

Fatty Acid Methyl Esters from Air-Dried Wood, Bark, and Leaves of *Brachychiton diversifolius* R. Br: Antibacterial, Antifungal, and Antioxidant Activities

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The composition of methylated fatty acids from wood, bark, and leaves of *Brachychiton diversifolius* was analyzed for the first time using gas chromatography (GC). The results indicated that the major methyl ester of fatty acids found in wood, bark, and leaves were: myristic acid (8.32%), palmitic acid (15.66%), and palmitic acid (9.95%), respectively. In accordance to the biological effects of fatty acid fraction, they were moderately effective against *Bacillus subtilis* and *Sarcina lutea*, but they did not show any effect against the growth of *Staphylococcus aureus* and *Pectobacterium carotovorum* at a concentration of 2000 µg/mL. The maximum percentages of inhibition of fungal mycelial growth against *Penicillium selerotigenum* (60.35%), *Paecilomyces variotii* (70.80%), and *Aspergillus niger* (70.50%) were shown by the fatty acids from leaves, bark, and bark, respectively. The total antioxidant activity (TAA %) of fatty acids from wood, bark, and leaves, were 40±3.13%, 80±5.14%, and 60±4.50%, respectively. In accordance to the results, the different parts of *B. diversifolius* could provide important components, such as fatty acids with antimicrobial and antioxidant activities for future studies or uses.

Keywords: *Brachychiton diversifolius*; Wood; Bark; Leaves; Methylated fatty acids; GC; Antibacterial; Antioxidant; Antifungal

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INTRODUCTION

Nowadays, there is large potential to expand the usage of biomass that is available in large volumes of unused residues such as wood, bark, and leaves. Materials left over from pruning processes are found in large quantities and can be used as a feedstock for energy production and extraction of valuable chemicals (Demirbas 2001).

Brachychiton diversifolius R. Br. belongs to the Sterculiaceae family and is a part of the Malvaceae family, originated in northern Australia (Brock 2001). In earlier work, the essential oils from the seeds of *B. discolor*, *B. diversifolius*, and *B. acerifolius*, also members of the Sterculiaceae family, were shown to contain compounds such as α -pinene, β -pinene, linalool, and hexadecanol (Rao *et al.* 1989). In the study of Smith (1970), the cyclopropene fatty acids (CPFA) were identified and estimated by using a combination of spectroscopic, chemical, and chromatographic analyses. These compounds are constituents of seed oils in the families of Sterculiaceae, Malvaceae, and

Bombacaceae, which have been shown to have adverse biological effects in animal feeding trials (Phelps *et al.* 1965; Lee *et al.* 1971).

Additionally, James and Forbes-Ewan *et al.* (1982) reported that the seeds of *B. diversifolius* are indigenous food eaten by Australian Aborigines. Fatty and amino acid compositions of *B. diversifolius*, *B. discolor*, and *B. acerifolius* seeds, combined with malvalic acid, were detected in greater amounts than sterculic acid, while dihydrosterculic acid was found in very small amounts (0.3 to 0.7%) (Rao *et al.* 1989). Consequently, the presence of CPFA in seeds was consistent with their general distribution in the Malvaceae, Bombacaceae, and Sterculaceae families (Smith 1970). In previous works, the inhibition zones observed in the case of different extracts from *B. diversifolius* wood branches against the growth of some pathogenic bacteria ranged from 7 ± 0.7 to 17 ± 1.4 mm (Abdel-Megeed *et al.* 2013; Ngomdir *et al.* 2007).

Fatty acids are widely seen in natural fats and dietary oils. They play important roles such as acting as nutritious substances and as metabolites in living organisms and are known to have antibacterial and antifungal properties (Agoramoorthy *et al.* 2007; Cakir 2004; Russel 1991). Moreover, fatty acids have been reported to possess the ability to impede bacterial growth (Benkendorff *et al.* 2005; McKellar *et al.* 1992). For example, linoleic acid was reported as a model compound of unsaturated fatty acids, which selectively inhibits the FabI enzyme in *Staphylococcus aureus* and *Escherichia coli* (Waller *et al.* 1998). In the present research we present data on the fatty acid methyl esters in air-dried wood, bark, and leaves of *Brachychiton diversifolius*. Information is provided on its antibacterial, antifungal, and antioxidant properties.

EXPERIMENTAL

Plant Samples Preparation

A sample of wood, bark, and leaves of *B. diversifolius* was collected from the Antoniadis Garden at the Horticultural Research Institute in Alexandria, Egypt during the month of August, 2013. The plant was identified by the Department of Forestry and Wood Technology, Faculty of Agriculture at Alexandria University. The wood, bark, and leaves were air-dried under shade at room temperature and then ground into powder to obtain particles in the 40 to 60 mesh range.

Methylation of Lipid

Ten grams from each source; wood, bark, and leaves, were weighed out into a conical flask containing 10 mL of concentrated HCl and boiled in a water bath until all the sample had been dissolved. The fats were extracted by adding 30 mL of diethyl ether to the solution, thorough shaking, and then the extract was collected into a weighed flask after allowing the layers to separate. The extraction was repeated three more times, the solvent was distilled off, and then the fat was dried at 100 °C, cooled, and weighed (Kirk and Sawyer 1991). This fraction was used for the biological assays.

For methylation of the extracted lipids from the wood, bark, and leaves, a sample of 50 mg of lipid from each source was weighed in a tube. Three chemicals were then added to each tube, 50 mL of the mixture from 1 mL concentrated sulfuric acid, 100 mL methanol, and 2 mL of benzene. The tube was sealed completely and placed in a water bath at 90 °C for an hour and a half. The tube then was cooled, and 8 mL water and 5 mL petroleum ether were added. Subsequently, the tube was thoroughly shaken, and the

ethereal layer was separated out and evaporated (Agoramoorthy *et al.* 2007). After the fatty acid methyl esters were prepared, they were subjected to the gas liquid chromatography analysis. The compositions of fatty acids were achieved by GC analysis using a HP (Hewlett Packard) 6890 GC (Table 1) (Central Lab. Unit in High Institute of Public Health, Alexandria, Egypt). The presented fatty acids were identified according to matching their retention times with standard fatty acids (C₂-C₂₅) chromatographed under the same conditions (Mohamed and Awatif 1998).

Table 1. GC Conditions 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
	1	10	200
	2	5	250
			Hold time
			-
			9 min

Antibacterial and Antifungal Activities of Fatty Acids

The fatty acid fraction from wood, bark, and leaves of *B. diversifolius* with a concentration of 2000 µg/mL were evaluated as antibacterial agents against the growth of *Bacillus subtilis* ATCC 6633, *Sarcina lutea* ATCC 9341, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, and *Pectobacterium carotovorum* subsp. *carotovorum* (strain No. ippbc038) using the Kirby-Bauer disc diffusion test (Bauer *et al.* 1966).

The diameters of the inhibition zones (IZs) were measured in millimeters. Control discs were impregnated with 20 µL of dimethyl sulfoxide (DMSO, Sigma-Aldrich) solution. Tetracycline (20 µg/disc) was used as a positive control with the tested bacteria. The experiment was done in triplicate, and the means ± standard deviations were reported. Minimum inhibitory concentrations (MICs) were determined by serial dilution (8, 16, 32, 64, 126, 250, 500, 1000, 2000, 4000, and 5000 µg/mL) of fatty acids using the method of Eloff (1998) and was recently adopted by Salem *et al.* (2014). The antifungal activity was assayed against the growth of *Penicillium selerotigenum*, *Paecilomyces variotii*, and *Aspergillus niger* (Salem *et al.* 2014; Satish *et al.* 2007). The percentage inhibition of mycelial growth, in terms of fungitoxicity of the extracts, was calculated using the following formula:

$$\% \text{ inhibition} = [(Mc - Mt) / Mc] \times 100, \quad (1)$$

where *Mc* is the average increase in mycelial growth in control and *Mt* is the average increase in mycelial growth in treatment (Singh and Tripathi 1999). The experiment was performed in triplicate.

Antioxidant Activity of Fatty Acids

The percent of the total antioxidant activity (TAA %) of fatty acid fraction from wood, bark, and leaves of *B. diversifolius* was evaluated by the 2,2-diphenyl-1-picrylhydrazyl method (DPPH, Sigma-Aldrich) (Salem *et al.* 2013). The TAA% was calculated using the following formula,

$$\text{TAA (\%)} = (\text{A-control} - \text{A-sample} / \text{A-control}) \times 100 \quad (2)$$

where A-control is the absorbance of the control reaction (containing all reagents except the test compound) and A-sample is the absorbance of the test compound. The measurements of DPPH radical scavenging activity were carried out for three replicates. Tannic acid was used as a positive antioxidant agent.

RESULTS AND DISCUSSION

Methyl Ester of Fatty Acids from Wood, Bark and Leaves

The total concentrations of FA presented in wood, bark, and leaves were 0.119%, 0.216%, and 0.145%, respectively. Mostly, the lipophilic components from wood, bark, and leaves are commonly composed of mainly fatty acids, and fatty acid esters (Bikovens *et al.* 2013). By using standard fatty acids ranging from C₂ to C₂₅, the GC analyses of methylated fatty acids from wood, bark, and leaves of *B. diversifolius* are presented in Table 2.

The fatty acids methyl ester constituents found in wood were as follows: methyl ester of myristic acid (C14:0) 8.32%, methyl ester of erucic acid (C22:1, *cis*-13) 7.31%, methyl ester of tridecanoic acid (C13:0) 6.74%, methyl ester of 14-pentadecenoic acid (C15:1) 6.18%, methyl ester of tetradecenoic acid (C14:1) 5.41%, methyl ester of pentadecanoic acid (C15:0) 4.64%, methyl ester of palmitic acid (C16:0) 3.23%, methyl ester of hexadecenoic acid (C16:1) 2.43%, methyl ester of heneicosanoic acid (C21:0) 1.97%, and methyl ester of caprylic acid (C8:0) 0.16%.

The bark contained the following methylated fatty acids: methyl ester of palmitic acid (C16:0) 15.66%, methyl ester of myristic acid (C14:0) 8.10%, methyl ester of erucic acid (C22:1, *cis*-13) 5.58%, methyl ester of heneicosanoic acid (C21:0) 5.52%, methyl ester of tetradecenoic acid (C14:1) 5.33%, methyl ester of tridecanoic acid (C13:0) 4.80%, methyl ester of pentadecanoic acid (C15:0) 2.96%, methyl ester of 14-pentadecenoic acid (C15:1) 2.94%, methyl ester of hexadecenoic acid (C16:1) 0.89%, and methyl ester of caprylic acid (C8:0) 0.51%.

The fatty acids composition detected in leaves were, methyl ester of palmitic acid (C16:0) 9.95%, methyl ester of myristic acid (C14:0) 9.21%, methyl ester of erucic acid (C22:1, *cis*-13) 7.93%, methyl ester of tridecanoic acid (C13:0) 6.03%, methyl ester of oleic acid (C18:1) 3.73%, methyl ester of pentadecanoic acid (C15:0) 3.51%, methyl ester of 14-pentadecenoic acid (C15:1) 3.16%, methyl ester of linoleic acid (C18:2, *cis*-9,12) 2.87%, methyl ester of caprylic acid (C8:0) 1.22%, methyl ester of tetradecenoic acid (C14:1) 1.18%, and methyl ester of hexadecenoic acid (C16:1) 1.16%.

It can be concluded that the major methyl ester of fatty acids found in wood, bark, and leaves were: myristic acid (8.32%), palmitic acid (15.66%), and [myristic acid (9.21%) and palmitic acid (9.95%)], respectively. Previously, the major fatty acid was

identified as oleic in seed oil of *B. acuminatus* and linoleic in the *B. rupestris*, *B. australis*, and *B. gregori* (Rao 1991).

Malvalic and sterculic, as cyclopropene fatty acids, were present in appreciable concentrations (6.6-10.6% and 0.5-2.2%) (Rao 1991). However, in the present study linoleic and oleic acids were found in small amounts in leaves with 2.87% and 3.73%, respectively.

Biological Activity of Fatty Acids from Wood, Bark, and Leaves of *Brachychiton diversifolius*

The tested fatty acids fraction from wood, bark, and leaves exhibited different degrees of antibacterial, antifungal, and antioxidant activities, which are shown in Table 3. The results showed that the fatty acids were moderately effective against *B. subtilis*, and *S. lutea*, but they did not show any effect against the growth of *S. aureus* and *P. carotovorum* at a concentration of 2000 µg/mL. On the other hand, the fatty acids had shown a great antibacterial effect against the growth of *E. coli* with IZs of 22.63±2.53 mm, 16.39±1.54 mm, and 18.34±1.52, for fatty acids from wood, bark, and leaves, respectively. The maximum percentages of inhibition of fungal mycelial growth against *P. selerotigenum* (60.35%), *P. variotii* (70.80%), and *A. niger* (70.50%) were shown by the fatty acids from leaves, bark, and bark, respectively.

In previous studies, palmitic and oleic acids have been shown to have a potent antiviral property (Orhan *et al.* 2011), and linoleic and oleic acids were reported to be potent antibacterial agents (Zheng *et al.* 2005; Dilika *et al.* 2000). Linoleic and oleic acids from *Pelagonium* sp. possessed antibacterial activity against *Mycobacterium aurum*, *M. phlei*, *M. smegmatis*, and *M. fortuitum* (Galbraith *et al.* 1971). It has been stated that the antibacterial and antifungal activities of fatty acids are associated with chain length and unsaturation degree (Benkendorff *et al.* 2005; Knapp and Melly 1986). For example, the unsaturated fatty acids with long-chain exhibited great effects against many bacteria, even including methicillin-resistant *S. aureus* (Farrington *et al.* 1992). Additionally, Isaac *et al.* (1995) demonstrated that the fatty acid and monoglycerides with 8 to 12 carbons were more strongly antimicrobial (virus and bacteria) when added to milk than long chain monoglycerides.

In the present study, the total antioxidant activity (TAA %) of fatty acids from wood, bark, and leaves, were 40±3.13%, 80±5.14%, and 60±4.50%, respectively. The value of bark is close to the value of the positive control (tannic acid; 85±5.45%). Oleic and linoleic acids as well as their derivatives were reported to have a potent antioxidant effect (El-Din *et al.* 2007; Hur *et al.* 2007; Ceffarelli *et al.* 2005). Additionally, trans-fatty acids derivatives widely found in the oils such as *Corylus avellana* L. (hazelnut), and *Juglans regia* L. (walnut), could be possibly responsible for the antioxidant activity of these oils (Orhan *et al.* 2011).

Table 2. Fatty Acid Composition (wt%) of Wood, Bark, and Leaves of *Brachychiton diversifolius*

Fatty acid (FA)	FA (g/100g lipid)			FA % ^a			FA (g/100g sample)		
	Wood	Bark	Leaves	Wood	Bark	Leaves	Wood	Bark	Leaves
Methyl ester of n-Carpoic acid (C6:0)	-	0.0003	0.001	-	0.085± 0.01	0.204± 0.005	-	0.0002	0.0004
Methyl ester of caprylic acid (C8:0)	0.0004	0.0019	0.003	0.1640± 0.003	0.510± 0.023	1.225±0 .095	0.001	0.001	0.0025
Methyl ester of capric acid (C10:0)	0.0001	-	0.000	0.0467± 0.001	-	0.037±0 .001	0.0003	-	0.0001
Methyl ester of lauric acid (C12:0)	0.0005	0.0061	0.001	0.2075± 0.004	1.638± 0.045	0.222±0 .011	0.001	0.0032	0.0005
Methyl ester of tridecanoic acid (C13:0)	0.0162	0.0178	0.016	6.7477± 0.051	4.809± 0.091	6.032±0 .022	0.041	0.0094	0.0124
Methyl ester of tetradecenoic acid (C14:1)	0.0130	0.0198	0.003	5.4134± 0.145	5.330± 0.020	1.187±0 .030	0.033	0.0105	0.0024
Methyl ester of myristic acid (C14:0)	0.0199	0.0301	0.025	8.3219± 0.010	8.106± 0.025	9.209±0 .020	0.051	0.0159	0.0189
Methyl ester of 14-pentadecenoic acid (C15:1)	0.0148	0.0109	0.009	6.1874± 0.016	2.940± 0.036	3.162±0 .030	0.038	0.0058	0.0065
Methyl ester of pentadecanoic acid (C15:0)	0.0111	0.0110	0.010	4.6452± 0.020	2.960± 0.030	3.517±0 .001	0.028	0.0058	0.0072
Methyl ester of hexadecenoic acid (C16:1)	0.0058	0.0033	0.003	2.4378± 0.015	0.893± 0.020	1.164±0 .005	0.015	0.0018	0.0024
Methyl ester of palmitic acid (C16:0)	0.0078	0.0581	0.027	3.2365± 0.025	15.66± 0.030	9.953±0 .025	0.020	0.0308	0.0205
Methyl ester of heptadecenoic acid (C17:1)	0.0017	0.0024	-	0.7073± 0.040	0.639± 0.003	-	0.004	0.0013	-
Methyl ester of linolenic acid (C18:3)	0.0012	-	-	0.5171± 0.002	-	-	0.003	-	-
Methyl ester of linoleic acid (C18:2, <i>cis</i> -9,12)	-	-	0.008	-	-	2.876±0 .055	-	-	0.0059
Methyl ester of oleic acid (C18:1)	-	-	0.010	-	-	3.732±0 .025	-	-	0.0077

Table 2. Continued

Fatty acid (FA)	FA (g/100g lipid)			FA % ^a			FA (g/100g sample)		
	Wood	Bark	Leaves	Wood	Bark	Leaves	Wood	Bark	Leaves
Methyl ester of archidic acid (C20:0)	-	0.0033	0.002	-	0.892± 0.015	0.599±0 .035	-	0.0018	0.0012
Methyl ester of 8,11,14-ecosatrienoic acid (C20:3)	0.0005	-	-	0.1921± 0.020	-	-	0.001	-	-
Methyl ester of heneicosanoic acid (C21:0)	0.0047	0.0205	-	1.9773± 0.020	5.527± 0.035	-	0.012	0.0109	-
Methyl ester of docosadienoic acid (C22:2)	0.0004	0.0022	0.001	0.1675± 0.002	0.591± 0.015	0.425±0 .014	0.001	0.0012	0.0009
Methyl ester of erucic acid (C22:1, <i>cis</i> -13)	0.0175	0.0207	0.022	7.3190± 0.175	5.583± 0.095	7.931±0 .123	0.045	0.0110	0.0163
Methyl ester of behenic acid (C22:0)	0.0012	-	-	0.5188± 0.055	-	-	0.003	-	-

a- Values are means ± standard deviations

Table 3. Antibacterial, Antifungal, and Antioxidant Activities of Fatty Acids from Wood, Bark, and Leaves of *Brachychiton diversifolius*

Fatty acid fraction	IZ of bacterial strains (mm) ^a					% inhibition of fungal mycelial growth			TAA%
	<i>B. s.</i>	<i>S. l.</i>	<i>S. a.</i>	<i>E. c.</i>	<i>P. c.</i>	<i>P. s.</i>	<i>P. v.</i>	<i>A. n.</i>	
Wood	9.36 ± 1.12 (1000)	13.00 ± 0.00 (500)	na (>5000)	22.63 ± 2.53 (64)	na (>5000)	50.44	54.07	0	40± 3.13
	12.62 ± 1.53 (>250)	12.23 ± 2.15 (500)	na (>5000)	16.39 ± 1.54 (126)	na (>5000)	50.53	70.80	70.50	80± 5.14
Leaves	13.13 ± 1.50 (250)	14.66 ± 2.50 (126)	na (>5000)	18.34 ± 1.52 (32)	na (>5000)	60.35	60.90	0	60± 4.50
	Negative control	na	na	na	na	0	0	0	
Positive Control ^c	19	20	21	22	nt	nt	nt	nt	(85± 5.45) ^b

a - Zone of inhibition in mm (include 5 mm disc).

b - TAA% (Total Antioxidant Activity) of the Tannic acid as a positive antioxidant agent.

na - not active. nt – not tested.

c - Tetracycline (20 µg/disc). Values in parentheses are MICs values (µg/mL).

Negative Control discs were impregnated with 20 µL of dimethyl sulfoxide (DMSO).

B.s.- *Bacillus subtilis*, *S.l.*- *Sarcina lutea*, *S.a.*- *Staphylococcus aureus*, *E.c.*- *Escherichia coli*, *P.c.*- *Pectobacterium carotovorum subsp. carotovorum*, *P.s.*- *Penicillium selerotigenum*, *P.v.*- *Paecilomyces variotii*, and *A.n.*- *Aspergillus niger*.

CONCLUSIONS

In the present study, the methylated fatty acids from wood, bark, and leaves of *Brachychiton diversifolius* R.Br were analyzed for the first time using GC as compared to the previously injected standard fatty acids (C₂-C₂₅). The following points can be drawn from the results:

1. The total concentrations of the methyl ester of fatty acids present in wood, bark, and leaves were 0.119%, 0.216%, and 0.145%, respectively.
2. The major methyl ester of fatty acids constituents found in wood were myristic acid (C14:0) 8.32%, erucic acid (C22:1, *cis*-13) 7.31%, tridecanoic acid (C13:0) 6.74%, 14-pentadecenoic acid (C15:1) 6.18%, and tetradecenoic acid (C14:1) 5.41%.
3. The major methyl ester of fatty acids constituents found in bark were those involving palmitic acid (C16:0) 15.66%, myristic acid (C14:0) 8.10%, erucic acid (C22:1, *cis*-13) 5.58%, heneicosanoic acid (C21:0) 5.52%, and tetradecenoic acid (C14:1) 5.33%.
4. The major methyl ester of fatty acids composition detected in leaves were those incorporating palmitic acid (C16:0) 9.95%, myristic acid (C14:0) 9.21%, erucic acid (C22:1, *cis*-13) 7.93%, and tridecanoic acid (C13:0) 6.03%.

5. In accordance to the results of antioxidant, antibacterial, and antifungal activities, the different parts of *Brachychiton diversifolius* could provide important components, such as fatty acids with antimicrobial (bacteria and fungi), and antioxidant activities.

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