat (Cucmis melo var. tibish) seeds.

No	Constituent	RT*	Percentage
1	Hexenal	4.83	1.33
2	Cyclotetrasiloxane, octamethyl	8.95	0.52
3	Nonanal	11.34	1.16
4	2,4-Decadienal, (E,E)-	16.18	0.45
5	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)	17.60	0.51
6	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	19.81	9.21
7	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	20.25	0.41
8	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)	20.35	2.94
9	α-Sesquiphellandrene	20.67	0.78
10	Diethyl Phthalate	21.98	9.23
11	3-Cyclohexene-1-methanol, α,4-dimethyl-α-(4-methyl-3-pentenyl)	22.44	0.54
12	3-Cyclohexene-1-methanol, α,4-dimethyl-α-(4-methyl-3-pentenyl)	22.75	0.31
13	2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-α,α,4a,8-tetramethyl	23.22	0.33
14	Cyclohexane, 1,1,2-trimethyl-3,5-bis(1-methylethenyl)	23.77	1.53
15	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one	24.14	0.62
16	Bicyclo[3.1.1]heptane, 6-methyl-2-methylene-6-(4-methyl-3-pentenyl)	24.34	0.30
17	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl	25.14	0.93
18	Cyclopropanemethanol, α,2-dimethyl-2-(4-methyl-3-pentenyl)	25.41	0.31
19	Dibutyl phthalate	26.07	0.36
20	α-Bisabolol	26.13	0.58
21	2-Nonadecanone	26.41	0.87
22	6-(3,3-Dimethyl-oxiran-2-ylidene)-5,5-dimethyl-hex-3-en-2-one	26.56	0.34
23	Hexadecanoic acid, methyl ester	26.66	2.50
24	n-Hexadecanoic acid	27.01	2.06
25	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	27.05	3.81
26	Hexadecanoic acid, ethyl ester	27.35	1.69
27	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	27.80	0.69
28	9,12-Octadecadienoic acid, methyl ester	28.30	15.27
29	Octadecanoic acid, methyl ester	28.56	0.91
30	1,E-11,Z-13-Octadecatriene	28.63	1.92
31	13-Octadecenal, (Z)	28.67	3.52
32	2H-Pyran-2-one, tetrahydro-6-nonyl	28.72	14.60
33	9,12-Octadecadienoic acid, ethyl ester	28.91	10.06
34	Ethyl 9-octadecenoate, (E)	28.95	4.44
35	Pentadecanoic acid, ethyl ester	29.19	0.77
36	Tetracontane, 3,5,24-trimethyl	29.25	0.84
37	Heneicosane	30.31	1.85
38	10-Undecyn-1-ol	30.55	0.38
39	1-Hexadecyne	30.63	0.35
40	2H-Pyran-2-one, tetrahydro-6-nonyl	31.04	0.81

* Retention time

The MIC values varied from 0.5 to 5 mg/mL for both Gram-positive and negative bacteria. The antibacterial activity of essential oil of seinat seeds was lower than those of the standard antibiotic (penicillin and streptomycin).

Table 2. Results of antibacterial activity of the essential oil of seinat (Cucmis melo var. tibish) seeds.

Tested bacteria	Diameter of zone of inhibition (mm)		MIC of the essential oil concentration (mg/mL)
	Essential oil (100µL/disk)	Refer-ence*	
Gram positive bacteria			
Streptococcus pyogenes	21.42±0.43	25.17±0.73	0.5
Staphylococcus aureus	22.13±0.07	27.49±0.61	2.5
Bacillus subtilis	19.39±0.66	23.68±0.08	0.5
Gram negative bacteria			
Salmonella typhimurium	16.53±1.63	21.16±0.46	2.5
Shigella dysen-teriae	15.67±1.33	19.66±0.67	5.0
Escherichia coli	16.41±1.41	20.71±0.80	2.5

Values (diameter in mm, including diameter of 6 mm) are expressed as mean±standard deviation, analyzed individually in triplicate.* Reference: Penicillin (20 µg/mL) for Gram-positive bacteria; Streptomycin (40 µg/mL) for Gram-negative bacteria.MIC: minimum inhibitory concentration. Inhibition zones: <9 mm no active, 9 –12 mm less active, 13–18 mm active, >18 mm very active [10]. The reasonable explanation might be attributed to the presence of the volatile components in the essential oil. As reported by Stevens, [14] and Smeesters et al. [15] Streptococcus pyogenes and Staphylococcus aureus are Gram-positive, non-spore forming, facultative anaerobic bacteria that are able to invade via the broken skin or mucous membrane. The infections caused by Streptococcus pyogenes include pharyngitis, localized skin infections, rheumatic fever, rheumatic heart disease and streptococcal toxic shock syndrome.

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