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Identification of Fatty Acids from *Schinus terebinthifolius* Raddi Leaves using Standard Fatty Acids with C_2-C_{25}

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In the present study the fatty acids from leaves of *Schinus terebinthifolius* Raddi were analyzed using GC. The total concentrations of fatty acids presented in leaves were 0.551%. The total amount found in 100 g of sample of leaves was 1.403 g/100g. The fatty acids constituents found in leaves were; methyl ester of heptadecenoic acid (C17:1) 22.56%, methyl ester of stearic acid (C18:0) 11.47%, methyl ester of oleic acid (C18:1) 11.18%, methyl ester of pentadecanoic acid (C15:0) 5.65%, methyl ester of hexadecenoic acid (C16:1) 5.28%, methyl ester of archidic acid (C20:0) 7.57%, methyl ester of tetradecenoic acid (C14:1) 6.71%, methyl ester of myristic acid (C14:0) 5.21%, methyl ester of tridecanoic acid (C13:0) 4.69%, methyl ester of palmitic acid (C16:0) 4.06%, methyl ester of 14-pentadecenooic acid (C15:1) 4.91%, methyl ester of caprylic acid (C8:0) 1.72%, methyl ester of lauric acid (C12:0) 1.96%, methyl ester of heneicosanoic acid (C21:0) 1.97%, methyl ester of erucic acid (C22:1, *cis*-13) 2.32%, Methyl ester of linoleic acid (C18:2, *cis*-9,12) 1.27%, methyl ester of margarinic acid (C17:0) 0.44%, methyl ester of capric acid (C10:0) 0.18%, and methyl ester of docosadienoic acid (C22:2) 0.17%.

Key words: *Schinus terebinthifolius*, leaves, standard fatty acids, GC.

The Brazilian pepper tree *Schinus terebinthifolius* var. *terebinthifolius* Raddi (*S. terebinthifolius*) belongs to family Anacardiaceae is an evergreen tree, shrub and occasionally a liana or epiphyte with 10 to 43 feet (3-13 m) tall, found in Northeast region of Brazil and has been used in popular medicine to treat respiratory infections (Lima *et al.*, 2006). It has been introduced and naturalized in many countries of the world (Taylor, 2005). It typically has a multiple-stemmed trunk with most stems less than 4 inches (<10 cm). The fruit is becoming red in December and January with 17 cm-leaves height, with 5-13 leaflets (Lemke, 1992; Spector and Putz, 2006).

This specie is highly aromatic and as consequence, numerous investigations of their volatiles oils have been undertaken. The previous studies reported that essential oils of this plant have antibacterial activity against *Escherichia coli*, *Shigella dysenteriae*, *Bacillus subtilis* and *Staphylococcus albuns* (Siddiqui *et al.*, 1995) and recently reviewed by Silva *et al.*, (2010). Additionally, the ancient phytochemical studies carried out by Lloyd *et al.*, (1977) was revealed the presence of triterpene alcohols, ketones, acids, monoterpenes and sesquiterpenes in the bark,

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leaves and fruits. Furthermore, the leaves essential oil from the different regions around the world has shown a number of differnt chemotypes by GC/MS analysis. For example, α-pinene (51,82%) in Indian plants (Chowdhury and Tripani, 2001), α-phellandrene (24,2%) in Egypt plants (Ibrahim *et al.*, 2004), limonene (17,7%) and p-cymene (15,7%) in Reunion Island plants (Vernin and Parkanyi, 2003). Almost all parts of *S. terebinthifolius*, including leaves, bark, fruit, seeds, resin, and oleoresin (or balsam), have been used medicinally by indigenous peoples throughout the tropical regions. In South Africa, a leaf tea is used to treat colds, and a leaf decoction is inhaled for hypertension, depression, and irregular heart beat (El-Massry *et al.*, 2009). Traditionally, *S. terebinthifolius* was also used as an antibacterial, antiviral, diuretic, digestive stimulant, tonic, wound healer, anti-inflammatory, and hemostatic as well as a medicament to treat urinary and respiratory infections (Molina-Salinas *et al.*, 2006). Leaf extracts were reported to possess antioxidant (Woraratphoka *et al.*, 2012). Schinol, biphenyl 4'-ethyl-4-methyl-2,2',6,6'-tetrahydroxy[1,1'-biphenyl]-4,4'-dicarboxylate obtained from leaves and stems, showed marked antifungal activity against *P. brasiliensis* (MIC = 15.6µg.mL-1) (Johann *et al.*, 2010a) as well as quercetin and kaempferolwere were isolated from *S. terebinthifolius* and showed good Antifungal activity against the pathogenic fungus *Paracoccidioides brasiliensis* (Johann *et al.*, 2010a). Quercetin was previously exhibited activity against Phytophthora megasperma and Cylindrocarpon destructans (Báidez *et al.*, 2006). Crude extracts from leaves were showed activity against clinical isolates of *P. brasiliensis*, *Cryptococcus neoformans*, *Sporothrix schenkii*, and five clinical relevant species of *Candida* ((Johann *et al.*, 2010b; Johann *et al.*, 2007). The aim of the present study was to identify the fatty acids composition of dried leaves of *Schinus terebinthifolius* Raddi using GC apparatus.

MATERIALS AND METHODS

Leaves 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 at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University. The leaves were air-dried under shade at room temperature and then pulverized into powder to obtain a 40–60 mesh.

Fatty Acids Determination

Leaves 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 blown 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, added 8 mL water and 5 mL petroleum ether. Subsequently, the tube was strongly-shacked and 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 (C_2-C_{25}) were previously injected with the same condition used by GC (Radwan 1978).

RESULTS AND DISCUSSION

Fatty acids detected in leaves of *Schinus terebinthifolius*

The total concentrations of FA presented in leaves was 0.551%. Additionally, the total amount found in 100 g of sample leaves was 1.403 g/100g leaves sample.

Mostly, the lipophilic components from wood, bark and leaves are commonly composed mainly of fatty acids, and fatty acid esters. GC analyses of fatty acids of leaves of *S. terebinthifolius* are presented in Table 2. The fatty acids constituents found in leaves (Figure 1) were;

methyl ester of heptadecenoic acid (C17:1) 22.56%, methyl ester of stearic acid (C18:0) 11.47%, methyl ester of oleic acid (C18:1) 11.18%, methyl ester of pentadecanoic acid (C15:0) 5.65%, methyl ester of hexadecenoic acid (C16:1) 5.28%, methyl ester of archidic acid (C20:0) 7.57%, methyl ester of tetradecenoic acid (C14:1) 6.71%, methyl ester of myristic acid (C14:0) 5.21%, methyl ester of tridecanoic acid (C13:0) 4.69%, methyl ester of palmitic acid (C16:0) 4.06%, methyl ester of 14-pentadecenooic acid (C15:1) 4.91%, methyl ester of caprylic acid (C8:0) 1.72%, methyl ester of lauric acid (C12:0) 1.96%, methyl ester of heneicosanoic acid (C21:0) 1.97%, methyl ester of erucic acid (C22:1, *cis*-13) 2.32%, Methyl ester of linoleic acid (C18:2, *cis*-9,12) 1.27%, methyl ester of margarinic acid (C17:0) 0.44%, methyl ester of capric acid (C10:0) 0.18%, and methyl ester of docosadienoic acid (C22:2) 0.17%.

Family Anacardiaceae are showed to present the substances like; triterpenes, phenolic lipids and bioflavonoids, phenols and cinnamic acid derivatives (Correia *et al.,* 2006). Terpenoids and fatty acids are commonly presnent in *S. terebinthifolius* and *S. molle* (Lloyd *et al.*, 1977, Terhune *et al.,* 1974). The leaves showed the presence of phenols, flavones, flavonoids, xanthones, leucoanthocyanidins, flavanones and

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 splitless mode.		
Oven program	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 leaves of *Schinus terebinthifolius*

Fatty acid (FA)	FA (g/100g lipid)	FA %	FA (g/100g sample)
Methyl ester of caprylic acid (C8:0)	0.0041	1.72	0.011
Methyl ester of capric acid (C10:0)	0.0004	0.18	0.0011
Methyl ester of lauric acid (C12:0)	0.0047	1.96	0.012
Methyl ester of tridecanoic acid (C13:0)	0.011	4.69	0.028
Methyl ester of tetradecenoic acid (C14:1)	0.044	6.71	0.114
Methyl ester of myristic acid (C14:0)	0.031	5.21	0.78
Methyl ester of 14-pentadecenooic acid (C15:1)	0.019	4.91	0.05
Methyl ester of pentadecanoic acid (C15:0)	0.028	5.65	0.07
Methyl ester of hexadecenoic acid (C16:1)	0.022	5.28	0.056
Methyl ester of palmitic acid (C16:0)	0.009	4.06	0.024
Methyl ester of heptadecenoic acid (C17:1)	0.131	22.56	0.33
Methyl ester of margarinic acid (C17:0)	0.001	0.44	0.003
Methyl ester of linoleic acid (C18:2, *cis*-9,12)	0.003	1.27	0.007
Methyl ester of oleic acid (C18:1)	0.092	11.18	0.233
Methyl ester of stearic acid (C18:0)	0.092	11.47	0.234
Methyl ester of archidic acid (C20:0)	0.032	7.57	0.082
Methyl ester of heneicosanoic acid (C21:0)	0.0047	1.97	0.012
Methyl ester of docosadienoic acid (C22:2)	0.0004	0.17	0.001
Methyl ester of erucic acid (C22:1, *cis*-13)	0.017	2.32	0.044
Methyl ester of behenic acid (C22:0)	0.0012	0.52	0.003

free steroids (Lima *et al.*, 2006). The leaves of *S. terebinthifolius* are rich in tannins and essential oils (Jorge and Markmann, 1996). The ethyl acetate fraction of the ethanolic extract from leaves of *S. terebinthifolius* are rich of the chemical substances like; ethyl gallate, myricetrin, quercitrin, methyl gallate and myricetin and were responsible for the anti-free radical (Ceruks *et al.*, 2007). Solutions obtained from leaf decoction or crushed dried leaves acted as an antiseptic agent when applied directly to wounds or ulcers (Morton, 1978). In addition, the oil extracted from the barks has also been used for the treatment of tumors and corneal diseases (Bornhausen, 2010).

Alkaloids, fatty acids, flavonoids, isoflavonoids, 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). Biologically, Johann *et al.*, (2007) reported that ethyl acetate fraction from leaves of *S. terebinthifolius* inhibited the growth of three *C. albicans* strains, as well as their adhesion to buccal epithelial cells at concentrations of 7.8 µg.mL-1 and 15 µg.mL-1, respectively. Recently, schinol and a (4'- ethyl – 4 – methyl – 2, 2', 6, 6'- tetrahydroxyl(1.1'- biphenyl) – 4,4'- dicarboxylate), isolated from hexane and dichloromethane fractions obtained from leaves and stems, showed marked antifungal activity against *P. brasiliensis* (MIC = 15.6µg.mL-1) (Johann *et al.*, 2010b). Compounds like, Myricetin 3-O-b-D-glucuronide (Farag, 2008), (+) Catechin (Ceruks, et al., 2007), myricetin (Cavalher-Machado, *et al.*, 2008), Gallic acid (Cavalher-Machado, et al., 2008), [Syringic acid, Coumaric acid, Ellagic acid, Caffeic acid] (El-Massry, *et al.*, 2009), and Simiarenol (Lloyd, *et al.*, 1977) were also reported. On the other hand, ethanolic extracts from the leaves showed positive results for phenols, flavones, flavonoids, xanthones, leucoanthocyanidins, flavanones and free steroids (Lima *et al.*, 2006).

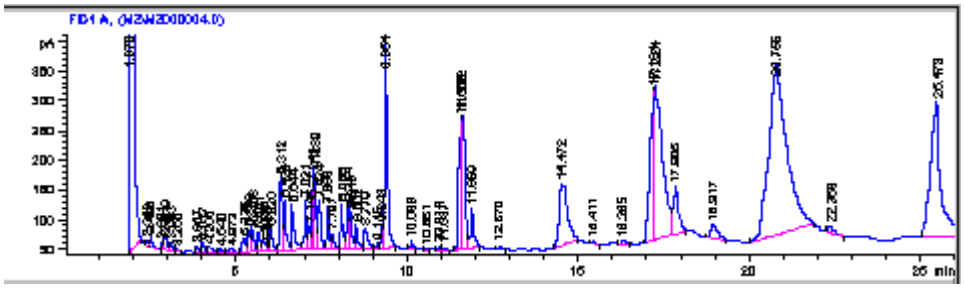

Fig. 1. GC chromatogram of the fatty acids from *Schinus terebinthifolius* leaves

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CONCLUSION

In the present study, the total concentrations of fatty acids presented in leaves of *Schinus terebinthifolius* were 0.551%. The fatty acids constituents found in leaves were; methyl ester of heptadecenoic acid (C17:1) 22.56%, methyl ester of stearic acid (C18:0) 11.47%, methyl ester of oleic acid (C18:1) 11.18%, methyl ester of pentadecanoic acid (C15:0) 5.65%, methyl ester of hexadecenoic acid (C16:1) 5.28%, methyl ester of archidic acid (C20:0) 7.57%, methyl ester of tetradecenoic acid (C14:1) 6.71%, methyl ester of myristic acid (C14:0) 5.21%, methyl ester of tridecanoic acid (C13:0) 4.69%, methyl ester of palmitic acid (C16:0) 4.06%, and methyl ester of 14-pentadecenooic acid (C15:1) 4.91%.

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