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GC-MS analysis of unpolar fraction from *Ficus carica* L. (fig) leaves

[1*]Ivanov, I., [2]Dincheva, I., [2]Badjakov, I., [1]Petkova, N., [1]Denev, P. and [3]Pavlov, A.

[1]Department of Organic Chemistry and Inorganic Chemistry, University of Food Technologies, 26 Maritza Blvd., Plovdiv, Bulgaria
[2]AgroBioInstitute, Agricultural Academy, 8 Dr. Tsankov Blvd., Sofia, Bulgaria
[3]Department of Analytical Chemistry and Physical Chemistry, University of Food Technologies, 26 Maritza Blvd., Plovdiv, 4002, Bulgaria; Laboratory of Applied Biotechnologies, Institute of Microbiology, BAS, 135 Ruski Blvd., Plovdiv, Bulgaria

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Abstract

Moraceae is very common in the Mediterranean region and in the countries with dry and warm climate. Since ancient times the figs have been used for human consumption, but it was only recently that their nutritive and pharmacological value has been investigated. The aim of the current study was to investigate the metabolites profile of the different extracts (diethyl ether, petroleum ether, n-hexane, acetone and ethanol) from *Ficus carica* leaves. The highest yield of the extractable components has been obtained with n-hexane – 5.0 g/100 g dw. Thirty-seven unpolar compounds have been identified by GC-MS analysis. In the investigated extracts were mainly detected the following phytochemical compounds: saturated and unsaturated fatty acids (18% of TIC) – myristic acid, palmitic acid, margaric acid, linoleic acid, α-linolenic acid, stearic acid, arachidic acid, behenic acid, lignoceric acid, cerotic acid; fatty alcohols (35% of TIC) – stearyl, behenyl, pentacosan-1-ol, ceryl, montanyl, melissyl alcohol and diterpenic alcohol – phytol; phytosterols (18% of TIC) – stigmasterol, β-sitosterol, lanosterol and cycloartenol; triterpenes (11% of TIC) – squalene, α-amyrin, β-amyrin, germanicol and lupeol. Phytol (32% of TIC), β-sitosterol (12% of TIC), α-linolenic acid (8% of TIC), cycloartenol (5% of TIC), lupeol (4% of TIC), α-amyrin (3% of TIC) and β-amyrin (2% of TIC) were established as the dominants compounds in *Ficus carica* leaves. The obtaining extracts of fig leaves were evaluated as a valuable source of high biological active lipid components for protecting the skin from environmental stress conditions and reactive oxygen species. These extracts can be potentially used for medical and natural cosmetics and caring protection against premature skin aging.

Introduction

Ficus carica L. is a tree commonly known as an edible fruit. This plant traditionally used in folk medicines to treat cancer, pneumonia, diarrhoea and treat inflammation and indigestion. Previously chemical studies of figs reported the isolation of phenolic acids, coumarins and triterpenoids (Chawla *et al.*, 2012). Their biological activity and application in traditional medicine is due to different compounds. The pharmacological investigations of fig demonstrated antioxidant (Ivanov *et al*., 2015), antimicrobial (Aref *et al*., 2010; Chawla *et al*., 2012; Al-Yousuf, 2012), anticancer (Hashemi *et al*., 2011; Khodarahmi *et al*., 2011) and hepatoprotective activities (Gond and Khadabadi, 2008; Aghel *et al*., 2011). Also, in Bulgaria fig leaves are commonly consumed as herbal infusions and decoctions, because of their diuretic and anti-inflammatory activities.

Pentacyclic triterpenes are distributed in the plant kingdom and long times have been known to possess a number of biological effects (Ikeda *et al*., 2008). The compounds α-amyrin, β-amyrin and lupeol, found commonly in medicinal plants, demonstrate many bio-active properties and pharmacological effects – analgesic, anti-inflammatory, anti-ulcerogenic, and anti-hyperglycemic activities. The anti-lipoxygenase and anti-arthritic activities of amyrin derivatives are also well documented (Lavoie and Stevanovic, 2005; Hernanddez-Vazquez *et al.*, 2012).

Phytosterols are cholesterol-like molecules found in many plant foods for human nutrition with the highest concentration of vegetable oils. Phytosterols inhibit the absorption of intestinal and endogenous biliary cholesterol. Plants contain free and esterified phytosterols that can be acylated with β-sitosterol, campisterol and stigmaserol. These phytochemical compounds play significant function in cellular processes such as regulation of membrane fluidity, adaptation of membranes to temperature, participation in cellular differentiation and proliferation (MacKay and Jones, 2011).

*Corresponding author.
Email: *ivanov_ivan.1979@yahoo.com*

Oliveira *et al.* (2010) investigated metabolite profile of *Ficus carica* latex and they identified triterpenes (lupeol, α-amyrin, β-amyrin), sterols (lanosterol and β-sitosterol), fatty acids (from C14 to C24) and amino acids. Phytosterols and fatty acids were identified by GS-MS analysis in fig fruits, bark, steam and pith (Jeong and Lachance, 2001).

Nowadays, there is a worldwide tendency of continuously growing demand for natural products used as pharmaceuticals, nutraceuticals, food and cosmetic additives produced from plant. To the best of our knowledge the photochemical profile of Ficus carica leaves were not enough investigated. In this reason, the aim of the current study was to investigate the chemical constituents of the different extracts from *F. carica* leaves. Therefore, this research was carried out in order to have an insight into the chemical basis for some of the pharmacological and cosmetical properties reported for the plant in traditional medical. In the present study commonly used solvent diethyl ether, petroleum ether, n-hexane, acetone and ethanol were applied for extracting the phytochemicals from Ficus carica leaves.

Materials and Methods

Plant materials

Leaves from several randomly chosen plants of *Ficus carica* L. were collected in Jun 2015 from Plovdiv, Bulgaria. The plant material was air-dried at room temperature.

Extraction procedure

Each dry plant sample (25 g) was extracted two times with 100 mL diethyl ether, petroleum ether, n-hexane, acetone, 95% ethanol at room temperature for 24 h. The biomass was removed by filtration through filter paper, and the combined extracts were removed to dryness. The dry extracts were saponified with 2 mol/L KOH (dissolved in 50% ethanol) under reflux for 1.5 h. After cooling, the obtained extracts were separated in triplicate by liquid-liquid extraction with n-hexane. The n-hexane fraction was evaporated to dryness and then used for the further analyses.

GC-MS analysis

GC-MS analysis were carried out on gas chromatograph Agilent Technology Hewlett Packard 7890 A, coupled with mass detector Agilent Technology 5975 C inert XL EI/CI MSD at 70 eV). Separation of metabolites was performed on HP-5MS column (30 m x 0.25 mm x 0.25 μm) at temperature program: from 100°C to 180°C with the step of 15°C/min and from 180°C to 300°C with the step of 5°C/min then hold on 300°C for 10 min. The injector temperature was 250°C and the flow rate of carrier gas (helium) of 1.0 mL/min was used. The injection volume was 1μL.

Identification of the metabolites

The obtained mass spectra were analysed using 2.64 AMDIS (Automated Mass Spectral Deconvolution and Identification System, National Institute of Standardization and Technology (NIST), Gaithersburg, MD, USA). The separated polar and non-polar compounds were identified by comparison of their GC-MS spectra and Kovach retention index (RI) with referent compounds in NIST 08 database (NIST Mass Spectral Database, PC-Version 5.0, 2008). The RIs of compounds were recorded with standard n-hydrocarbon calibration mixture (C10-C40, Fluka) using 2.64 AMDIS software.

Result and Discussion

The results from the GC-MS analysis of the fig leaves extracts were summarized in Table 1. Thirty-seven compounds were identified in unpolar fraction (Table 1). Phytol (10) (from 21 to 26% of TIC), β-sitosterol (30) (from 11 to 14% of TIC), α-amyrin (31) (from 3 to 5% of TIC), β-amyrin (34) (from 1.5 to 2.2% of TIC), lupeol acetate (35) (from 4 to 6% of TIC), cycloartenol acetate (37) (from 5 to 9% of TIC) and germanicol (32) (from 1 to 5% of TIC) were the major compounds found in the unpolar fraction from *Ficus carica* L. leaves (Table 1). The identified constituents of the investigated extracts from fig leaves could be classified into five categories - alkenes, fatty acids, fatty alcohols, phytosterols and triterpenes. The results from obtained extracts with different solvents were compared and revealed the presence of similar compounds. Only petroleum ether fraction showed the absence of sterols (Table 1).

The current study demonstrated the high content of free fatty acids in fig leaves, as eleven representatives were identified (C14 to C26). The most abundant saturated fatty acids were palmitic acid C16:0 (7) (from 4% to 14% of TIC) followed by stearic acid C18:0 (13) (from 1% to 4% of TIC). The highest value of polyunsaturated fatty acid α-linolenic acid C18:3 (12) (from 5% to 13.8% of TIC) and linoleic acid C18:2 (11) (from 1.8% to 3.9% of TIC) were established in all extracts (Table 1). The polyunsaturated acids content ranged from 7% to 20% of TIC, but saturated ones – from 7% to 30% of TIC. It is well known that polyunsaturated fatty acids can influence some physical properties of the cellular membranes such as fluidity and permeability

Table 1. Phytochemical constituents of unpolar fractions obtaining from fig (*Ficus carica* L.) leaves

	Compound	Rt	RI	Diethyl ether	Petroleum ether	*n*-Hexane	Acetone	Ethanol
1	n-Dodecane	4.98	1197	0.3*	3.1	4.5	1.1	0.9
2	Glycerol	5.78	1240	tr	tr	0.1	0.1	0.1
3	n-Tridecane	5.97	1250	0.8	6.6	7.3	2.1	1.9
4	n-Tetradecane	6.91	1299	0.5	4.6	4.6	1.2	1.4
5	3-Octadecene, (E)-	11.32	1792	0.2	nd	nd	nd	nd
6	n-Tetradecanoic acid (Myristic acid)	12.14	1849	tr	nd	tr	0.4	0.3
7	n-Hexadecanoic acid (Palmitic acid)	15.18	2047	4.4	14.5	4.3	9.0	10.1
8	n-Heptadecanoic acid (Margaric acid)	16.75	2146	0.2	1.3	0.5	0.3	0.2
9	1-Octadecanol (Stearyl alcohol)	16.93	2158	0.2	0.5	0.1	0.1	tr
10	Phytol	17.32	2182	23.1	nd	32.5	21.3	26.4
11	Linoleic acid	17.84	2215	1.8	nd	2.7	4.5	3.9
12	α-Linolenic acid	17.96	2222	5.2	nd	7.8	13.8	12.3
13	Stearic acid	18.33	2245	1.9	4.2	1.1	2.8	2.7
14	n-Eicosanoic acid (Arachidic acid)	21.41	2444	1.9	7.2	0.8	2.0	1.0
15	1-Docosanol (Behenyl alcohol)	23.05	2553	0.4	1.1	0.3	0.1	tr
16	1-Hexadecanoylglycerol (1-Palmitoylglycerol)	23.81	2602	0.1	0.1	0.2	0.1	tr
17	n-Docosanoic acid (Behenic acid)	24.36	2621	1.7	6.3	0.6	0.8	0.4
18	Tetracosan-1-ol (Lignoceryl alcohol)	25.89	2675	1.7	3.9	1.2	0.4	0.3
19	Squalene	27.00	2730	0.7	nd	0.6	0.7	0.7
20	Tetracosanoic acid (Lignoceric acid)	27.13	2740	0.8	3.4	0.3	0.5	0.3
21	Pentacosan-1-ol	27.24	2748	tr	nd	nd	nd	tr
22	n-Octacosane	27.93	2800	7.5	22.8	nd	4.4	2.8
23	1-Hexacosanol (Ceryl alcohol)	28.55	2847	0.5	1.2	0.3	0.2	0.1
24	Hexacosanoic acid (Cerotic acid)	29.75	2939	0.1	0.7	tr	tr	tr
25	Heptacosan-1-ol	29.83	2945	tr	nd	nd	tr	tr
26	Octacosan-1-ol (Montanyl alcohol)	31.07	3044	1.5	3.3	0.8	0.6	0.5
27	α-Tocopherol	31.22	3056	0.1	nd	0.3	0.1	0.1
28	Stigmasterol	33.06	3205	1.0	nd	1.0	0.9	0.9
29	Triacontan-1-ol (Melissyl alcohol)	33.55	3241	1.0	1.8	tr	0.4	0.4
30	β-Sitosterol	33.89	3265	14.4	nd	11.9	11.2	11.4
31	α-Amyrin	34.02	3275	5.8	nd	3.2	4.5	4.1
32	Germanicol	34.12	3282	2.6	4.9	0.7	1.5	1.1
33	Lanosterol	34.39	3302	1.5	nd	0.8	1.2	1.3
34	β-Amyrin	34.55	3312	2.2	nd	1.5	1.7	1.7
35	Lupeol acetate	34.68	3320	6.7	8.3	4.0	5.4	6.4
36	β-Amyrin acetate	35.44	3367	nd	nd	0.7	nd	nd
37	Cycloartenol acetate	36.02	3403	9.0	nd	4.9	6.3	6.1
	Fatty acid			18.0	37.6	18.3	34.0	31.1
	PUFA			8.9	7.2	11.3	20.3	17.1
	SFA			9.1	30.4	7.0	13.7	14.0
	Fatty alcochols			28.6	11.7	35.3	23.2	27.9
	Ttiterpenes			18.0	13.2	10.7	13.8	14.0
	Sterols			26.0	nd	18.6	19.7	19.7
	Alkanes			9.2	37.2	16.4	9.0	7.0
	Total			99.8	99.8	99.3	99.7	99.7
	UFA/SFA			0.97	0.23	1.61	1.48	1.22
	g/100g dw			1.8	2.2	5.0	1.5	1.7

* – TIC (total ion current); nd – not detected; tr – trace <0.1% of TIC; PUFA – polyunsaturated fatty acid; SFA – saturated fatty acid; UFA – unsaturated fatty acid; Rt – retention time; RI – retention index (Kovats' retention index)

(Ward and Singh, 2005). Moreover, the benefits of polyunsaturated fatty acids in some diseases, such as cardiovascular diseases and autoimmune disorders, have been reported (Reiffel and McDonald, 2006). Results showed that the polyunsaturated/saturated ratio of *F. carica* leaves varied from 0.2 (petroleum ether) to 1.6 (n-hexane). The ω-3 (12) and ω-6 (11) polyunsaturated fatty acids are essential components of the human diet. Moreover, essential fatty acids are absolutely necessary for the healthy condition of human skin (Boelsma *et al.*, 2001). Results clearly showed that the fig leaves contained more unsaturated fatty acids as α-linolenic acid (12) and linoleic acid (11). Moreover, in the different extracts from the fig leaves were identified fatty alcohols (C18-C30), as well (Table 1). Their content varied from 11% to 35% of TIC (Table 1). These natural components have been used as co-emulsifiers, emollients and thickeners in cosmetics and food industry. The relative concentration of acyclic diterpene alcohol phytol was the highest in investigated fig extracts (from 21% to 32% of TIC) (Table 1). Due to the strong antioxidant and antimicrobial activity of phytol in the fig fractions can be used in the fragrance and cosmetics industry (McGinty *et al.*, 2010; Pejin *et al.*, 2014).

It was established that β-sitosterol (30) presented the main phytosterol in the fig leaves – 12% to 14% of TIC (Table 1). This compound is a non-competitive inhibitor of 5-α-reductase and it possesses proven anti-inflammatory effect due to inhibition of 5-lipoxygenase pathways of arachidonic acid (Cabeza *et al.*, 2003; Prieto *et al.*, 2006). It is well-known that phytosterols were used as a sunscreen emulsion and anti-age additives in cosmetics (Dweck, 2003). In addition, it was found that the unpolar fractions from fig leaves contained also stigmasterol (28),

germanicol (32) and lanosterol (33) (Table 1). In the skin phytosterols absorbed the UV radiation and after UV irradiation expressed in the cells the synthesis of collagen type I. Phytosterols stimulate also the differentiation of cells and inhibit angiogenesis, contribute to the strengthening of the dermal structure by inhibiting the enzymatic degradation of the dermis fibres and proteoglycans (Quirin, 2011).

In vivo and *in vitro* triterpenes blocked matrix metalloproteinase, which catalyzes the degradation of many components of connective tissue including proteoglycans and different types of collagen (Hartog *et al.*, 2009). These constituents were identified in unpolar fig leaves – 10%-18% of TIC (Table 1). The plant-derived pentacyclic triterpenes α-Amyrin (31), β-amyrin (34) and lupeol (35) possessed proven anti-inflammatory effect. Therefore, these compounds inhibited inflammatory mediators - tumor necrosis factor–α (α-TNF), PGE 2 and interleukin–1β (Aragao *et al*., 2007). Lupeol (35) possesses anti-inflammatory activity greater than of the indomethacin (Saleem, 2009). In combination lupeol and amyrins have a pronounced anti-edematous effect and suppressed hypersensitivity reactions of IgE type (Israel, 2014).

Due to the revealed metabolite profile of unpolar fractions of fig leaves, this plant material showed the potential for application in cosmetic formulas. From all investigated extracts the application of n-hexane as solvent gave the highest yield (5.0 g/100g dw) and the various number of phytocomponents with high biological activity. These results could be explained with the possibility of n-hexane to dissolve and extract more unpolar molecules in comparison with other solvents. Moreover n-hexane is industrially applied for refining process of fat and oils for food purposes. Therefore, *Ficus carica* leaves could be used as a promising source of extracts with high biologically value for application of pharmaceutical and cosmetic sectors.

Conclusion

The current study demonstrates that the highest yield of phytochemical compounds from *Ficus carica* leaves were isolated by extraction with n-hexane. Petroleum ether was evaluated as the other promising solvent for the fractionation of lipid compounds. In general all investigated fig leaves fractions contained natural compounds as terpenes, phytosterols, fatty acid with well-pronounced bioactivity. Therefore, the presence of these biologically active substances in Ficus carica leaves reveals their potential application in functional food, pharmaceutical and cosmetics formulas with potential health benefits.

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