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Fatty Acid Composition and Antimicrobial Activity Against Sensitive and Multi-drug Resistant Bacteria of Tunisian *Allium cepa* seed extract

Mohamed Derbali[1]*, Ameur Elaissi[2], Imed Cheraief[3], Mahjoub Aouni[1]

[1]Laboratory of Transmissible Diseases and Biologically Active Substances, Faculty of Pharmacy, Monastir, Tunisia

[2]Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Monastir, Tunisia

[3]Laboratory of Biochemistry, Faculty of Medicine, University of Monastir, Tunisia

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***For Correspondence**

Mohamed Derbali, Laboratory of Transmissible Diseases and Biologically Active Substances, Faculty of Pharmacy, Monastir, Tunisia, Tel: +216 97571180.

E-mail: mohamed.edderbali@gmail.com

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ABSTRACT

Objective: This study is about *Allium cepa* (*A. cepa*) seed oil extraction, Fatty acids identification and its antibacterial activity.

Methods: Soxhlet extraction method using methanol (MetOH), Fatty acid profile of seed oil using gas-chromatography with flame ionization detector (GC-FID). Disc diffusion and plate micro dilution for the determination of the antibacterial activity.

Results: Extraction's yield was of 24.86 ± 0.98 (% of V/W). Seed extract shows that Linoleic acid (C18:2, ± 70.8%), oleic acid (C18:1, 20.14%) and palmitic acid (C16, ± 4.96%) were the dominant fatty acids in the *A. cepa* seed oil. Antimicrobial potential was detected against reference bacteria and clinical isolates pathogens.

Conclusion: Those results show that *A. cepa* is a promising oil seed crop with high level of unsaturated fatty acids and antibacterial potential. A bivalent role: nutritive and antibiotic, of *A. cepa* seed oil prove its importance in food and pharmaceutical applications.

INTRODUCTION

Plants seed oils have multiple applications, being used in various fields from cosmetics to cooking. Seeds oils are gaining more importance as studies prove they are a source of tocopherols and polyphenols [1-3], known for their antioixidant [4] and anti-inflammatory capacities [5]. Seed oil is known to be rich with fatty acids. Polyunsaturated acids such as linoleic acid are essential for the human metabolism due to the lack of enzymes responsible for their biosynthesis [6]. PUFA play a key role promoting cardiovascular health, enhancing anti-tumoral activity [7,8] and prevent atherosclerosis [9,10].

Onion: *Allium cepa* L. is known to be an ingredient of food over the world. Researches demonstrated that *A. cepa* is involved in many biological activities such as antioxidant potential [11], hepatoprotective [12] and antiallergic [13] activities. *A. cepa* extracts were efficient in reversing organ toxicity [14], treating human leukemia [15]. The seeds of *A. cepa* L. were used in folk medicine by the Uygur nationality in China and improve the functions of internal organs, treat diarrhea, fervescence, calenture, and puffiness of the face and eyes, and promote blood flood [16]. In this report we focused on *A. cepa* seed oil extraction with organic solvent, fatty acid profile determination, major compounds identification and testing the antibacterial activity of seed oil against sensitive and multi drug resistant bacteria.

MATERIAL AND METHODS

Chemical and materials

Seeds of *A. cepa* were recolted from our local field in Jelma, Sidi Bouzid, Tunisia. All chemicals used in this study were of analytical grade (Sigma–Aldrich ® 90, St. Louis, Mo., USA) and the reagents used were standardized.

Oil extraction

Seeds were fine powdered using high speed mixer. The oils were extracted from the seeds with soxhlet extractor using MetOH for 4 h. The ratio of solids to solvent used was 1:10. The oil was then recovered by evaporating off the solvent using rotary evaporator.

Fatty acid methyl esters (FAMEs) synthesis and GC-FID

FAMEs were prepared, following the procedure described by AOAC [17]. Aliquot of 10 mg lipid for each solvent extract were esterified after addition of internal standard (0.1 ml of C17:0, 1 mg/ml) and 1 ml of BF3-etherate, the samples were boiled for 30 min at 70°C. The FAMEs were extracted from a salt saturated mixture with hexane (1.0 ml). For drying FAMEs, anhydride Na2SO4 was added and centrifuged (10 min, 3.56 g), then the upper part was poured in specific cell. FAMEs analysis was carried out according to the European Union Commission modified Regulation EEC 2568/91 on a Hewlett-Packard GC (Hewlett-Packard, Palo Alto, CA), fitted with a FID and a split–splitless injector, set at 270°C. The carrier gas was nitrogen (1 ml/min), and elution was performed with a fused silica Agilent DB23 capillary column (60 m length, 0.32 mm inner diameter, and 0.25 m film thicknesses). Conditions were as follows: injector temperature, 270°C; FID, 280°C; injector split ratio, 1:50; the initial column temperature, 130°C; step 1, 6.5°C/min to 170°C; step 2, 2.8°C/min to 215°C and maintained for 12 min; step 3, 40°C/min to 230°C and maintained for 20 min. FAMEs were identified by comparing their relative and absolute retention times to those of authentic cis-fatty acid (CFA) and TFA standards [18].

Antimicrobial activity

Microorganisms

The microorganism strains employed in the biological assays are listed in **Table 1.** Different American Type Culture Collection (ATCC) reference bacteria were used as well as multi-drug resistant strains from clinical isolates.

Table 1. Zone inhibition and MIC of *Allium cepa* seed extract.

Strain	Zone inhibition (mm)	MIC (µg/ml)	Gentamycin
GRAM negative			
Enterobacter cloacae MDR	9,33 ± 0.57	500	R
Escherichia coli ATCC25922	12.66 ± 0.57	250	24
Escherichia coli MDR	12.33 ± 0.57	250	24
Klebsiella pneumoniae MDR	14 ± 1	250	R
Proteus mirabilis MDR	10,33 ± 0.57	500	19
Pseudomonas aeruginosa ATCC 27853	10.66 ± 0.57	500	20
Pseudomonas aeruginosa MDR	11 ± 1	500	20
GRAM positive			
Staphylococcus aureus ATCC 25923	18,33 ± 0.57	125	26
Staphylococcus aureus MDR	18 ± 1	125	26
Staphylococcus epidermidis ATCC12228	14,33 ± 0.57	250	25
Streptococcus pseudopneumonaie MDR	14 ± 1	250	24
Bacillus subtilus ATCC 6633	20.66 ± 0.57	125	28
Enterococcus faecalis ATCC 29212	16 ± 1	125	17

Note: MDR: multi-drug resistant, MIC: minimal inhibitory concentration, R: gentamycin resistant.

Disk diffusion assay

Antimicrobial activity was carried out using disc-diffusion method [19]. Petri plates were prepared with 20 ml of sterile Mueller–Hinton Agar [20] for bacteria. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. *A. cepa* seed oil was diluted in 10% dimethyl sulfoxide (DMSO) and 20 µl was loaded on discs (1 mg per disc). Discs of Gentamycin (30 µg) were used as positive control. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. The plates were incubated at 37°C. After 24 h, zones of inhibition were recorded in millimeters.

Determination of minimum inhibitory concentration

The minimal inhibitory concentration (MIC) preventing visible bacterial growth was measured by the broth dilution method (microdilution using 96-well microplates) [21]. Extract stock solution were prepared by dissolution in 10% DMSO. The MIC of each extract was defined as the lowest concentration which inhibited bacterial growth, after incubation at 37°C between 18 and 24 h.

Statistical analysis

All the experiments were performed in triplicate, and statistical significance was calculated by one-way analysis of variance (ANOVA). A value of $p<0.05$ indicated statistical significance using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Fatty acid profile

Results shown in **Table 2** shows that *A. cepa* is rich of MUFA and PUFA responsable of lipid regulation [22] and their protective effects against seizures, cognitive impairment and hippocampal oxidative DNA damage [23] especially oleic acid and linoleic acid. MUFA and PUFA represent more than 92% of total fatty acid profile, providing omega-3 and omega-6 fatty acids. Those essential fatty acids should be provided by food intake since humans have lack of enzymes responsible of their synthesis [24,25]. In fact, Linoleic acid is an essential fatty acid from which the whole omega-6 fatty acid family is derived. These fatty acids are important components of the cell membranes and are precursors of other substances involved in many physiological responses. By contrast, palmitic acid is considered as an atherogenic compound, when consumed in high amount [26].

Table 2. Fatty acid composition of *Allium cepa* seed extract as % of total fatty acid profile.

S. No.	Fatty acids	Yield (%)
	SFA	
1.	Butyric acid C4 :0	0.004
2.	Caproic acid C6 :0	0.003
3.	Capric acid C10 :0	0.006
4.	Lauric acid C12 :0	0.058
5.	Tridecylic acid C13 :0	0.002
6.	Myristic acid C14 :0	0.052
7.	Pentadecylic acid C15 :0	0.001
8.	Palmitic acid C16 :0	4.968
9.	Margaric acid C17 :0	0.040
10.	Stearic acid C18 :0	1.772
11.	Arachidic acid C20 :0	0.213
12.	Heneicosylic acid C21 :0	0.033
13.	Behenic acid C 22 :0	0.135
14.	Tricosylic acid C23 :0	0.001
	MUFA	
15.	Myristoleic acid C14 :1	0.022
16.	Pentadecenoic acid C15 :1	0.005
18.	Palmitoleic acid C16 :1	0.049
19.	Heptadecenoic acid C17 :1	0.038
20.	Oleic acid C18 :1	20.14
21.	Vaccenic acid C18 :1	0.914
22.	Icosenoic acid C20 :1	0.313
23.	Erucic acid C22 :1	0.008
	PUFA	
24.	Linoleic acid C18 :2	70.80
25.	Alpha-linolenic acid C18 :3	0.080
26.	Gamma-linolenic acid C18 :3	0.002
27.	Eicosadienoic acid C20 :2	0.054
28.	Dihomo-gamma-linolenic acid C20:3	0.007
29.	Eicosatrienoic acid C20 :3	0.016
30.	Arachidonic acid C20 :4	0.001
31.	Docosadienoic acid C22 :2	0.008
32.	Docosatrienoic acid C22 :3	0.051
33.	Docosatetraenoic acid C22 :4	0.003
34.	Docosapentaenoic acid C22 :5	0.042
	Total SFA	7.29
	Total MUFA	21.50
	Total PUFA	71.21

Note: MetOH, Methanolic extract; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Antibacterial activity

The in vitro antimicrobial activity of the metOH seed extract from *A. cepa* against tested microorganisms including multidrug resistant pathogens was qualitatively and quantitatively assessed by the inhibition zones and the minimum inhibitory concentration were determined using serial microdilutions technique.

According to the results shown in Table 1, the seed oil exhibited a potent inhibitory effect with diameter of inhibition zones

ranging from 9 to 20 mm. MIC values vary from 125 to 500 µg/ml for ATTCC microorganisms and multidrug resistant pathogens. The MetOH extract were more effective against GRAM negative strains. *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* MDR, *Bacillus subtilus* ATCC 6633 and *Enterococcus faecalis* ATCC 29212 were the most affected GRAM negative bacteria by the MetOH seed extract with an MIC value of 125 µg/ml. *Escherichia coli* ATCC25922, *Escherichia coli* MDR and *Klebsiella pneumoniae* MDR were the most affected GRAM positive tested strains to the MetOH seed extract with MIC value of 250 µg/ml.

Those findings are original concerning MetOH seed extract of *A. cepa* and they are in accordance with antimicrobial activity of aerial part extracts and essential oil of *A. cepa* [27-30]. Those results took a highly importance regarding the inhibitory effects especially against tested multidrug resistant bacteria from clinical isolates from Tunisian University Hospitals concerning their potential danger and their threat to the human health.

CONCLUSION

This report revealed that *A. cepa* seed oil is well rich with MUFA and PUFA and it possess an important antibacterial activity against ATTCC strains and multi drug resistant pathogens. Taken together *A. cepa* seed oil combines the nutritive and the pharmaceutical aspects wich testifies it's highly utilities as food additive and food preservative from bacterial pathogens **(Figures 1 and 2).**

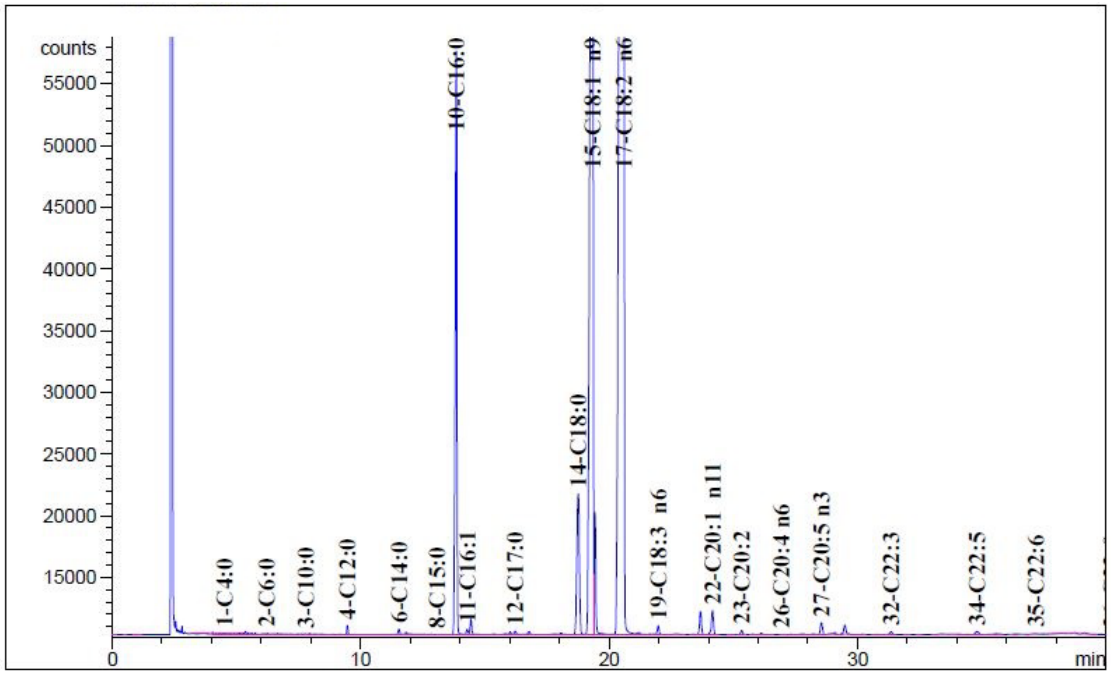
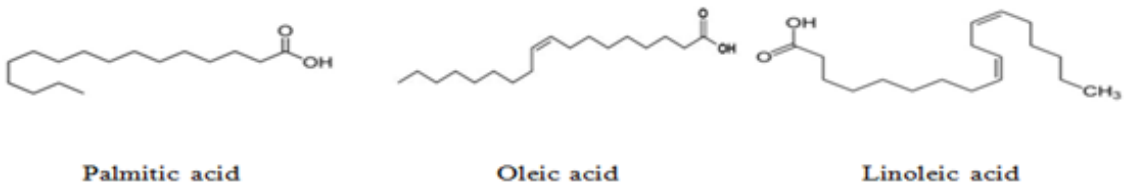


Figure 1. Fatty acids chromatogramm of *Allium cepa* seed extract.



Palmitic acid Oleic acid Linoleic acid

Figure 2. Chemical structure of major fatty acids of *Allium cepa* seed extract.

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REFERENCES

1. Butinar B, et al. New vitamin E isomers (gamma-tocomonoenol and alpha-tocomonoenol) in seeds, roasted seeds and roasted seed oil from the Slovenian pumpkin variety 'Slovenska golica'. Food Chem. 2011;128:505-512.
2. Bustamante A, et al. Microencapsulation of H. pluvialis oleoresins with different fatty acid composition: Kinetic stability of astaxanthin and alpha-tocopherol. Food Chem. 2016;190:1013-1021.
3. Seiquer I, et al. Assessing the bioavailability of polyphenols and antioxidant properties of extra virgin argan oil by simulated digestion and Caco-2 cell assays, Comparative study with extra virgin olive oil. Food Chem. 2015;188:496-503.

4. Tang Y, et al. Characterisation of fatty acid, carotenoid, tocopherol/tocotrienol compositions and antioxidant activities in seeds of three Chenopodium quinoa Willd. Genotypes. Food Chem. 2015;174:502-508.

5. Shahzad M, et al. Black seed oil ameliorates allergic airway inflammation by inhibiting T-cell proliferation in rats. Pulm Pharmacol Ther. 2009;22:37-43.

6. Hanganu A, et al. The compositional characterisation of Romanian grape seed oils using spectroscopic methods. Food Chem. 2014;134:2453-2458.

7. Schacky CV. Omega-3 fatty Acids in cardiovascular disease -An uphill battle. Prostag Leukotr Ess. 2015;92:41-47.

8. Corrales GP and Rivero NL. Role of hake as a source of omega-3 fatty acids in cardiovascular disease prevention. Clin Nutr. 2013;32:660.

9. Machado RM, et al. Omega-6 polyunsaturated fatty acids prevent atherosclerosis development in LDLr-KO mice, in spite of displaying a pro-inflammatory profile similar to trans fatty acids. Atherosclerosis. 2012;224:66-74.

10. Sekikawa A, et al. Recent findings of long-chain n-3 polyunsaturated fatty acids (LCn-3 PUFAs) on atherosclerosis and coronary heart disease (CHD) contrasting studies in Western countries to Japan. Trends in Cardiovascular Medicine. 2015;25:717-723.

11. Roldán E, et al. Characterisation of onion (Allium cepa L.) by-products as food ingredients with antioxidant and antibrowning properties. Food Chem. 2008;108:907-916.

12. Kumar KE, et al. In vitro antioxidant activity and in vivo hepatoprotective activity of aqueous extract of Allium cepa bulb in ethanol induced liver damage in Wistar rats. Food Science and Human Wellness. 2013;2:132-138.

13. Sato A, et al. Antiallergic activities of eleven onions (Allium cepa) were attributed to quercetin 4′-glucoside using QuEChERS method and Pearson's correlation coefficient. J Funct Foods. 2015;14:581-589.

14. Ige SF and Akhigbe RE. Common onion (Allium cepa) extract reverses cadmium-induced organ toxicity and dyslipidaemia via redox alteration in rats. Pathophysiology. 2013;20:269-274.

15. Han MH, et al. Polyphenols isolated from Allium cepa L. induces apoptosis by suppressing IAP-1 through inhibiting PI3K/Akt signaling pathways in human leukemic cells. Food Chem Toxicol. 2013;62:382-389.

16. Li CJ, et al. Furostanol saponins from the seeds of Allium cepa L. Fitoterapia. 2014;99:56-63.

17. AOAC. Official Methods of Analysis. Fatty Acids in Oils and Fats. Preparation of Methyl Esters, Method 969.33, and Methyl Esters of Fatty Acids in Oils and Fats, Method. 15th ed. Assoc Off Ana Chem, Arlington, Virginia; 1990.

18. Mekni M, et al. Aroma volatile components, fatty acids and antibacterial activity of four Tunisian Punica granatum L. flower cultivars. Ind Crop Prod. 2013;48:111-117.

19. Murray PR, et al. Manual of clinical microbiology. 6th ed. ASM Press, United States of America; 1998.

20. Ericsson HM and Sherris JC. Antibiotic sensitivity testing, reportofan international collaborative study. Acta Path Micro Im B. 1971;217:1-90.

21. Berche P, et al. Mesure de l'activité antibacterienne. Bactériologie, bactéries des infections humaines. Flammarion. 1991;7:12-16.

22. Khaire A, et al. Vitamin B12 and omega-3 fatty acids together regulate lipid metabolism in Wistar rats. Prostag Leukotr Ess. 2015;99:7-17.

23. Wahab BA, et al. Omega-3 polyunsaturated fatty acids in large doses attenuate seizures, cognitive impairment, and hippocampal oxidative DNA damage in young kindled rats. Neurosci Lett. 2015;584:173-177.

24. Meyer BJ, et al. Assessing long-chain ω-3 polyunsaturated fatty acids: A tailored food-frequency questionnaire is better. Nutrition. 2013;29:491-496.

25. Rahmawati S, et al. Effect of replacing bread, egg, milk, and yogurt with equivalent ω-3 enriched foods on ω-3 LCPUFA intake of Australian children. Nutrition. 2014;30:1337–1343.

26. Lai TNH, et al. Nutritional composition and antioxidant properties of the sim fruit (Rhodomyrtus tomentosa). Food Chem. 2015;168:410-416.

27. Benmalek Y, et al. Antimicrobial and antioxidant activities of Illicium verum, Crataegus oxyacantha ssp monogyna and Allium cepa red and white varieties. Bioengineered. 2013;4:244-248.

28. Ye CL, et al. Antimicrobial and antioxidant activities of the essential oil from onion (Allium cepa L.). Food Control. 2013;30:48-53.

29. Rahman S, et al. Antibacterial activity of natural spices on multiple drug resistant Escherichia coli isolated from drinking water, Bangladesh. Ann Clin Microbiol Antimicrob. 2011;10:10.

30. Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). Lebensm Wiss Technol. 2004;37:263-268.