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Fatty acid profile of four *Ganoderma* species collected from various host trees with chemometric approach

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ABSTRACT

Ganoderma species have been used in traditional medicine, particularly for cancer therapy. To evaluate the similarities and the differences between four *Ganoderma* species, fatty acid constituents of five *Ganoderma lucidum*, seven *G. adspersum*, one *G. applanatum* and one *G. resinaceum* collected from different trees and localities were studied using GC-MS. The fatty acids were obtained by three different techniques; namely, maceration, soxhlet and ultrasonic extractions. The Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) for selected fifteen fatty acids of 14 samples of 4 *Ganoderma* species were performed using Minitab statistical software 16.2.1 to classify the samples. Linoleic acid (ranged from 9.80 to 3467 μg/g), oleic acid (16.9–3356 μg/g), palmitic acid (15.5–1272 μg/g), tricosanoic acid (1.11–406 μg/g), tetracosanoic acid (1.11–408 μg/g), lauric acid (2.17–314 μg/g) and stearic acid (1.11–286 μg/g) were the major fatty acids determined in all samples. The host tree and the extraction technique were discriminated the *Ganoderma* species from each other.

1. Introduction

It is known that the usage of mushrooms as therapeutic agents is not new. The statistical data show that over 10,000 mushroom species are known, of which 2000 are safe for human health, and 300 of them are used for medicinal properties (Sheena et al., 2005). Among them, *Ganoderma* species are considered as an important medicinal mushroom genus (Baby et al., 2015; Kohno et al., 2008; Ríos et al., 2012; Sanodiya et al., 2009). In the old times in the Asian countries such as China, Japan, Korea, and in some part of Africa *Ganoderma* species were used by the local people as foods and medicines. Nowadays, Ganoderma species are used to find a cure for various cancers and strengthen the immune system. The *Ganoderma* species especially, *Ganoderma lucidum* has a big market all over the world. *Ganoderma lucidum* is consumed as tea, in coffee and in dietary supplements both by the healthy and sick people. On the other hand, it is cultivated by several people for marketing purposes to meet the demands. *Ganoderma* mushrooms have a high content of carbohydrates, proteins, amino acids, vitamins, minerals and fats (Li et al., 2013; Tel-Çayan et al., 2016). The extracts and compounds isolated from *Ganoderma* species also exhibited antibiotic, antiviral and anti-hypertensive and blood lipid lowering effects besides their anticancer and immunomodulatory activities (Grienke et al., 2015; Li et al., 2013). The genus *Ganoderma* comprises more than 50 species with health-promoting and therapeutic properties (Chen et al., 2010).

Other useful components of mushrooms are fatty acids which comprise 6–8% of the dry weight. Particularly, the essential polyunsaturated fatty acids are essential for human's basal metabolism and have many beneficial effects on human health (Parikh et al., 2005). Lack of dietary essential fatty acids or their inefficient metabolism has been implicated in etiology of disease including cardiovascular disease and progression of it (Brown, 2005).

Fingerprinting is an analytical technique designed to inform about the characterizing or authenticating of medicinal natural products such as mushroom and herbs (Cuadros-Rodríguez et al., 2016). Besides the identification utility of medicinal natural products, fingerprint studies can also be used for modeling bioactivities. The required step of this approach is to develop a chromatogram with a maximal peak capacity (Alaerts et al., 2007).

The morphological criteria are generally used to classify mushrooms. In addition to this, the fatty acid composition can be also used for characterization, differentiation, and sorting of mushrooms using the combination of multivariate statistical analyses such as principal component analysis and hierarchical cluster analysis (Marekov et al., 2012).

The composition of fatty acids of mushrooms varies because of the differences in environment, climate conditions, geographic origin and/or other factors (Chen et al., 2008). Since the medicinal usages of *Ganoderma lucidum* are well known, it was aimed to study to distinguish

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the *Ganoderma* samples collected from different geographical localities and from different trees using hierarchical cluster analysis (HCA) and principal component analysis (PCA).

2. Materials and methods

2.1. The mushroom material and extraction

A total of 14 samples of *G. lucidum, G. adspersum, G. applanatum* and *G. resinaceum* fruiting bodies growing on various trees were obtained from Muğla and Izmir, Turkey (Table 1). The mushroom species were identified by Cansu Korkmaz, Mugla Sitki Kocman University. All the samples were dried using a fruit drier machine and sliced into small particles (2–6 mm).

All samples were divided into three parts. Maceration extraction, soxhlet extraction, and ultrasonic extraction methods were used for each. For the maceration extraction method, the mushroom was extracted (24 h × 5 times) with petroleum ether: chloroform (4:1, v/v) mixture. For the soxhlet extraction method, the mushroom was extracted with petroleum ether: chloroform (4:1, v/v) mixture with soxhlet apparatus for four cycles. For the ultrasonic extraction method, the mushroom was extracted (20 min × 3 times) with petroleum ether: chloroform (4:1, v/v) mixture in an ultrasonic bath at 35 °C for 20 min. The rotary evaporator was used to evaporate all solvents. **M**, **S**, **U** letters were used as a suffix to discriminate the extraction type for the maceration, soxhlet and ultrasonic extraction methods, respectively.

2.2. Preparation of methyl esters of fatty acids

25.0 ± 0.1 mg of extract was weighted carefully in a 25 mL volumetric flask. To the flask, 1.5 mL of 0.5 M methanolic sodium hydroxide was added and heated in a water bath at 50 °C for five minutes. Then, 1.5 mL of BF_3-methanol (Merck, Darmstadt, Germany) was added and the mixture was heated for 5 min at 80 °C. After cooled, the flask was completed with saturated sodium chloride solution. Then the entire mixture was transferred to a separatory funnel, and about 5 mL of hexane was added. The funnel was shaken vigorously for 1 min and the layers were then allowed to separate. The lower aqueous layer is drained off and discarded. The hexane layer is drained through filter paper into a 50-ml. flask. The solvent was removed using a rotary evaporator. Before analysis with GC-MS, the mixture was diluted with hexane (1:25, v/v) (Öztürk et al., 2014; Tel et al., 2013).

2.3. Analysis of methyl esters of fatty acids by GC–MS

GC-MS analyses of fatty acids were performed using a Varian Saturn 2100T equipped with an electron ionizer, and an ion trap analyzer and DB-1 MS fused silica nonpolar capillary column (30 m × 0.25 mm i.d.,

Table 1
The Collection dates, regions, and host tree type, and extraction yields of *Ganoderma* samples.

Number	Mushroom species	Voucher number	Host tree	Collection region/time	Extraction technique	Codes	Extraction amounts (g)	Extraction yields (%)
1	*G. lucidum*	AT-21072	Sweetgum (*Liquidambar orientalis*)	Mugla, Fethiye/September 2014 36° 38′ 5.7″ N. 29° 10′ 15.8″E. 46 m	Maceration	**1.GL.M**	0.43	0.87
					Soxhlet	**1.GL.S**	0.42	0.85
					Ultrasonic	**1.GL.U**	0.09	0.89
2	*G. lucidum*	AT-21021	Sweetgum (*Liquidambar orientalis*)	Mugla, Koycegiz/September 2014, 36°57′11.2″N. 28°36′38.3″E. 8 m	Maceration	**2.GL.M**	2.06	1.21
					Soxhlet	**2.GL.S**	0.64	1.28
					Ultrasonic	**2.GL.U**	0.05	0.54
3	*G. lucidum*	AT-21042	Sweetgum (*Liquidambar orientalis*)	Mugla, Marmaris/November 2014 37°00′09.5″N 28°18′46.5″E 95 m	Maceration	**3.GL.M**	2.64	1.76
					Soxhlet	**3.GL.S**	1.31	2.61
					Ultrasonic	**3.GL.U**	0.28	2.84
4	*G. lucidum*	AT-21051	Sweetgum (*Liquidambar orientalis*)	Mugla, Ula/November 2014 37°00′30.2″ N.28°27′27.5″ E 95 m.	Maceration	**4.GL.M**	2.91	1.82
					Soxhlet	**4.GL.S**	0.87	1.74
					Ultrasonic	**4.GL.U**	0.12	1.19
5	*G. lucidum*	AT-21010	Mulberry (*Morus alba*)	Mugla, Koycegiz/November 2014 36°57′04.1″N. 28°37′17.8″E. 8 m	Maceration	**5.GL.M**	0.77	0.43
					Soxhlet	**5.GL.S**	0.19	0.37
					Ultrasonic	**5.GL.U**	0.05	0.48
6	*G. adspersum*	AT-22001	Sweetgum (*Liquidambar orientalis*)	Mugla, Fethiye/September 2014 36° 38′ 5.7″ N, 29° 10′ 15.8 E, 46 m	Maceration	**6.GA.M**	0.99	0.57
					Soxhlet	**6.GA.S**	0.19	0.38
					Ultrasonic	**6.GA.U**	0.03	0.33
7	*G. adspersum*	AT-22051	Walnut (*Juglans regia*)	Izmir, Balcova/October 2014 38°24′01.7″N 27°03′10.3″E. 10 m	Maceration	**7.GA.M**	0.85	0.66
					Soxhlet	**7.GA.S**	0.40	0.80
					Ultrasonic	**7.GA.U**	0.05	0.54
8	*G. adspersum*	AT-22071	Peach (*Prunus persica*)	Mugla, Ula/October 2014 37° 06′ 05.1″ N, 28° 24′ 47.9 E, 606 m	Maceration	**8.GA.M**	0.90	0.53
					Soxhlet	**8.GA.S**	0.27	0.55
					Ultrasonic	**8.GA.U**	0.02	0.23
9	*G. adspersum*	AT-22092	Plum (*Prunus domestica*)	Mugla, Fethiye/September 2014 36° 38′ 5.7″ N, 29° 10′ 15.8 E, 46 m	Maceration	**9.GA.M**	0.29	0.57
					Soxhlet	**9.GA.S**	0.25	0.49
					Ultrasonic	**9.GA.U**	0.03	2.87
10	*G. adspersum*	AT-22012	Sweetgum (*Liquidambar orientalis*)	Mugla, Marmaris/November 2014 37°00′09.5″N 28°18′46.5″E 95 m	Maceration	**10.GA.M**	1.44	0.62
					Soxhlet	**10.GA.S**	0.26	0.52
					Ultrasonic	**10.GA.U**	0.03	0.27
11	*G. adspersum*	AT-22021	Mulberry (*Morus alba*)	Mugla, Koycegiz/November 2014 36°57′41.2″N. 28°36′27.3″E. 8 m	Maceration	**11.GA.M**	1.01	0.80
					Soxhlet	**11.GA.S**	0.32	0.65
					Ultrasonic	**11.GA.U**	0.06	0.60
12	*G. adspersum*	AT-22031	Mulberry (*Morus alba*)	Muğla, Central/November 2014 37° 11′29.6″ N, 28°24′ 12.8 E, 660 m	Maceration	**12.GA.M**	1.16	1.45
					Soxhlet	**12.GA.S**	0.36	0.72
					Ultrasonic	**12.GA.U**	0.06	0.64
13	*G. applanatum*	AT-23010	Mulberry (*Morus alba*)	Mugla, Koycegiz/September 2014 36°55′53.1″N. 28°37′08.8″E. 8 m	Maceration	**13.GAp.M**	1.39	0.35
					Soxhlet	**13.GAp.S**	0.11	0.22
					Ultrasonic	**13.GAp.U**	0.02	0.15
14	*G. resinaceum*	AT-24530	Mulberry (*Morus alba*)	Mugla, Fethiye/November 2014 36° 38′ 5.7″ N, 29° 10′ 15.8 E, 46 m	Maceration	**14.GR.M**	1.18	0.47
					Soxhlet	**14.GR.S**	0.31	0.63
					Ultrasonic	**14.GR.U**	0.03	0.31