## Chemical Composition of Fatty Acids from the Air-dried Bark of Schinus terebinthifolius Raddi (Anacardiaceae)

### Mohamed Z.M. Salem<sup>1</sup>, Ashraf A. Hatamleh<sup>2</sup> and Hayssam M. Ali<sup>2,3\*</sup>

<sup>1</sup>Forestry and Wood Technology Department, Faculty of Agriculture (EL-Shatby), Alexandria University, Alexandria, Egypt.

<sup>2</sup>Botany and Microbiology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia.

<sup>3</sup>Timber Trees Research Department, Sabahia Horticulture Research Station, Horticulture Research Institute, Agriculture Research Center, Alexandria, Egypt.

(Received: 25 November 2013; accepted: 01 February 2014)

Recently and over the past few years, there are increasing in the studies of the diversity of secondary metabolites present in the Anacardiaceae family and these compounds are closely related to their biological active. In the present study the fatty acids from the air-dried bark of *Schinus terebinthifolius* Raddi were analyzed using GC. The total concentrations of fatty acids presented in bark were 0.113%. The major fatty acids constituents found in bark were; methyl ester of tridecanoic acid (C13:0) 7.05%, methyl ester of myristic acid (C14:0) 10.42%, methyl ester of palmitic acid (C16:0) 4.57%, and methyl ester of erucic acid (C22:1, *cis*-13) 6.57%.

Key words: Schinus terebinthifolius, air-dried bark, fatty acids, GC.

From the past studies, Schinus species are characterized by pungent-smell chemical constituents concentrated especially in fruits (Bendaoud et al., 2010), which are used to treat respiratory disorders and mycosis. According to (Lima et al., 2009), these properties are attributed to the presence of high levels of monoterpenes in these species. Schinus terebinthifolius Raddi (Anacardiaceae) is largely found in the Brazilian coast, and is distributed from the northeast to the south part of the country (Carvalho et al., 2013; Corrêa, 1974). The biological applications of this plant have been known for many years, and its properties have been described since the first edition of the Brazilian Pharmacopoeia, published in 1926 (Carvalho et al., 2013). S. terebinthifolius produce resin-like materials which contain monoterpenes to defend themselves against the penetrations of the attacking pathogens (Byers,

1995). Tannins, saponins and polyphenol S. terebinthifolius could be the major components that inhibited the growth of Jurkat cells (Woraratphoka et al., 2012; Queires et al., 2006). S. terebinthifolius has long been used in traditional Brazilian medicine, especially to treat inflammatory and haemostatic diseases (Lima et al., 2009). Several constituents have been identified like; phenols (Ceruks et al., 2007), terpens (Lloyd et al., 1977), tannins and flavonoids (Kassem et al., 2004; Degáspari et al., 2005) and anthraquinones, xanthones and free steroids (Lima et al., 2006). There are reports in the literature that patients suffering from rheumatism attained great relief after taking hot baths with decoction from the barks of S. terebinthifolius (Carvalho et al., 2013). Bark ethanolic extract showed good activity against resistant strains of Staphylococcus aureus (Lima et al., 2006). Additionally, the oil extracted from bark has been used for the treatment of tumors and corneal diseases (Bornhausen, 2010). Ouercetin and kaempferolwere were isolated from S. terebinthifolius and showed good Antifungal

<sup>\*</sup> To whom all correspondence should be addressed. Tel.: +966563772132; Fax: +966114675833; E-mail: hayssam77@hotmail.com

activity against the pathogenic fungus Paracoccidioides brasiliensis (Johann et al., 2010). (Melo Junior et al., 2000) was reported that the in vitro and in vivo of the ethanolic extract of bark showed to be effective against Enterococcus, viridans, Streptococcus Streptococcus hemoliticus, **Bacillus** corineforme, Staphylococcus aureus and gram-positive bacteria present in alveolitis in comparison to the one treated with gentamicin. The aim of the present study was to analyze the chemical composition of fatty acids from the air-dried bark of Schinus terebinthifolius Raddi by using the means of GC apparatus.

#### **MATERIALAND METHODS**

Bark samples of *Schinus terebinthifolius* was collected from pruning process to the tree species at Al-Diriyah City located on the northwestern outskirts of the Saudi capital, Riyadh, Saudi Arabia, and provided by the laboratory of Botany and Microbiology Department, College of Science, King Saud University, during the month of August 2013. The plant was kindly identified and authenticated at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University. The bark was air-dried under shade at room temperature and then pulverized into powder to obtain a 40–60 mesh.

#### **Fatty Acids Determination**

Bark sample of 10 g were weighted out into a conical and 10 mL of concentrated HCl and boiled in water bath until all the sample has dissolved. The conical was allowed to cool and the fats were extracted by shaking with 30 mL of diethyl ether and the extract was collected into a weighed flask after allowing the layers to separate. The extraction was repeated three times more and distilled off the solvent then the fat was dried at 100 °C, cooled and weighed (Kirk and Sawyer 1991).

#### **Methylation of Lipid**

Sample of 50 mg of lipid was weighted in a tube, and 50 mL of methanolic sulfuric acid (1 mL concentrated sulfuric acid and 100 mL methanol) and 2 mL of benzene were added. The tube was well-closed and placed in water bath at 90 °C for an hour and half. The tube then was cooled, and 8 mL water and 5 mL petroleum ether were added. Subsequently, the tube was strongly-shacked and

J PURE APPL MICROBIO, 8(1), FEBRUARY 2014.

the ethereal layer was separated out and evaporated to dryness. Table 1 is showing the condition used for characterization of fatty acids by GC. Standard fatty acids (C2-C25) were previously injected with the same condition used by GC (Radwan 1978).

#### **RESULTS AND DISCUSSION**

# Fatty acids detected in bark of Schinus terebinthifolius

The total concentrations of fatty acids presented in bark were 0.113%. Additionally, the total amount found in 100 g of bark sample was 0.053 g/100 g sample.

GC analyses of fatty acids of bark of S. terebinthifolius are presented in Table 2. The major fatty acids constituents found in bark (Figure 2) were; methyl ester of tridecanoic acid (C13:0) 7.05%, methyl ester of tetradecenoic acid (C14:1) 5.23%, Methyl ester of myristic acid (C14:0) 10.42%, methyl ester of palmitic acid (C16:0) 4.57%, methyl ester of erucic acid (C22:1, cis-13) 6.57%, methyl ester of 14-pentadecenooic acid (C15:1) 3.55%, methyl ester of pentadecanoic acid (C15:0) 3.69%, and methyl ester of hexadecenoic acid (C16:1) 1.72%. The minor amounts were reported by methyl ester of caprylic acid (C8:0) 0.71%, methyl ester of linoleic acid (C18:2, cis-9,12) 1.00%, methyl ester of oleic acid (C18:1) 0.88%, methyl ester of stearic acid (C18:0) 0.94%, methyl ester of heneicosanoic acid (C21:0) 0.39%, methyl ester of caprylic acid (C8:0) 0.71%, and methyl ester of undecanoic acid (C11:0) 0.60%. The most substances present in the family Anacardiaceae are triterpenes, phenolic lipids and biflavonoids and other classes of substances, such as phenols and cinnamic acid derivatives (Correia et al., 2006). Additionally, genus of Schinus revealed the presence of terpenoids and fatty acids in S. terebinthifolius and S. molle (Lloyd et al., 1977, Terhune et al., 1974). Other studies revealed that the ethanolic extract from the bark of S. terebinthifolius showed phenols, triterpenes and anthraquinones and the leaves showed the presence of phenols, flavones, flavonoids, xanthones, leucoanthocyanidins, flavanones and free steroids (Lima et al., 2006). The barks of S. terebinthifolius are rich in tannins and essential oils (Jorge and Markmann, 1996). The responsesurface models showed that ternary mixtures with equal portions of all the three solvents (water, ethanol and acetone) were better than the binary mixtures in generating crude extracts with the highest yield ( $22.04 \pm 0.48\%$ ), total polyphenol content ( $29.39 \pm 0.39\%$ ), and antioxidant capacity ( $6.38 \pm 0.21$ ) (DiCiaula *at al.*, 2013).

Previously, the oil extracted from the barks has also been used for the treatment of tumors and corneal diseases (Bornhausen, 2010). Carlini *et al.*, (2010) reported that extracts from barks showed a marked protective effect against gastric ulcerations induced by immobilization stresses at low temperature in rats. Saponins have antiinflammatory properties and are restricted to the bark, where it also showed positive reactions for flavonoids (Jorge and Markmann 1996). The results obtained by (Lima et al., 2009) showed that the oral administration of dried extracts of bark from S. terebinthifolius during 45 days in Wistar rats of both genders did not induce any toxic effect. Alkaloids, polyphenols, fatty acids, flavonoids, tannins, coumarins, glucosides, terpenes, polyacethylenes and essential oils are originated from various metabolic routes (Ali et al., 2013a; Ali et al., 2013b; Abdel-Megeed et al., 2013; Salem et al., 2013). Tannins are the major compounds of S. terebinthifolius bark and have been shown several biological activities such as anti-inflammatory, antibacterial, antifungal and anticancer are attributed to tannins (Matos, 1994). In addition, Varela-Barca et al., (2007) showed that flavonoidenriched fractions of *S. terebinthifolius* stem barks

Table 1. GC condition for analysis of fatty acids

Device model	HP (Hewlett Packard) 6890 GC			
Column	HP-5 (5% diphenyl, 95% dimethyl polysiloxane), 30 m, 0.32 mm. ID, 0.25 μm film thickness.			
Carrier gas/gas flow	Nitrogen/1 mL/min.			
Detector/temperature	FID (Flame Ionization Detector)/250 °C.			
Injector temperature, Injection volume	220 °C, 2 µL in a	220 °C, 2 μL in a splitless mode.		
Oven program	Initial Temp. 150	Initial Temp. 150 °C for 2 min.		
Ramps	Rate °C/min	Final Temp. °C	Hold time	
1	10	200	-	
2	5	250	9 min	

Table 2. Fatty	acid concentration	in bark of Schinus	terebinthifolius

Fatty acid (FA)	FA (g/100g lipid)	FA %	FA (g/100g sample)
Methyl ester of n-carpoic acid (C6:0)	0.0002	0.10	0.0001
Methyl ester of caprylic acid (C8:0)	0.0013	0.71	0.0006
Methyl ester of capric acid (C10:0)	0.0001	0.05	0.00005
Methyl ester of undecanoic acid (C11:0)	0.0013	0.60	0.0006
Methyl ester of lauric acid (C12:0)	0.0069	3.12	0.0033
Methyl ester of tridecanoic acid (C13:0)	0.0158	7.05	0.0075
Methyl ester of tetradecenoic acid (C14:1)	0.0117	5.23	0.0055
Methyl ester of myristic acid (C14:0)	0.0233	10.42	0.011
Methyl ester of 14-pentadecenooic acid (C15:1)	0.0079	3.55	0.0038
Methyl ester of pentadecanoic acid (C15:0)	0.0082	3.69	0.0039
Methyl ester of hexadecenoic acid (C16:1)	0.0038	1.72	0.0018
Methyl ester of palmitic acid (C16:0)	0.0102	4.57	0.0085
Methyl ester of linolenic acid (C18:3, <i>cis</i> -9,12,15)	0.0003	0.15	0.00016
Methyl ester of linoleic acid (C18:2, <i>cis</i> -9,12)	0.0022	1.00	0.00106
Methyl ester of oleic acid (C18:1)	0.0019	0.88	0.00093
Methyl ester of stearic acid (C18:0)	0.0021	0.94	0.001
Methyl ester of heneicosanoic acid (C21:0)	0.0008	0.39	0.00041
Methyl ester of erucic acid (C22:1, cis-13)	0.014	6.57	0.0069

J PURE APPL MICROBIO, 8(1), FEBRUARY 2014.

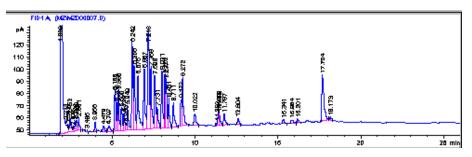


Fig. 1. GC chromatogram of the fatty acids from Schinus terebinthifolius bark

were capable of breaking phosphodiester bonds in DNA, generating lesions that could potentially lead to mutations. In other study, the bacterial action and broad spectrum of antifungal preservative system containing the combination of the powder from the bark of the *Schinus terebintifolius* and essencial oil of *Syzygium aromaticum* L. and the incorporation of the essential oil favored including the antifungal inhibiting the growth of *A. brasilienses* were evaluated by Machado *et al.*, (2012).

#### ACKNOWLEDGEMENTS

This project was supported by the King Saud University, Deanship of Scientific Research, College of Science Research Center.

#### CONCLUSION

In the present study, the total concentrations of fatty acids presented in bark of Schinus terebinthifolius were 00.113%. The major fatty acids detected in bark were; methyl ester of tridecanoic acid (C13:0) 7.05%, methyl ester of tetradecenoic acid (C14:1) 5.23%, Methyl ester of myristic acid (C14:0) 10.42%, methyl ester of palmitic acid (C16:0) 4.57%, methyl ester of erucic acid (C22:1, cis-13) 6.57%, methyl ester of 14pentadecenooic acid (C15:1) 3.55%, methyl ester of pentadecanoic acid (C15:0) 3.69%, and methyl ester of hexadecenoic acid (C16:1) 1.72%. The minor amounts were reported by methyl ester of caprylic acid (C8:0) 0.71%, methyl ester of linoleic acid (C18:2, cis-9,12) 1.00%, methyl ester of oleic acid (C18:1) 0.88%, methyl ester of stearic acid (C18:0) 0.94%, methyl ester of heneicosanoic acid (C21:0) 0.39%, methyl ester of caprylic acid (C8:0) 0.71%, and methyl ester of undecanoic acid (C11:0) 0.60%.

#### REFERENCES

- Abdel-Megeed, A., Salem, M.Z.M., Ali, H.M., Gohar, Y.M. *Brachychiton diversifolius* as a Source of Natural Products: Antibacterial and Antioxidant Evaluation of Extracts of Wood Branches. *J Pure Appl Microbio.*, 2013; 7(3):1843-1850.
- Ali, H.M., Salem, M.Z.M., Abdel-Megeed, A. In-vitro Antibacterial Activities of Alkaloids Extract from Leaves of *Conocarpus lancifolius* Engl. *J Pure Appl Microbio*, 2013a; 7(3):1903-1907.
- Ali, H.M., Salem, M.Z.M., Al Sahli, A.A., Performance of antioxidant activity of methanolic extracts from different parts of some tree species using DPPH radical-scavenging assay. *J Pure Appl Microbio*, 2013b; 7(Spl. Edn.): 131-137.
- Báidez, A.G., Gómez, P., Del Río, J.Á., Ortunõ, A., Antifungal capacity of major phenolic compounds of Olea europaea L. against Phytophthora megasperma Drechsler and Cylindrocarpon destructans (Zinssm.) Scholten. Physiol. Mol. Plant Pathol., 2006; 69:224-229.
- Bendaoud, H., Romdhane, M., Souchard, J. P., Cazaux, S., Bouajila, J. Chemical composition and anticancer and antioxidant activities of *Schinus Molle* L. and *Schinus terebinthifolius* Raddi berries essential oils. *J. Food Sci.*, 2010; **75**: 466-72.
- Bornhausen, R., Ervas do sítio. Http:// www.Ervasdositio.Com.br/enciclopedia/ enciclopedia.Asp 2010;.
- Byers, J.A., Chemical ecology of insects 2, Chapman and Hall, New York, 1995; 154-213.
- Carlini, E. A., Duarte-Almeida, J. M., Rodrigues, E., Tabach, R. Antiulcer effect of the pepper trees *Schinus terebinthifolius* Raddi (aroeira-dapraia) and *Myracrodruon urundeuva* Allemao, Anacardiaceae (aroeira-do-sertao). *Revista Brasileira De Farmacog*, 2010; 20: 140-6.
- 9. Carvalho, M.G., Melo, A.G.N., Aragão, C.F.S.,

J PURE APPL MICROBIO, 8(1), FEBRUARY 2014.

Raffin, F.N., Moura, T.F.A.L. Schinus terebinthifolius Raddi: chemical composition, biological properties and toxicity. *Rev. Bras. Pl. Med., Botucatu,* 2013; 15(1):158-169.

- Ceruks, M., Romoff, P., Fávero, O.A., Lago, J.H.G. Constituintes fenólicos polares de Schinus terebinthifolius Raddi (Anacardiaceae). *Química Nova* 2007; 30: 597–599.
- Corrêa, M.P. Dicionário de plantas úteis do Brasil e das exóticas cultivadas, vol. 3. Imprensa Nacional, Rio de Janeiro, *Brazil*, 1974; 125–126.
- Correia, S. J., David, J. P., David, J. M. Metabólitos secundários de espécies de Anacardiaceae. *Química Nova*, 2006; 29: 1287-1300.
- Degáspari, C.H., Waszczynskyj, N., Prado, M.R.M. Atividade antimicrobiana de Schinus terebinthifolius Raddi. *Ciência e Agrotecnologia Lavras*. 2005; 29: 617–622.
- DiCiaula, M. C., Lopes, G. C., Scarminio, I. S., de Mello, J. C. P. Optimization of solvent mixtures for extraction from bark of Schinus terebinthifolius by a statistical mixture-design technique and development of a UV-VIS spectrophotometric method for analysis of total polyphenols in the extract. Quim. Nova, Vol. XY, No. 00, 1-6, (In press).http://quimicanova. sbq.org.br/qn/Prelo\_QN\_NTecnica.htm
- Johann, S., Sá, N. P., Lima, L. A.R.S., Cisalpino, P. S., Cota, B. B., Alves, T. M.A., Siqueira, E. P., Zani. C. L., Antifungal activity of schinol and a new biphenyl compound isolated from *Schinus terebinthifolius* against the pathogenic fungus Paracoccidioides brasiliensis. *Ann. Clin. Microbiol. Antimicrob.*, 2010; 9:30
- Jorge, L. I. F., Markmann, B. E. O. Exame químico e microscópico de Schinus terebinthifolius Raddi (Aroeira). Revista De Ciências Farmacêuticas, 1996; 17: 139-45,
- Kassem, M.E.S., El-Desoky, S.K., Sharaf, M.,. Biphenyl esters and bioflavonoids from the fruits of Schinus terebinthifolius. *Chem. Nat. Compd.*, 2004; **40**:447–450.
- Kirk, R. S., Sawyer, R. "Pearson's Chemical Analysis of Foods' 9<sup>th</sup> Ed.," (Longman Scientific and Technical; Harlow, Essex, UK). 1991.
- Lima L.B., Vasconcelos, C.F.B. Maranhão, H.M.L., Leite, V.R., Ferreira, P.A., Andrade, B.A., Araújo, E.L., Xavier, H.S., Lafayette, S.S.L., Wanderley, A.G. Acute and subacute toxicity of Schinus terebinthifolius bark extract. *J. Ethnopharmacol.*, 2009; **126**: 468–473.
- 20. Lima, M.R.F., Luna, J.S., Santos, A.F., Andrade, M.C.C., Sant'ana, A.E.G., Genet, J.P., Marquez,

B., Neuville, L., Moreau, N. Anti-bacterial activity of some Brazilian medicinal plants. *J. Ethnopharmacol.*, 2006; **105**: 137–147.

- Lloyd, H. A., Jaouni, T. M., Evans, S. L., Morton, J. F. Terpenes of *Schinus* terebinthifolius. Phytochemistry, 1977; 16: 1301– 1302.
- Machado, J. A., Rebelo, M. A., Favaro, L. I.L., Vila, M. M.D.C., Gerenutti, M., In vitro evaluation of the antimicrobial potential association of *Schinus terebinthifolius* Raddi and *Syzygium aromaticum* L. iosr. *J. Pharma*. 2012; 2(3): May-June, 438-443.
- 23. Matos, F. J. A. Farmácias vivas. Fortaleza: EUFC, 1994.
- Melo Junior, E. J. M., Raposo, M. J., Sant'ana, A. E. G., Lisboa Neto, J. A., DINIZ, M. F. A. Estudo de plantas medicinais com atividade antimicrobiana sobre microrganismos presentes na alveolite. *Revista ABO Nacional*, 2000; 8: 220-6.
- Queires, L.C.S., Fauvel-Lafève, F., Terry, S., De la Taille, A., Kouyoumdjian, J.C., Chopin, D.K., Vacherot, F., Rodrigues L.E.A., Crépin, M., Polyphenols purified from the Brazilian aroeira plant (*Schinus terebinthifolius*, Raddi) induce apoptotic and autophagic cell death of DU145 cells. *Anticancer Res.*, 2006; **26**: 379-388.
- 26. Radwan, S.S. Coupling of Two-Dimensional Thin-Layer Chromatography with Gas Chromatography for the Quantitative Analysis of Lipid Classes and their Constituent Fatty Acids. J. Chrom. Sci. 1978; **16**: 538-542.
- Salem, M.Z.M., Ali, H.M., El-Shanhorey, N.A., Abdel-Megeed A. Evaluation of extracts and essential oil from *Callistemon viminalis* leaves: antibacterial and antioxidant activities, total phenolic and flavonoid contents. *Asian Pac. J. Trop. Med.*, 2013; 6: 785–791.
- Terhune, S. J., Hogg, J. W., Lawrence, B. M. â-Spathulene: a new sesquiterpene in *Schinus molle* oil. *Phytochemistry*, 1974; 13: 865-6,
- Varela-Barca, F. N., Agnez-Lima, L. F., Medeiros, S. R. Base excision repair pathway is involved in the repair of lesions generated by flavonoid-enriched fractions of pepper tree (*Schinus terebinthifolius* Raddi) stem bark. Environmental and Molecular Mutagenesis, 2007; 48: 672-81.
- Woraratphoka, J., Intarapichet, K-O., Indrapichate, K., Antioxidant Activity and Cytotoxicity of Six Selected, Regional, Thai Vegetables. *Amer. Euras. J. Toxicolo. Sci.*, 2012; 4(2): 108-117.

J PURE APPL MICROBIO, 8(1), FEBRUARY 2014.