FATTY ACIDS PROFILE AND ANTIMICROBIAL ACTIVITIES OF THE SEED OIL OF *MALVA SYLVESTRIS* L. FROM ALGERIA

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Abstract— In the current study, the oil obtained from the seed oil of *Malva sylvestris* L. was tested for it content, fatty acids composition and antimicrobial activity against the standard strains of *Staphylococcus* ATCC 25923, *Listeria monocytogenes* ATCC19115, *Bacillus cereus* ATCC11778, *Enterococcus feacalis* ATCC29212, *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Klebsiella pneumonia* ATCC700603, as well as the fungi *Candida albicans* ATCC 10231 by agar diffusion method. The oil yield from the seed was 9%. linoleic acid was the major fatty acids (49,906%) followed by palmitic acid (22,115%) and the third main fatty acid was oleic acid (15,273 %).
The oils displayed inhibitory potentials towards *Enterococcus feacalis* and *Listeria monocytogenes;* 14.6 mm, 13.6 mm diameters respectively.

Keywords— antimicrobial activity, fatty acids, *Malva sylvestris* L., oil seed.

I. INTRODUCTION

Oils extracted from plants have been used in many cultures since ancient times and many vegetable oils are consumed directly or used as food ingredients. Vegetable oil consumption throughout the world rose to 87.8 million metric tons (MMT) from 62.6 MMT between 1993 and 2000 [1].

The debate over the beneficial effects of saturated versus unsaturated fatty acids has been a topic of research among the world's leading nutritional experts. A diet containing fats of the unsaturated variety has been shown to be beneficial in the prevention of atherosclerosis and coronary heart disease [2].

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Malva sylvestris L. plant selected for this study is a species of mallow belong to family of Malvaceae known as common mallow. It is an annual or perennial herb, growing to a height of four feet [3]. It is an annual or perennial herb, attaining a height of four feet and is grown widely in ALGERIA.
The high mucilage content of *Malva sylvestris* makes it an excellent demulcent that can be used for many applications. In the digestive tract the fruit mucilage can be used to heal and soothe inflammations such as gastritis, peptic ulcers, enteritis, and colitis [3]. It is also shown that the extracts of some *Malva* species protected rats from gastric lesions induced by ethanol. The antiulcerogenic activity may be associated with the high mucilage content from the plant species [4].
In the present study, our object was to determine the oil content as well as the fatty acid composition, the physicochemical properties and to conduct an antimicrobial screening study on the oils obtained from the seed of *Malva sylvestris* L., to evaluate their potential for nutritional and medicinal applications.

II. MATERIALS AND METHODS

A. Malva sylvestris L. Seed Oil Extracted By Soxhlet Method

The sample was powdered and weighed accurately. Then it subjected to extraction with *n*-hexane for 8 h using a Soxhlet apparatus accordind to Ibironke (2006) [5].

B. Determination Of The Physicochemical Properties Of The Oil

Refractive index N_d^t

It consists in determining the ratio of the sine of the angle of incidence and the sine of the angle of refraction of a light beam of given wavelength passing from air into the oil at a constant temperature (20 ° C), using a refractometer according to Wolff (1968) [6].

Density index d_{20}

It consists in determining the ratio of the mass of a given volume of oil at 20 ° C and the weight of an equal volume of distilled water at the same temperature, using a pycnometer with a thermometer graduated and calibrated at 20 ° C according to AFNOR (1978) [7].

Saponification index IS

It consists in determining the number of milligrams of potassium hydroxide required to form 1gramme of ester according to Lion (1955) [8].

C. Determination of fatty acid composition of M sylvestris L. seed oil by gas chromatography

The fatty acid composition of the oil was determined by gas phase chromatography (Applied Sciences Labs, State College, PA, USA). Fatty acid standards were from Nu-Check-Prep (Elysian, MN, USA) according to the method described by Slover et Lanza, 1979 [9].

D.Antimicrobial activity

Bacterial and yeast strains

Microorganisms used in this study included reference strains: standard (ATCC; American Type Culture Collection) *Staphylococcus aureus* ATCC25923, *Listeria monocytogenes* ATCC19115, *Bacillus cereus* ATCC11778 , *Enterococcus feacalis* ATCC29212, *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Klebsiella pneumonia* ATCC700603 and yeast: *Candida albican* ATCC10231.

Susceptibility tests

The susceptibility tests were performed by the Mueller Hinton agar diffusion method. The bacterial and yeast strains grown on nutrient broth at 37°C for 18 to 20 h were suspended in a saline solution (0, 9 % NaCl, w/v) [10] to a turbidity of 0.5 Mac Farland standards (10^8 UFC/ml) [11] which was standardized by adjusting the optical density to 0.08-0.1 at 625nm (JENWAY 6405UV/Vis spectrophotometer). The suspension was used to inoculate 90 mm diameter Petri dishes. After, sterilized discs (Whatman n°1, 6 mm diameter) were impregnated with 15 µl, 20 µl, and 30 µl of oil. Plates were left for 2h at 4 °C to allow the diffusion of oil, and then they were incubated at 37°C for 24 h. Antimicrobial activities were evaluated by measuring inhibition zone diameters on millimeter [12].

III. Results and discussion

A. Levels of total fatty matter

In our study oil content of dried *seed from M. silvestris* powder was found to be 9% which is comparable of TEŠEVIĆ et al. (2012) [13] study (9.60%). Previously, the total oil content was found to be 16.6% [14]. The difference observed is probably due to the origin of the seed.

B. Determination of the physicochemical properties of the oil

No detailed study on the physicochemical properties of the oil of the seeds of *M. silvestris L.* has been found. The refractive index is also an important criterion of purity of oil is proportional to the molecular weight of the fatty acids. It varies depending on interesting degree of unsaturation of lipids and can give us an idea of the prevalence of such an unsaturated fatty acid in the oil [15]. The density index is considered a physical criterion that allows control of the purity of the extracted oil. The saponification index gives the information length of the carbon chain of the fatty acids which constitute triglycerides (major fraction of a fat).

The results of the determination of refractive indices, density and saponification index of the oil yield values estimated to 1.468, 0.8618, and 13.142 respectively.

C. Fatty acids profile

Fatty acid profiles of seed oil are presented in Table I. Ten fatty acids were identified, wherein the analysis of oil gave the proportion of linoleic acid as the major fatty acids comprising 49,906% of total fatty matter followed by palmitic acid (22,115%). The third main fatty acid was oleic acid which amounted to 15,273 % of total fatty matter, wherein palmitoleic acid (0,741%) was still the lowest fatty acid in this fraction. Our study was similar with that obtained by TEŠEVIĆ et al.(2012) [13]; they reveal the presence of linoleic acid (44.16%), palmitic acid (24.29%),oleic acid (13.66%) and stearic acid (3.68%) and others, while those of Mukarram (1984) [14] was quite different, they showed that contains 5.6% sterculic, 11.0% malvalic, 1.6% vernolic, 15.6% lauric, 6.6% myristic, 26.6% palmitic, 5.6% palmitoleic, a trace of stearic, 23.0% oleic and 4.0% linoleic acids. By cons, Emets et al., 1994 [16] has been represented 20 compounds, among which malvic and sterculic acids were detected from the Ukrainian seed oils. Bohannon and Kleiman (1978) [17] determined the fatty acid compositions of seed oils from eleven from Malvaceae. Each of the seed oils contains varying amounts of both malvalic and sterculic acids accompanied by one or both of the corresponding cyclopropane fatty acids. The fatty acid composition and high amounts of PUFA makes the *M. silvestris L.* lipids a special component for nutritional applications.

Moreover, the variations in the fatty acid composition of seed oil can be attributed to various factors, including the geographical origin of samples.

TABLE I
FATTY ACID PROFILE OF *M. SILVESTRIS* L. (CONTENT IN % OF TOTAL FATTY MATTER).

Fatty acids	CONSTITUENT	Seed oil
14:0	myristic acid	1,575
16:0	Palmitic acid	22,115
18:0	Stearic acid	4 ,009
	Total SFA	27,699
14 :1	Myristoleic acid	0,768
16 :1	Palmitoleic acid	0,741
18 :1	Oleic acid	15,273
	Total MUFA	16,782
18:2N6	Linoleic acid	49,906
18:3N6	Linolenic acid	3,085
20:4N6	Arachidonic acid	1,232
20:5N3	timnodonic acid (EPA)	1,294
	Total PUFA	55,517
	Total MUFA+ PUFA	72,299

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA =polyunsaturated fatty acids.

D.Antimicrobial activity

The examination of antimicrobial activity of the oil seed by the agar diffusion method revealed that the seed oil inhibited the growth of all microorganisms tested (Table II) except the Gram-negative bacteria (*P. aeruginosa*). The maximum inhibition zone obtained with 30µl/disc was that against *Enterococcus feacalis* 14.6 mm. On the other hand, the Oil seed led to the highest inhibition of Gram positive bacteria, wherein the inhibition zones were between 13.6mm and 7.0mm at the concentration of 30 µl/disc. We noted also that it was active against yeasts. No study was found about antimicrobial activity of the oil seed.

Since the fatty acid analysis indicated the presence of other fatty acids that had been also reported, the antimicrobial activity found in seed oil could be linked to the synergic effect of all these fatty acids.

It was hypothesised that lipids kill microorganisms by leading to disruption of the cellular membrane(s) [18]. Lipids kill the gram-positive bacteria, fungi and yeast because they can penetrate the extensive meshwork of peptidoglycan in the cell wall without visible changes and reach the bacterial membrane leading to its disintegration. This can probably be explained by the strong fabric of the cell wall of gram-positive bacteria, which maintains its structure in spite of substantial hydrostatic turgor pressure within the bacteria [19].

TABLE II
ANTIMICROBIAL ACTIVITY AT DIFFERENT CONCENTRATIONS OF SEED OIL OF *M SYLVESTRIS* L. (DIAMETER OF INHIBITION IN MM).

Bacteria and Yeast	*15 µl*	*20 µl*	*30 µl*
St. aureus	/	/	/
L. monocytogenes	7	9.6	13.6
B. cereus	9	10	12.6
Ent. feacalis	10.3	11.3	14.6
E. coli	8	9	11
P. aeruginosa	/	/	/
Kl. pneumonia	/	/	7
C. albican	7.33	8.16	8.83

IV. CONCLUSIONS

M sylvestris L. seed oils serves as a new source of oil, the extraction by Soxhlet method resulted different types of fatty acids essentially unsaturated fatty acids. Based on the obtained results, seed oil may play potential roles as health-promoting agents, as well as providing valuable natural antimicrobial for the pharmaceutical industry. Further studies are needed to evaluate other biological activities of seed oil to identify and characterize the active components which are responsible for antimicrobial activity in these oils, other than fatty acids.

ACKNOWLEDGEMENTS

We thank all the volunteers for their cooperation. We also thank the UPRES Laboratory of Lipids and Nutrition, Faculty of Sciences Gabriel, Burgundy University (Dijon, France). All authors read and approved the manuscript. S. F., M. B., S. S.

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