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Chemical composition of two non-conventional oils in Morocco: *Melia azadirachta* and *Silybum marianum (L.)*

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Abstract
A comprehensive lipid profiling has been carried out on the seed oils of two non-conventional oils in Morocco, Neem (*Melia azadirachta*) and Milk thistle (*Silybum marianum (L.)*), in order to evaluate their potential uses. The paper reports the proximate evaluation of the two oils. The major fatty acid (FAs) of the Neem and Milk thistle oil was linoleic (69.2 and 48.26%) followed by oleic (18.9 and 27.7%), the phytosterol marker β-sitosterol accounted for 66.7 and 27.6% of total sterols contained in Neem and Milk thistle oil respectively. The main tocopherol for Neem oil is γ- tocopherol (59.2%) contrary to Milk thistle oil where α-tocopherol is the main tocopherol with 82.42%. Oxidative stability of both oils was measured at 110 °C at the air flow rate of 20 L/h, Neem and Milk thistle oil was less stable than other Moroccan oils with 5.07 and 5.84 hours respectively. This oil, therefore, has a potential for its use in human nutrition or industrial applications.

Keywords: Neem; Milk thistle; fatty acid; phytosterol; tocopherol; Oxidative stability

1. Introduction
Vegetable oils are essential in meeting global nutritional demands and are utilized for many food and other industrial purposes [1]. Despite the broad range of sources for vegetable oils, the world consumption is dominated by soybean, palm, rapeseed, and sunflower oils with 31.6, 30.5, 15.5, and 8.6 million tons consumed per year, respectively [2]. These conventional sources of vegetable oil no longer meet the ever increasing demands of domestic and industrial sectors [1]. Therefore, the need exists to look for other.
To meet the increasing demand for vegetable oils, improvements are being made, with conventional crops in the one hand and great interest in newer sources of non-conventional edible oils has recently grown in the other hand. Indeed, no oil from a single source has been found to be suitable for all purposes because oils from different sources generally differ in their composition [3]. Several plants are now grown, not only for food and fodder, but also for a variety of products with applications in industry, including oils and pharmaceuticals.
Among these plants, Milk thistle (*Silybum marianum (L.)*) and Neem (*Melia azadirachta*). Few data on seeds of Milk thistle (MT) are available [4] and those existing for *Melia azadirachta* (Neem) are rather old and/or incomplete [5, 6].

Silybum marianum (L.) is an annual or biennial medicinal herb that has been widely used in European traditional medicine [7], particularly in the treatment of various liver diseases [8], and belong to the family composite. It's abundantly available as weed in Morocco that mature in June, mostly grow wild on unutilized lands along roadside and is suitable for the control of environment pollutants [9]. *Melia azedarach* L. (Meliaceae), a deciduous tree native to northeastern India, has been naturalized in tropical and subtropical countries. Extracts and components of this plant are reputed for their insecticidal; *Melia azedarach* is also the source of many other important bioactive compounds [10]. *M. azedarach* is adapted in the Middle East and North Africa (MENA) region, little is known about its potential benefits as a source of natural products in the region.
Therefore, in the course of our investigation on the composition of locally available but less known seeds, either for food or for industrial uses [11, 12], we report here on the chemical composition of Neem and MT seed oils from Morocco: Fatty acid, sterol, tocopherol composition and oxidative stability, The results are then compared to those of the published works available in the literature. Data about the oxidative stability are the first reported about MT and Neem to our knowledge. The aim of the present work is to update and to widen available knowledge in order to check and confirm the interest of these seeds readily available as by-products.

2. Material and methods

2.1 Plant material

Fresh Neem (*Melia azadirachta*) and Milk thistle (*Silybum marianum* (L.)) seeds were collected respectively from Rabat (33°58'35.1"N 6°51'59.7"W) in the region of - Rabat Salé Kenitra -. The seeds were separated manually, cleaned for any adhering flesh and dried at 50 ◦C for 48 h. The dried seeds (1.5 kg) were ground in a mill and screened through a mesh of 0.5 mm.

2.2 Neem and Milk thistle Seed Oil Analysis

Oil extraction and yield quantification: Twenty g of ground seeds were extracted in a Soxhlet apparatus with 150 mL of boiling n-hexane for 8 h. The organic phase was collected, concentrated under vacuum, and dried for 5 min at 105°C. Extraction yield was determined using the official recommendation (ISO 659) [13], and the oil was used for the analyses.

Fatty acid composition was determined using method ISO 5508 [14]. Before analysis, fatty acids (FAs) were converted to fatty acid methyl esters (FAMEs) by shaking a solution of 60 mg oil and 3 mL of hexane with 0.3 mL of 2N methanolic potassium hydroxide. FAs were analyzed by gas chromatography using a Varian CP-3800 (Varian Inc.) chromatograph equipped with a FID. The column used was a CP-Wax 52CB column (30 m×0.25 mm i.d.; Varian Inc., Middelburg, The Netherlands). The carrier gas was helium and the total gas flow rate was 1 mL/min. The initial and final column temperature was 170 °C and 230 °C, respectively, and the temperature was increased by steps of 4°C/min. The injector and detector temperature was 230°C. Data were processed using a Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA). Results were expressed as the relative percentage of each individual FA present in the sample.

Sterol composition was determined using method ISO 6799 [15], after trimethylsilylation of the crude sterol fraction, using a Varian 3800 instrument equipped with a VF-1 ms column (30 m 9 0.25 mm i.d.) and helium (flow rate 1.6 mL/mn) as carrier gas, column temperature was isothermal at 270°C, injector and detector temperature was 300°C. Injected quantity was 1 µL for each analysis. Data were processed using Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA).

Tocopherols composition was determined using the method ISO 9936 [16]. High performance liquid chromatography (HPLC) was used for the determination of tocopherols, using a solution of 250 mg of oil in 25 mL of n-heptane. Tocopherols were analyzed by HPLC using Shimadzu CR8A instruments (Champ sur Marne, France) equipped with a C18-Varian column (25 cm×4 mm; Varian Inc., Middelburg, The Netherlands). Detection was performed using a fluorescence detector (excitation wavelength 290 nm, detection wavelength 330 nm). Eluent used was a 99:1 isooctane/isopropanol (V/V) mixture, flow rate of 1.2 mL/min.

2.3 Oxidative stability

Oxidative stability of seed oils Induction time was determined using the method ISO 6886 [17].The oxidative stability was evaluated by the Rancimat method. Stability was expressed as the oxidative induction period (IP, hours) measured at 110 °C on a Rancimat 743 (Metrohm Co, Basel) apparatus using 3 g of oil sample with an air flow of 20 L/h. Volatile oxidation products were stripped from the oil and dissolved in cold water, whose conductivity increased progressively. The time taken to reach a level of conductivity was measured.

2.4 Statistical analysis

Values reported in tables are the means ± SE of three replications. The significance level was set at P=0.05. Separation of means was performed by the Tukey's test at the 0.05 significance level.

3. **Results and discussion**

3.1 Oil yield

The oil yield of MT and Neem seeds is much lower than reported for other well-known oilseeds such as argan kernels (50 g/100 g), sesame seeds (54 g/100 g), nigella seeds (34 g/100 g), sunflower (44 g/100 g), and olive fruits (20 g/100 g) [18- 20]. This comparison shows that the use of MT and Neem for oil production is not useful from an economical point of view for a large scale industrial processing.

This value was lower than that found for Ikhtar Khan et al. (26.05%) for MT [21]. For Neem oil, our finding was higher that of Aoudia et al [22], but lower than the other variety of Neem oil (*Azadirachta indica*) (44%) [23].

Table 1: *Oil content in Neem and MT seeds.*

Neem (%)	13,55 ± 0.5
Milk Thistl (MT) (%)	19,01± 1.5

3.2 Fatty Acid Composition

The fatty acid (FA) composition is an essential indicator of the nutritional value of the oil. Neem and MT seed oil belongs to the linoleic-oleic acid group.

Table.2: *Fatty acid (%) composition of Moroccan Neem and MT seed oils.*

Fatty acid	*Milk Thistle*	*Neem*
Myristic C 14:0	0.07	0.04
Palmitic C 16:0	7.67	6.7
Palmitoleic C 16:1	0.05	0.06
Margaric C 17:0	0.07	0.1
Stearic C 18:0	4.97	3.3
Oleic C 18:1	27.71	18.9
Linoleic C 18:2	48.26	69.2
Linolenic C 18:3	0.22	0.33
Arachidic C 20:0	3.99	0.27
Eicosenoic C 20:1	1.11	0.32
Behenic C 22:0	3.85	0.66
Lignoceric C 24:0	1.05	0
MUFA	29.63	19.28
PUFA	48.48	69.53
SFA	21.67	11.07
P/S index	2.23	6.28

SFA: Saturated fatty acids. PUFA: Polyunsaturated fatty acids, MUFA: monounsaturated fatty acids

The polyunsaturated FAs (Table 2) make about half the composition, with the preponderance of Linoleic acid (48.26 and 69.2 % for MT and Neem, respectively). The saturated FAs primarily consist of palmitic (7.67, 6.7%)

and stearic acids (4.97, 3.3%); while oleic is also an important component (27.71, 18.9%), linolenic acid accounting for less than 1%. The low percentage of tri-unsaturated FA, less than 1%, should also play in favor of thermal and oxidative stability.
The relationship between saturated and polyunsaturated FA content is expressed as P/S index. This value is an important parameter for determination of nutritional value of certain oil. Oils and fats with higher value of P/S index than 1 are considered to have nutritional value. Both MT and Neem have a higher P/S index (2.23 and 6.28 respectively). Those values show that MT and Neem can have a good impact in the human health and it makes them suitable edible oils for mass consumption.
It should be noted that MT is found definitively lower in C18:2 with 48.26% in comparison to the 64.4% reported by Ikhtar Khan et al. [21] for MT from Pakistan and 53.3% from Egypt [4].
In contrary to our finding Hadjiakhoondi et al [24] report that Palmitic is the major fatty acid in Neem oil from Iran (18.8%), followed by linoleic acid (16.1%) this difference may be due to the region and climate effect. For the other variety of Neem the linoleic acid was the predominant with 48.26% [23].
These oils are close to other food oils of linoleic/oleic type: groundnut, cotton, olive [20, 25, 26].

3.3 Sterol Composition
Phytosterols are structurally similar to cholesterol and they are found naturally in plant seeds. The composition and the content of these compounds are very useful parameters for detecting adulterations or to proof authenticity, since it can be considered as a fingerprint of the oil. Besides, phytosterols are of major interest due to their antioxidant activity and impact on health [27, 28].

Table.3: *Sterol (%) composition of Moroccan Neem and MT seed oils.*

	Milk Thistle	*Neem*
Cholesterol	22.38	0.31
Campesterol	3.54	15.6
Stigmasterol	5.86	5.2
ß-sitosterol	27.06	66.7
Δ-5 Avenasterol	1.16	6.6
Δ -7 Avenasterol	19.74	1.01
Δ -7 Stigmastenol	16.39	1.16
Total sterol [mg/kg]	6273.3	2856.2

Total sterols amount 6273.3 mg/kg for MT, definitely closer to the value reported in the literature (6000 mg/kg). For Neem oil, total sterols amount was 2856.2 mg/kg, this value was higher than that reported by Djenontin et al (1880 mg/kg) [23]. These values are close to those reported for cotton seed oil (2920–3080 mg/kg) [29].
ß -sitosterol is the main sterol (66.7 and 27.06 % for Neem and MT) followed by Campesterol (15.6 %) for Neem oil, to the best of our knowledge, no results was found about sterol for this variety of Neem, but the same results was found by Djenontin et al. for the other variety (*Azadirachta indica*) [23]. On behalf of MT we were surprised by the high amount of Cholesterol (22.38%), this was not in agree with El-Mallah et al., for the Egyptian MT [4], who found that ß-sitosterol was followed by Δ7-Stigmasterol (20%). It is interesting to note that most of Moroccan seed oil is rich in ß-sitosterol, which has been shown to inhibit the absorption of dietary cholesterol [30].

3.4 Tocopherols composition
Owing to their role in the protection against oxidative deterioration of polyunsaturated fatty acids in plant material, tocopherols in seed oil are extremely important. They are natural lipophilic antioxidants mainly found in vegetable oils.

Table 4. *Tocopherol composition (%) of Moroccan Neem and MT seed oils.*

	α-T	*α-T3*	*β-T*	*γ-T*	*δ-T*	*δ-T3*	*Summe mg/100g*
Milk Thistle	82.42	0.22	11.64	4.34	0	1.32	38
Neem	35.6	0	0	59.2	5.1	0	27.6

T- Tocopherol
T3 - Tocotrienol

Now regarding the tocopherols in Table 4, the total content is 38 and 27.6 mg/100g, respectively, for MT and Neem. The total tocopherol in Neem oil is closer to the value of the other variety (29.8 mg/100g) [23]. For the MT our value was higher than the finding of El-Mallah et al (26 mg/100g) [4]. Nonetheless these values are lower than the content of most Moroccan edible oils, such as argan, olive, sesame and cactus oil (732, 182, 446, and 946 respectively) [19-20]. This is especially the case of Neem which is even poorer than primarily consists of γ, α, and δ -tocopherols, with the preponderance of the γ -tocopherol in Neem oil (59.2%), followed by α -tocopherol (35.6 %) and the absent of tocotrienol. MT shows α -tocopherol and β -tocopherol (82.42 and 11.64 %, respectively). We also detect a minor constituent of δ- and α- Tocotrienol (1.32 and 0.22 respectively).

3.5 Rancimat

Oxidation of lipid is a major cause of deterioration in the quality of edible oils. It is the cause of important deteriorative changes in their chemical, sensory and nutritional properties [31].

Table 5. *Rancimat induction period (h) at 110 °C of Neem, MT seed oil and other Moroccan vegetable oils.*

	Neem	*MT*	*Argan* [32]	*Sesame* [33]	*Olive* [20]	*Sunflower* [34]
Rancimat (h)	5.07	5.84	31	28.5	23	5.5

In our knowledge, the aspect of oxidation of Neem and MT oils has never been investigated in previous studies. To get a complete picture of these seeds oil oxidative stability, we decided to determine the induction period by Rancimat test. The induction time, evaluated by the Rancimat method is 5.07 and 5.84 h at 110 °C for Neem and MT respectively. Those last results cannot be compared to the literature data because Rancimat were not considered in relevant papers.

At the same temperature, we found the Rancimat induction time of 31, 28.5, 23and 5 h for argan, sesame, olive and refined sunflower oils, respectively (Table 5). Our results show that the stability of Neem and MT oil to oxidation is closer to that of sunflower oil. However, Neem and MT oil stability of much lower than that of sesame, olive and argan oils. The low stability oxidative of Neem and MT oil could be attributed to the low level of tocopherols and the high level of PUFA.

Conclusions

The study revealed that the fatty acid profile of Neem and Milk thistle oil showed four majority compounds, linoleic acid, oleic acid, stearic acid and palmitic acid. The analysis of the unsaponifiable fraction showed that the β-sitosterol is by far the major sterol for both oils. The major tocopherol was γ-tocopherol for Neem oil while it's α-tocopherol for milk thistle oil. The oxidative stability showed that the shelf life of both oils are short and can't be stored for long time.

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References

1. Idouraine A., Kohlhepp E. A. and Weber C.W., *J. Agric. Food Chem*. 44 (1996) 721–724.
2. Stevenson D. G., Eller F. J., Wang L., Jane J. L., Wang T., Inglett G. E., *J. Agric. Food Chem*. 55 (2007) 4005–4013.

3. Archana J, Singhal P. K. and Bachheti R. K.. *Int. J. Chem. Sci*., 10(4) (2012) 1951-1958.
4. El-Mallah M. H; El-Shami S M. and Hassanein M M. *Grasas y Aceites*, 54. 4 (2003) 397-402.
5. Kaushik, N.,Vir, S.,. *Biochem. Soc. Trans*. 28 (2000) 880–882.
6. Faye, M., (2010). PhD Thesis, University of Toulouse, 267p
7. Eichler O and Hahn M., *Arch Exp. Path Pharm*., 206 (1949) 674.
8. Ikram M., Ahmed N. and Bibi R., Bulletin No 4, PCSIR *Labs Pesh. Pak*. P.4 (1984)
9. Hussain I., Saleem M., Khan I., Khan S. A., Ullah W. and Khan H., *Jour. Chem. Soc. Pak*., 27 (2005) 373
10. Pimentel MR, Molina G, Dionísio AP, Maróstica Junior MR, Pastore GM. (2011). *Biotechnol Res Intern* Article, ID 576286, 11 pages
11. Gharby, S., Harhar, H., Guillaume, D., Roudani, A., Boulbaroud, S., Ibrahimi, M., Ahmad, M., Sultana, S., Ben Hadda, T., Chafchaouni-Moussaoui, I., Charrouf, Z., *J. Saudi Soc. Agric. Sci*. 14 (2015) 172–177
12. Asdadi A., Idrissi Hassani L.M., Chebli B., Moutaj R., Gharby S., Harhar H., Salghi R., EL Hadek M., *J. Mater. Environ. Sci.* 5 (3) (2014) 823-830
13. ISO 659, 2009. Oil seeds determination of oil content (reference method). EN ISO 659.
14. ISO 5508, 1990. Animal and vegetable fats and oils – analysis by CPG of methyl esters of fatty acids.
15. ISO 6799, 1991. Determination of the sterol fraction by gas chromatography.
16. ISO 9936, 2006. Animal fats and vegetable determination of tocopherols and tocotrienols by HPLC.
17. ISO – 6886, 2006. Animal and vegetable fats and oils-determination of oxidative stability.
18. Zine S, Gharby S, El Hadek M. *Biosci, Biotechnol Res Asia*. 10 (1) (2013) 1-7.
19. Harhar H, Gharby S, Guillaume D, Charrouf Z. *Eur J Lipid Sci Technol*. 112 (2010) 915-20.
20. Gharby S., Harhar H., Kartah B., Chafchauni I., Sibawayh Z., Charrouf Z., *J. Mater. Environ. Sci*. 4 (6) (2013) 935-942
21. Khan I, Khattak H, Ihsan Ullah I and Bangash F K., *Jour Chem. Soc. Pak*., 29, 6 (2007) 545-548.
22. Aoudia H, Oomah BD, Zaidi F, Zaidi-Yahiaoui R, Drover JCG, Harrison JE., (2013) *Inter J App Res Nat Prod*. 6 (2), 19-29.
23. Djenontina T S, Wotto V D., Avlessi F, Lozano P,. Sohounhloué D.K.C, Pioch D., *Ind Crop Prod* 38 (2012) 39– 45
24. Hadjiakhoondi A, Vatandoost H, Khanavi M, Sadeghipour-Roodsari H R, Vosoughid M, Kazemi M, Abai M R, *Iran J Pharma Sci*, 2(2) (2006) 97-102
25. Nile S H and Park S W., *Food Sci. Technol. Res*., 19, 6 (2013) 957 – 962,
26. Labuschagne M.T. and Hugo A., *Journal of Food Biochemistry* 34 (2010) 93–100.
27. Maestro-Duran, R., Borja-Padilla, R., *Grasas y Aceites* 44 (1993) 208–212.
28. Rigane, G., Ayadi, M., Boukhris, M., Sayadi, S., Bouaziz, M., *J. Sci. Food Agric*. 93, 3, (2013) 527–534.
29. Cercaci, L., M. T. Rodriguez-Estrada and G. Lercker. *J.Chromatogr. A.* 985 (2003) 211-220
30. Pegel, K.H.,. *S. Afr. J. Sci*. 93 (1997) 263–268.
31. Matthäus B., Guillaume D., Gharby S., Haddad A., Harhar H., and Charrouf Z. *Food Chemistry*. 120 (2010) 426-432.
32. Harhar. H, Gharby. S, Kartah. B, El Monfalouti. H, Guillaume. D and Charrouf. Z., (2011) *Plant Foods Hum Nutr* 66:163–168
33. Gharby S., Harhar H., Bouzoubaa Z., Asdadi A., El Yadini A., Charrouf Z., *J Saudi Soc Agri Sci* (2015) (In Press) doi:10.1016/j.jssas.2015.03.004
34. Gharby S., Harhar H., Bouzoubaa Z., Roudani A., Chafchaouni I., Kartah B., Charrouf Z., *J. Mater. Environ. Sci*. 5 (2) (2014) 464-469