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Research Article

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Chemical composition and anti-tubercular activity of the fixed oil of *Moringa oleifera* seed

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ABSTRACT

The seed oil of the highly promoted food plant Moringa oleifera Lam was extracted by solvent extraction using Soxhlet apparatus. The oil was methylated and analyzed on a Shimadzu GC-MS QP2010 SE.The oil was also screened against local strains of Mycobacterium tuberculosis by tetrazolium dye microbroth dilution assay and was found to be active at 25% (v/v). The result of GC-MS analysis of the methylated oil revealed 15 chemical components. The three major fatty components of the oil were oleic acid (58.88%), palmitic acid (26.16%) and glycerylmonooleate (5.27%). The anti-tubercular activity of the oil was attributed to the oleic acid and palmitic acid content. The oil could be a substitute for other oleic acid rich edible oil as well as a raw material for industrial production of oleic and palmitic acid derivatives and oleochemicals. The findings in this study could be exploited in new anti-tubercular drug research and drug design.

Keywords: *Moringa oleifera*, *Mycobacterium tuberculosis*, seed oil, oleic acid, palmitic acid, glycerylmonooleate.

INTRODUCTION

*Moringa oleifera*Lam commonly known as Moringa is a tropical and subtropical plant belonging to the Moringaceae family. It is believed to be native to India but is now grown in most tropical climate of the world [1]. The edible plant is widely consumed as a result of its highly attributed nutritional and medicinal value. The plant is known for its high nutrient value and had been reported to be rich in phytochemicals like β-carotene, flavonoids vitamin A, C and D, and essential amino acids, as well as minerals such as iron, potassium, calcium and phosphorous [2,3,4].The leaf is consumed in as vegetable in soups while the seed is consumed as vegetable as for refreshment and energy. The seed is also highly exploited for its highly nutritious oil which is believed to be as nutritive as olive oil. Moyo*et al.* reported 17 fatty acids from the leaf with α-Linolenic acid (44.57%) having the highest value followed by heneicosanoic (14.41%), γ-linolenic(0.20%) palmitic (0.17%) and capric acid (0.07%), as well as vitamin E (77 mg/100 g) and β-carotene (18.5 mg/100 g)[5]. A number of other bioactive compounds including alkaloids had been isolated and characterized from different parts of the plant. Some the compounds include niaziminin, niazinin, niazimicin, moringinine (benzylamine), quercetin, chlorogenic acid, aurantiamide acetate, etc., (Figure 1). Benzylamine and niaziminin had been reported with antidiabetic property [1]. Various parts of the plant are used in ethnomedicine for the management of various disease conditions. For instance, extracts of the leaf are drunk as tea for the management of diabetes, hypertension and HIV/AIDS. Within the last decade the plant has been promoted by

many herbalists as miracle tree for the treatment of numerous ailments[6]. The plant is also consumed by animals, especially livestock as foliage, whiles the trunk is deployed for various use in the community, e.g. for biogas, blue dye, etc.[7].

Scientifically, the leaf extract of the plant had been found to exhibit hypoglycemic, dyslipidemia and hepatoprotective activities in animal models [1,8,9]. The entire part of the plant include the seed oil had been reported to exhibit various biological and pharmacological activities including antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypo-cholesterolemic, and hypoglycemic activities. The plant had also been reported to be useful and efficacious in water purification by flocculation, sedimentation, antibiosis and reduction of *Schistosome cercariae* titer. The antibacterial and anti-ulcer activities have been attributed to benzyl isothiocyanate obtained from the dissociation of pterygospermin (Figure 1),a compound which was isolated and characterized from the plant. The seed oil had been reported to possess antifungal and antioxidant properties [7]. The oil had also been acclaimed with high energy and good oil quality. Despite the seemingly high profile of the plant and its current promotion for use in "food as medicine", not much work has been reported on moringa oil from varieties grown in Nigeria and the anti-tubercular potential of the plant. This study intended to determine the chemical composition of moringa seed oil from *Moringa oleifera* grown in NIPRD medicinal plant garden at Abuja Nigeria, and to determine its anti-tubercular activity.

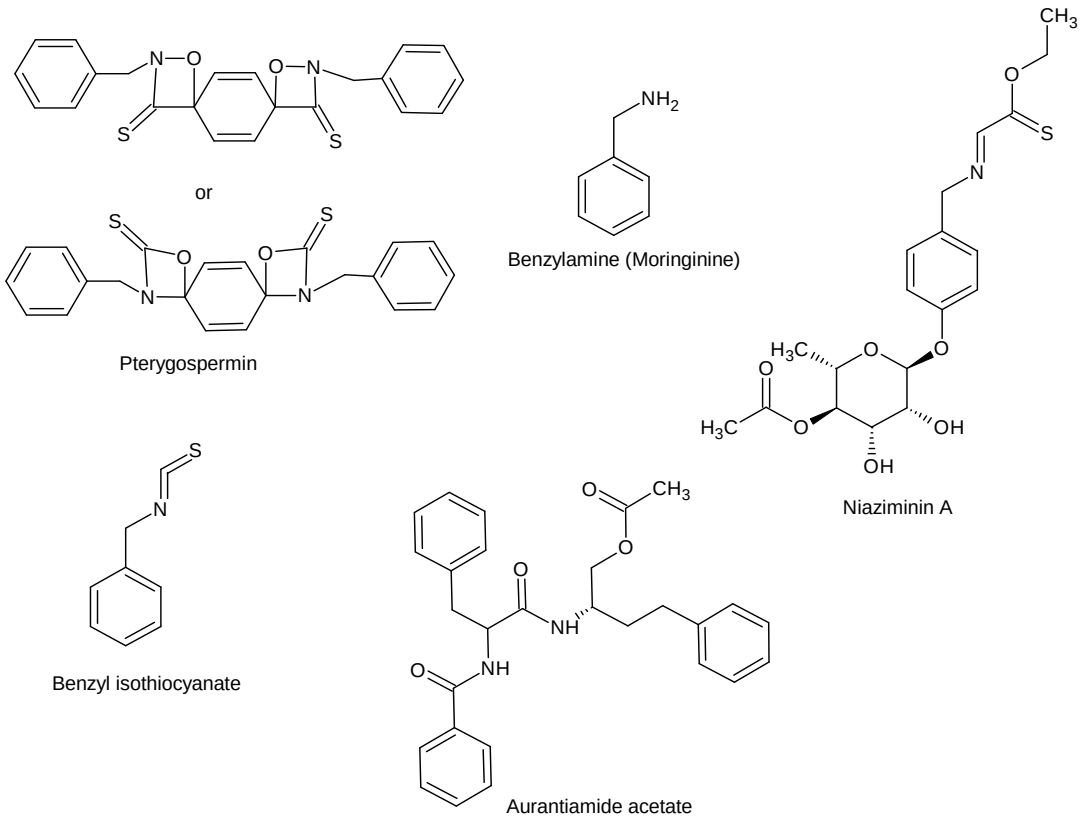

Figure 1: Structures of pterygospermin, moringinine, niaziminin A, benzyl isothiocyanate and aurantiamide

EXPERIMENTAL SECTION

Materials

All reagents used were of analytical grade. The chemical components of the seed oil were determined using a Shimadzu GC-MS model QP2010 SE.

Plant collection and extraction of seed oil

Dried seeds (200 g) of *M. Oleifera* harvested from NIPRD Medicinal Plant Garden at Idu, Abuja, were collected and pulverized with the aid of a mortar and pestle. The pulverized material was extracted in Soxhlet apparatus with hexane as solvent.The extract was concentrated under vacuum using a rotary evaporator at 50 °C. The oil obtained was kept in a sealed clean sterile sample bottles and stored in the dark at room temperature until required.

Methylation of seed oil for GC-MS analysis

Methanolic sodium hydroxide was prepared by dissolving 2 g of sodium hydroxide (NaOH) in 100 ml of methanol. The mixture was stirred for about 2 minutes till a clear solution of methanolic sodium hydroxide was obtained. 0.2g of oil was weighed into a separate quick fit conical flask and 6ml of methanolic sodium hydroxide was added, and refluxed for 10minutes on a steam bath. 10 ml of solution methanol and concentrated hydrochloric acid (30ml conc.HCl in 20ml methanol) was added and refluxed for another 10 minutes on the steam bath. This was followed by the addition of 10 ml of hexane and further reflux for 2 minutes. The mixture was allowed to cool and 10ml of distilled water was added. The mixture was poured into a separating funnel and the organic layer was collected and dried with calcium chloride[10]. The dried organic layer was used for GC-MS analysis.

GC-MS analysis of Seed oil

The methylated seed oil was analyzed on a Shimadzu GC-MS model QP2010 SE (Japan) at the Shimadzu Training Centre for Analytical Instruments (STC) Lagos. GC-MS analysis was carried out on Optima 5ms column of length, 30 m, internal diameter, 0.25 mm, and film thickness 0.25 µm. Carrier gas was helium, flow rate 0.9 mL min-1 and split 1.0. The conditions for analysis were set as follows; column oven temperature was programmed from 60-280°C (i.e. temperature at 60°C was raised to 180°C at 10°C/min and held for 2 min, and then finally to 280°C at 15°C/min and held for 4 min). The Injector and detector temperatures were 250 and 280°C, respectively. The MS parameters were: m/z range was 45 – 600 Da, and ion source temperature was 200°C. Essential oil compounds were identified and confirmed by matching their mass spectra with NIST11 mass spectral library collection.

Anti-TB screening – Tetrazolium microplate assay (TEMA)

Clinical *M. tuberculosis* collected from Tuberculosis Research Unit of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria was confirmed by Ziehl-Neelsen stain, grown on niacin media and arylsulphatase test which was positive after 14 days. The stock was filter-sterilized with 0.22 µm membrane filter and 50 µL were dispensed into well 1 in triplicate. Dilution of stock (1:2) was made in well 2 through to well 9 with double strength Middlebrook 7H9 broth, also in triplicate. This was followed by addition of 50 µL prepared test organisms using suspensions of *M. tuberculosis* prepared by emulsifying growth from slants (7H11) with 100 µL of Tween 80 into 0.2% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.). The turbidity was adjusted to McFarland standard no. 1 (approximately 3×10^7 CFU/mL) by adding Tween 80 and bovine serum albumin. The medium sterility, stock and organism viability controls were included. After 5 days incubation at 37°C, 50 µL of the Tetrazolium-Tween 80 solution was dispensed each into few wells for colour change to indicate growth and colourless if there is no growth of the *Mycobacterium* strains. Rifampicin was used as the standard antibiotic. Organism viability and media sterility control were also set up. The least concentration at which there was no growth of *Mycobacterium* was taken as the minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

The extraction yield for the seed oil was about 16.14% (w/w).The result of GC-MS analysis revealed fifteen (15) major component including three major fatty acids components (Figure 2, Table 1). The three major components include oleic acid (58.88%), palmitic acid (26.16%) and glycerylmonooleate or α-monoolein (5.27%). The percentage composition was determined using the % peak area of the constituents. The three major constituents, which are C_{16} and C_{18} fatty acid (and derivative) constituted 94.39% of the total constituents of the seed oil (Table 1).The non-fatty acid components of the oil amounted to only 4.34%. The, oleic acid rich oil could be said to be an unsaturated oil, and like other oleic acid rich oils, it may be heart friendly. The biological activity of the oil may perhaps be due to the fatty acid components, especially the oleic and palmitic fatty acids.

Several reports on moringa seed oil, known commercially as “ben oil” had reported oil content ranging from about 19 to 47%, and extraction yield of over 25 - 40% and described as sweet non-sticky and non-drying oil which resist rancidity [11-15]. It has found use in salad, machine lubricants, and perfume/cosmetic hair-care products manufacture [7,11]. Wild *M. oleifera* seed oil with oleic acid (73.22%), palmitic (6.45%), stearic (5.50%), behenic

(6.16%) and arachidic acids (4.08%) had been reported from Pakistan. The oil was categorized as high-oleic oil. Similar oil of high oleic content,(comparable to those found in virgin olive oil) with high oxidative stability had been reported in *M. oleifera* seed oil from Kenya, Indiaand other parts of the world (Table 2) [11,16]. The slightly lower oleic acid (58.88%) content and higher palmitic acid (26.16%) content in this study may be indicative of geographic differences or genetic variation.

The result of anti-tubercular activity showed that the oil was active against locally isolated strain of *Mycobacterium tuberculosis* at 25% (v/v), while that of the control drug, rifampicin, was 0.09 µg/mL. The antibacterial activity of fatty acids and oily substances had been well reported [17-23].Also, the antioxidant, antibacterial and anti-mycobacterial activity of oleic acid and its derivatives had been reported by many workers. Orhan *et al.* found fixed oil rich in oleic and linoleic acid to possess strong antioxidant and antimicrobial activities[20]. Similarly, Esquivel-Ferriño*et al.* reported the activities of palmitic acid and decanal against *M. tuberculosis* at 50 µg/ml and 25 µg/ml, respectively, in a study of essential oil from *Citrus* species[24]. Esquivel-Ferriño and her coworkers attributed the anti-tubercular activity of the hexane extract of *C. sinensis* peel to the palmitic acid, decanal, caryophyllene oxide, and cis-limonene oxide contained in the extract [24].In another study Esquivel-Ferriño and coworkers also reported the anti-tubercular activity of linoleic and oleic acid at 100 µg/mL[25].The activity of bioactive fractions of linoleic and oleic acid from *Pelagonium sp.* against *Mycobacterium aurum* and *M. phlei* was also reported by Venkatesalu *et al.*[23]. The compound (-)-Z-9-octadecene-4-olide, an oleic acid derivative had been isolated from the dichloromethane extract of the stem bark of *Micromelum hirsutum*, and found to possess potent in vitro anti-tubercular activity against H37Rv and the Erdman strain of *M. tuberculosis* in a J774 mouse macrophage model with MIC of 1.5 µg/mL and EC_{90} of 5.6 µg/mL, respectively [26].

In their study, Orhan*et al.* found *C. avellana* and *J. regia* oils containing oleic acid as high as 77.4% and 47.2%, respectively, to be more active towards *Parainfluenza*-type 3 (PI-3) than those with lower oleic acid contents[20]. Sandoval-Montemayor *et al*. also reported the anti-tubercular activity of commercially available palmitic acid (MICs = 25–50 µg/mL) and oleic acid (MICs = 100 µg/mL) against *M. tuberculosis*[27]. Thus the anti-tubercular activity of the seed oil of *M. oleifera* in this study is attributable to the oleic acid and palmitic acid content which amounted to about 94% of the oil fatty acid/derivatives composition. Although the mode of action of fatty acids in inhibiting bacterial cell growth or its outright destruction is not well understood, their antimicrobial potentials had been exploited by different organisms and by man in application in medicine, foods and agricultural preservation. It is believed that the primary site of action of fatty acids is the cell membrane where it interferes with the energy transport system by disrupts the electron transport chain and oxidative phosphorylation. Fatty acids membrane action may also inhibit enzyme activity, impair nutrient uptake, generate hazardous products of peroxidation and auto-oxidation and / or directly lyse bacterial cells[28]. Some studies had been reported to attribute fatty acid activity on inhibition of oxygen uptake and stimulation of amino acid uptake into the cell [20].*M. oleifera* oil may be exhibiting its actions through one or more of these mechanisms.

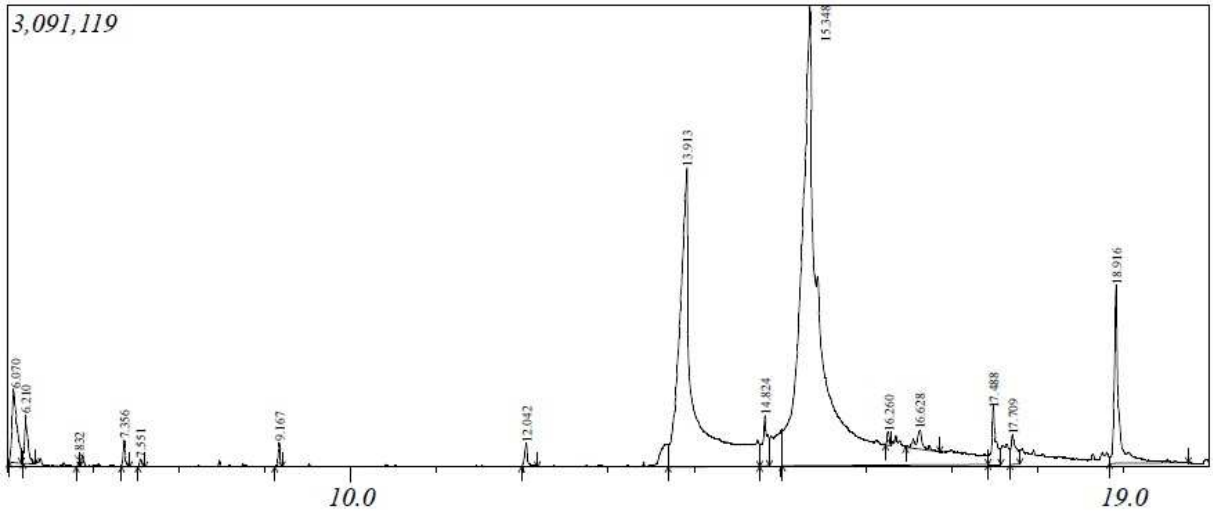

Figure 2: GC chromatogram of methylated seed oil of *M. oleifera*

Table 1: Result of GC-MS analysis of *Moringa oleifera* seed oil

S/N	Retention Time (min)	% Composition	MW (Da)	Names of identified compounds
1	6.070	2.27	128	Naphthalene
2	6.210	0.99	170	Dodecane
3	6.832	0.06	168	(Z)-9-methyl-3-Undecene
4	7.356	0.39	142	1-methyl-Naphthalene
5	7.551	0.12	142	2-methyl-Naphthalene
6	9.167	0.32	133	4-hydroxy-Benzeneacetonitrile
7	12.042	0.48	228	Myristic acid
8	13.913	26.16	256	Palmitic acid
9	14.824	1.55	296	Oleic acid methyl ester
10	15.348	58.88	282	Oleic acid
11	16.260	0.20	974	Triarachine
12	16.628	0.79	282	Butyl 9-tetradecenoate
13	17.488	1.57	884	(E,E,E)-1,2,3-propanetriyl 9-Octadecenoic acid ester
14	17.709	0.96	330	2-mono-Palmitin
15	18.916	5.27	356	GlycerylMonooleate
	C_{14} FA	1.27		
	C_{16} FA	27.12		
	C_{18} FA	67.27		
	C_{20} FA	0.2		
	Others	4.34		

FA = Fatty acids

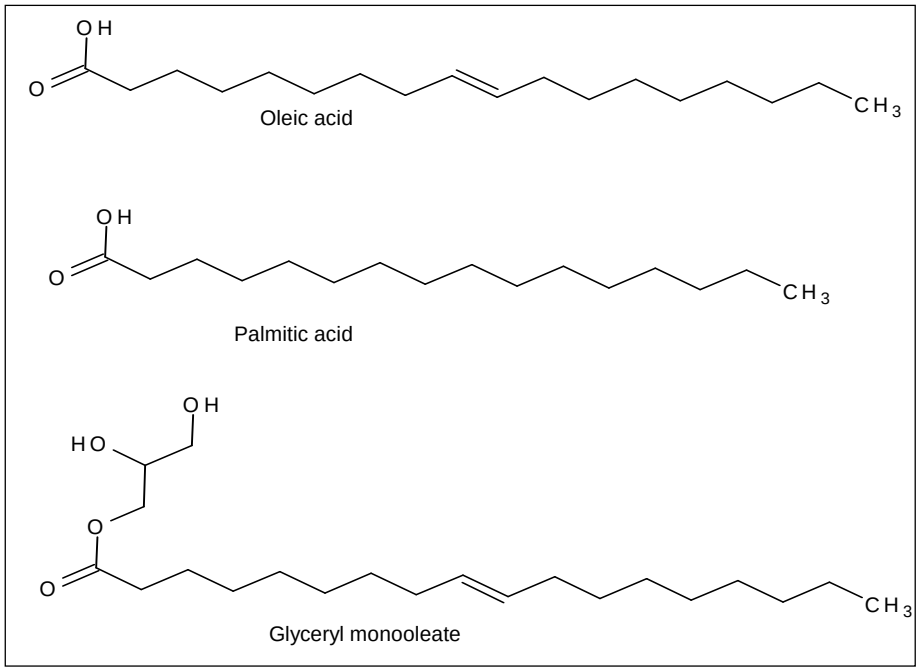

Figure 2: Structures of the major molecular components of *M. oleifera* seed oil

Table 2: Percentage C_{18}& C_{16} content in *M. oleifera* seed oil from different countries

Source Country/variety	% C_{18}	% C_{16}	% (C_{18} + C_{16})
Pakistan (Wild) [11]	80.29	7.42	87.71
Pakistan (Sindh) [11]	82.96	7.50	90.46
India (Periyakulam) [11]	77.92	7.82	85.74
Kenya (Mbololo) [11]	78.69	7.50	86.19
Malawi (Wild) [11]	74.57	6.61	81.18
Burkina Faso [12]	77.64	6.85	84.49
Malaysia [12]	69.20	10.00	79.20
Greece [12]	77.92	7.91	85.83
Nigeria (NIPRD)*	67.27	27.12	94.39

**This study.*

CONCLUSION

The study revealed that the *M. oleifera* seed oil from NIPRD grown Moringa plant is rich in oleic acid (58.88%), palmitic acid (26.16%) and glycerylmonooleate (5.27%). The oil was found to be active against *Mycobacterium tuberculosis* at 25% (v/v) concentration. The anti-tubercular activity of the oil was attributed to the high oleic acid and palmitic acid content due to the fact that both compounds had been previously reported to possess anti-tubercular activities. The oil could be a substitute for other oleic acid rich edible oil in the diets of TB patients as well as a raw material for industrial production of oleic and palmitic acid derivatives and oleochemicals. The findings in the study could be exploited in new anti-tubercular drug research and drug design.

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REFERENCES

[1] M Mbikay, *Frontiers Pharmacol*.,**2012**, 3 (Article 24), 1-12. doi:10.3389/fphar.2012.00024
[2] NK Amaglo; RN Bennett;RB LoCurto;EAS Rosa; VLoTurco; AGiuffrid; A LoCurto; FCrea; GMTimpo, *Food Chem*.,**2010**, 122, 1047–1054.
[3] R Gowrishankar; M Kumar; VMenon; SMDivi; MSaravanan; PMagudapathy;BK Panigrahi;KG Nair; K Venkataramaniah, *Biol. Trace Elem. Res*.,**2010**, 133, 357–363.
[4] PS Kumar; D Mishra; GGhosh; GS Panda, *Int.J. Phytomed*.,**2010**, 2, 210–216.
[5] B Moyo; PJ Masika; A Hugo;V Muchenje, *Afri. J. Biotechnol*.,**2011**, 10(60), 12925-12933.
[6] TG Monera; CCMaponga, *J. Int. AIDS Soc*.,**2010**, 13, 188.
[7] JW Fahey,*Moringa oleifera*: *Trees for Life J*.,**2005**,1(5), 1-15.
[8] JA Tende;I Ezekiel;AAU Dikko; ADTGoji, *Br. J. Pharm. Toxicol*.,**2011**, 2(1), 1-4.
[9] EE Efiong; GOIgile; BIA Mgbeje; EAOut; PEEbong, *J. Diabetes Endocrinol*.,**2013**, 4(4), 45-50.
[10] NB Kyriokiais; GDianysopoulos, *Analyst*,**1983**, 108(1287), 738-741.
[11] F Anwar; U Rashid, *Pak. J. Bot*.,**2007**, 39(5), 1443-1453.
[12] WR Compaoré;PA Nikièma; HINBassolé; ASavadogo; JMouecoucou; DJHounhouigan;SA Traoré, *Curr. Res. J. Biol. Sci*.,**2011**, 3(1), 64-72.
[13] Ahmad M.B., Rauf A., Osman S.M. Physicochemical analysis of seven seeds oils. Journal of the Oils Technologists' Association of India **1989**, 21(3):46-47.
[14] EN Ojiako;CC Okeke, *Asian J. Plant Sci. Res.*, **2013**, 3(3),1-4.
[15] A Roloff, H Weisgerber, U Lang, B Stimm.Moringa oleifera Lam, 1785; WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim ISBN: 978-3-527-32141-4. Enzyklopädie der Holzgewächse – 40. Erg.Lfg. 6/05. **2009**, Available at: https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwjfwIPv8r_JAhVI7xQKHS0KDkAQFggdMAA&url=http%3A%2F%2Fwww.wiley-vch.de%2Fbooks%2Fsample%2F3527321411_kap1.pdf&usg=AFQjCNHZVle9Lw5rEDkB68SZTkZzCXvkAg&sig2=5qy0UhCdWBmzF5kTzIcD5g [Accessed 03.12.2015].
[16] S Lalas; JTsaknis, *J. Food Composit. Analysis*,**2002**, 15, 65–77. doi:10.1006/jfca.2001.1042
[17] G Agoramoorthy; MChandrasekaran; V Venkatesalu; MJ Hsu, *Braz. J. Microbiol.*, **2007**, 38, 739-742.
[18] HO Egharevba; SKOkwute, *Pterodonta*. *Nat. Sci*.,**2014**, 12(1), 79-86.
[19] LJ McGaw; AK Jäger; J Van Staden, *Fitoterapia*,**2002**, 73,431-433.
[20] İ Orhan; BÖzçelik; BŞener, Evaluation of antibacterial, antifungal, antiviral, and antioxidant potentials of some edible oils and their fatty acid profiles. *Turk. J. Biol*.,**2011**, 35, 251-258. doi:10.3906/biy-0907-107.
[21] RS Santhosh; BSuriyanarayanan, *Planta Med*.,**2014**, 80, 9–21.
[22] V Seidel; PW Taylor, *Int. J. Antimicrob. Agen*.,**2004**, 23, 613-619.
[23] V Venkatesalu; PSundaramoorthy; MAnantharaj; MGopalakrishnan; MChandrasekaran, *Seaweed Res. Util*.**2004**, 26, 83-86.
[24] PC Esquivel-Ferriño; AF Clemente-Soto; MYRamírez-Cabriales; E Garza-González; LÁlvarez;MR Camacho-Corona, *J. Mex. Chem. Soc*..**2004**, 58(4), 431-434.

[25] PC Esquivel-Ferriño; JMJ Favela-Hernández; E Garza-González; N Waksman; MY Rios; MR Camacho-Corona,Antimycobacterial activity of constituents from *Foeniculumvulgare* Var. Dulce grown in Mexico. *Molecules*,**2012**, 17, 8471–8482.
[26] C Ma; RJCase; YWang; HJZhang; GTTan; NVHung; NMCuong; SGFranzblau; DDSoejarto; HHS Fong; GF Pauli, *Planta Med*.**2005**,71(3), 261–267. doi:10.1055/s-2005-837826.
[27] NE Sandoval-Montemayor; AGarcía; E Elizondo-Treviño; E Garza-González; L Alvarez; MRCamacho-Corona, *Molecules*,**2012**, *17*, 11173-11184.doi:10.3390/molecules170911173.
[28] AP Desbois; VJ Smith, *Appl. Microbiol. Biotechnol*.,**2010**, 85, 1629–1642.doi:10.1007/s00253-009-2355-3.