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Chemical Assessment and *in Vitro* Antioxidant Capacity of *Ficus carica* Latex

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Ficus species possess latex-like material within their vasculatures, affording protection and self-healing from physical attacks. In this work, metabolite profiling was performed on *Ficus carica* latex. Volatiles profile was determined by HS-SPME/GC−IT-MS, with 34 compounds being identified, distributed by distinct chemical classes: 5 aldehydes, 7 alcohols, 1 ketone, 9 monoterpenes, 9 sesquiterpenes and 3 other compounds. Sesquiterpenes constituted the most abundant class in latex (ca. 91% of total identified compounds). Organic acids composition was also characterized, by HPLC−UV, and oxalic, citric, malic, quinic, shikimic and fumaric acids were determined. Malic and shikimic acids were present in higher amounts (ca. 26%, each). The antioxidant potential of this material was checked by distinct *in vitro* chemical assays. A concentration-dependent activity was noticed against DPPH, nitric oxide and superoxide radicals. Additionally, acetylcholinesterase inhibitory capacity was evaluated, but a weak effect was found.

KEYWORDS: *Ficus carica* latex; volatiles; organic acids; antioxidant potential; acetylcholinesterase inhibition

INTRODUCTION

Latex is widely distributed in plants and consists of cytoplasmatic fluid of laticiferous tissues that contain the usual organelles of plant cells, such as nucleus, mitochondria, vacuoles and ribossomes, among others (*1*). This material contains various secondary metabolites, like terpenoids and phenolics, and proteins, namely, cysteine proteases (*2*, *3*). Many of these compounds provide resistance to hervibores via toxic or antinutritive effects, whereas others are involved in the stickiness that can mire insect hervibores (*2*).

Ficus carica L., the common fig, is a species of great commercial importance, comprising numerous varieties with significant genetic diversity. All *Ficus* species possess latex-like material within their vasculatures. *F. carica* latex has been traditionally used in the treatment of gout, ulcers and warts, among other situations (*4*, *5*), given its proteolytic and keratolytic effects, associated with its viscosity (*6*).

Plants show a constitutive emission of volatile compounds that are released from the surface of the leaf and/or accumulated in storage sites. Terpenes, as the largest class of plant secondary metabolites (*7*), have many volatile representatives. Monoterpenes (C_{10}), sesquiterpenes (C_{15}), and even some diterpenes (C_{20}), have high enough vapor pressures at normal atmospheric conditions to allow significant release into the air (*8*). These compounds play different roles in herbivore elimination, either by attraction of parasitoids that increase herbivore mortality (indirect defense) or by directly reducing herbivores (*7*).

Organic acids are primary metabolites, which can be found in great amounts in all plants, especially in the fruits. The type and content of organic acids found are extremely variable between species, developmental stages and tissues types, additionally playing an important role in pH regulation (*9*). These compounds also exert a protective role against various diseases, due to their antioxidant activity (*10*).

Antioxidant compounds, such as phenolics, organic acids, vitamin E and carotenoids, protect against oxidation, or cellular damage caused by reactive species, preventing the initiation of several diseases, like many types of cancer, heart disease, diabetes and neurodegenerative illnesses (*11*). Antioxidant activities have also been observed for volatile compounds, which are found in many plants, as well as in foods and beverages (*12*).

Recently, several studies have been developed to assess the ability of natural compounds for inhibiting acetylcholinesterase activity, since this is the first approach for the treatment of neurological disorders, such as Alzheimer's disease, senile dementia and ataxia (*13*).

Few studies have been reported in *F. carica* latex, to describe the presence of 6-*O*-acyl-β-D-glucosyl-β-sitosterols and their

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capacity to inhibit the proliferation of some human cancer cells (*4*, *14*, *15*), the characterization of ficin, a cysteine proteinase (*3*), antifungal and antihelmintic activities (*16*, *17*), as well as the characterization of protein genes (*1*). As far as we know, no study concerned the volatiles and organic acids composition of *F. carica* latex, or its antioxidant and acetylcholinesterase inhibitory potential.

This work aimed to contribute to the knowledge of the metabolic profile of *F. carica* latex and to evaluate some of its biological capacities. Volatile compounds and organic acids profiles were characterized, and antioxidant and acetylcholinesterase inhibitory capacities were checked.

MATERIALS AND METHODS

Standards and Reagents. All chemicals used were of analytical grade. The standards compounds were purchased from various suppliers: pentanal, heptanal, octanal, 1-butanol-3-methyl, 1-butanol-2-methyl, 1-pentanol, 1-heptanol, 6-methyl-5-hepten-2-one, limonene, terpinolene, *cis*-linalool oxide, linalool, cadinene, methyl salicylate, quinoline and psoralene were obtained from Sigma-Aldrich (St. Louis, MO); hexanal, benzaldehyde, phenylethyl alcohol, phenylpropyl alcohol were from SAFC (Steinheim, Germany); α-pinene, β-pinene and eucalyptol were from Extrasynthése (Genay, France), and 1-hexanol was from Fluka (Buchs, Switzerland). Sodium nitroprussiate dihydrate was purchased from Riedel-de Haën (St. Louis, MO). *N*-(1-Naphthyl) ethylene-diamine dihydrochloride, phosphoric acid and methanol were from Merck. Sulfanilamide, β-nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium chloride (NBT), phenazine methosulfate (PMS), 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), acetylthiocholine iodide and acetylcholinesterase (from electric eel, type VI-s) were obtained from Sigma-Aldrich. The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA).

Latex Sample. Immature green fruits from *F. carica* cultivar Pingo de Mel trees growing in Mirandela region (Northeast Portugal) were harvested in June of 2009. Latex was collected manually without using steel knives, by incising the stalk of the green fruit from the main branch. The sample was obtained drop-by-drop without squeezing, homogenized, weighted, separated by aliquots and kept at −20 °C until processed.

Volatile Compounds. *SPME Fibers*. Several commercial fibers can be used to extract volatiles. According to bibliography, recommendations of supplier (Supelco, Bellefonte, PA) and our own knowledge (*18*), the fiber used was coated with divinylbenzene/polydimethylsiloxane (DVB/PDMS), 65 μm.

Headspace Solid-Phase Microextraction (*HS-SPME*). *F. carica* latex was kept at 40 °C, for 5 min, to promote compounds' release. This was done without agitation because the sample had previously corroded the stirrer. The fiber was then exposed to the headspace for 60 min, at 40 °C. Afterward the fiber was pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption, for 1 min. The fiber was then removed and conditioned in another GC injection port for 15 min at 250 °C.

Gas Chromatography−Ion Trap Mass Spectrometry Analysis (*GC−IT-MS*). HS-SPME analysis was performed with a Varian CP-3800 gas chromatograph (USA) coupled to a VARIAN Saturn 4000 mass selective detector (USA) and a Saturn GC/MS workstation software version 6.8. A VF-5 ms 30 m × 0.25 mm × 0.25 μm (FactorFour) column from VARIAN was used in the analysis. The injector port was heated to 220 °C, and injections were performed in splitless mode. The carrier gas was helium C-60 (Gasin, Portugal), at a constant flow of 1 mL/min. Oven temperature was set at 40 °C (for 1 min), then increasing 2 °C/min to 220 °C and held for 30 min. All mass spectra were acquired in electron impact (EI) mode. Ionization was maintained off during the first minute. Transfer line, manifold and trap temperatures of the ion trap detector were set at 280, 50, and 180 °C, respectively. As a preliminary analysis of the matrix revealed the presence of low molecular weight compounds (below 350 *m*/*z*), in order to increase the method's sensibility covered mass ranged from 40 to 350 *m*/*z*, with a scan rate of 6 scan/s. The emission current was 50 mA, and the electron multiplier was set in relative mode to auto tune procedure. The maximum ionization time was 25,000 ms, with an ionization storage level of 35 *m*/*z*. The analysis was performed in Full Scan mode. Compounds were identified by comparing their retention times with those of authentic reference compounds analyzed under the same conditions, and by comparison of the retention indices (as Kovats indices) with literature data (*18*). The comparison of MS fragmentation pattern with those of pure compounds and mass spectrum database search was performed using the National Institute of Standards and Technology (NIST 05) MS spectral database. Confirmation was also accomplished using laboratory built MS spectral database, obtained from chromatographic runs of pure compounds performed with the same equipment and conditions. Peaks' areas were determined by reconstructed Full Scan chromatogram using for each compound some specific ions, quantification ions (**Table 1**). By this way some peaks which were coeluted in Full Scan mode (resolution value less than 1) could be integrated with a value of resolution higher than 1. Each sample was analyzed in triplicate.

HPLC/UV for Organic Acids Analysis. The preparation of the sample consisted in the acidification of 5 mL of latex with 5 mL of H_2SO_4 0.01 N, followed by filtration. Twenty microliters of the acidified sample was analyzed on an analytical HPLC unit (Gilson), using an ion exclusion column Nucleogel Ion 300 OA (300 × 7.7 mm), in conjunction with a column heating device set at 30 °C. Elution (70 min) was carried out at a solvent flow rate of 0.2 mL/min, isocratically, with sulfuric acid 0.01 N as the mobile phase. Detection was performed with a Gilson UV detector at 214 nm. The organic acids quantification was achieved by the absorbance recorded in the chromatograms relative to external standards.

Antioxidant Activity. *DPPH$^{\bullet}$ Assay*. Antiradical activity was determined spectrophotometrically in an Multiskan Ascent plate reader (Thermo Electron Corporation), by monitoring the disappearance of DPPH$^{\bullet}$ at 515 nm, according to a described procedure with some modifications (*10*). Latex was diluted with methanol and centrifuged at 13,000 rpm for 2 min (Biofuge Fresco, Heraeus). The reaction mixtures in the sample wells consisted of 25 μL of supernatant and 200 μL of 150 μM DPPH$^{\bullet}$ dissolved in methanol. The plate was incubated for 30 min at room temperature after the addition of DPPH$^{\bullet}$. Three experiments were performed in triplicate.

Nitric Oxide Radical Assay. Antiradical activity was determined in an Multiskan Ascent plate reader (Thermo Electron Corporation), according to a described procedure (*10*) with some modifications. Briefly, 100 μL of sodium nitroprusside 20 mM were incubated with 100 μL of sample for 60 min, at room temperature, under light. After this period Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine, in 2% phosphoric acid) was added and the mixture was incubated at room temperature for 10 min, with centrifugation at 4,000 rpm (Eppendorf centrifuge 5810R) during 5 min. The absorbance of the supernatant was read at 562 nm. Three experiments were performed in triplicate.

Superoxide Radical Assay. Superoxide radicals were generated by NADH/PMS system according to a described procedure (*10*). All components were dissolved in 19 mM phosphate buffer (pH 7.4). In each well, sample, NADH and NBT were added. The reaction was initiated by the addition of PMS and conducted at 560 nm, at room temperature for 2 min. Three experiments were performed in triplicate.

Acetylcholinesterase Inhibitory Activity. The effect on acetylcholinesterase activity was determined spectrophotometrically in a Multiskan Ascent plate reader (Thermo Electron Corporation) based on Ellman's method, according to a described procedure (*10*). The following buffers were used: buffer A, 50 mM Tris-HCl, pH 8; buffer B, 50 mM Tris-HCl, pH 8, containing 0.1% bovine serum albumin (BSA); buffer C, 50 mM Tris-HCl, pH 8, containing 0.1 M NaCl and 0.02 M $MgCl.6H_2O$.

In each well the mixture consisted of acetylthiocholine in water, DTNB in buffer C, buffer B, sample dissolved in a solution of 10% methanol in buffer A and acetylcholinesterase. The absorbance was measured at 405 nm before and after enzyme addition. The rates of reactions were calculated by Ascent Software version 2.6 (Thermo Labsystems Oy). The rate of the reaction before adding the enzyme was subtracted from that obtained after enzyme addition, in order to correct eventual spontaneous hydrolysis of substrate. Percentage of inhibition was calculated by comparing the rates of the sample with the control (10% methanol in buffer A). Three experiments were performed in triplicate.

RESULTS AND DISCUSSION

Volatile Compounds. The results obtained by HS-SPME/GC−IT-MS with *F. carica* latex are displayed in **Table 1**. As far as we know, this is the first study describing the volatile profile of *F. carica* latex.

Table 1. Volatile Composition of *F. carica* Latex

no.	compound	RI[a]	ID[b]	QI[c] (*m/z*)	area[d]/1000 (SD)
			Aldehydes		
1	pentanal	784	S, MS (74.3/87.8)	44/58/81	0.28 (0.1)
2	hexanal	891	S, MS (86.9/87.8)	56/57/67/72	3.56 (0.28)
3	heptanal	991	S, MS (78.1/80.1)	55/57/70	0.44 (0.09)
4	benzaldehyde	1057	S, MS (81.6/83.2)	77/105	33.67 (1.21)
5	octanal	1094	S, MS (79.8/90.9)	67/81/95	1.43 (0.16)
total of aldehydes					39.38
			Alcohols		
6	1-butanol-3-methyl	825	S, MS (81.7/85.7)	56/71	0.11 (0.01)
7	1-butanol-2-methyl	829	S, MS (82.0/89.6)	56/70	0.52 (0.07)
8	1-pentanol	859	S, MS (86.6/90.6)	55/70	2.01 (0.17)
9	1-hexanol	950	S, MS (78.8/80.1)	56/69	58.49 (0.57)
10	1-heptanol	1063	S, MS (82.8/83.3)	55/70/83	64.24 (0.21)
11	phenylethyl alcohol	1203	S, MS (86.5/91.3)	91/122	5.45 (0.11)
12	phenylpropyl alcohol	1312	S, MS (88.7/92.6)	91/117/136	3.81 (0.10)
total of alcohols					134.63
			Ketones		
13	6-methyl-5-hepten-2-one	1077	S, MS (86.5/85.8)	67/108	0.56 (0.09)
total of ketones					0.56
			Monoterpenes		
14	α-thujene	1016	MS (85.3/89.6)	77/93/136	2.05 (0.83)
15	α-pinene	1024	S, MS (88.5/89.6)	92/93/136	1.76 (0.18)
16	*β*-pinene	1079	S, MS (86.5/85.8)	93/121	22.18 (0.70)
17	limonene	1119	S, MS (73.4/81.0)	68/93	17.70 (2.00)
18	eucalyptol	1123	S, MS (78.4/83.6)	81/93/108	0.33 (0.01)
19	terpinolene	1175	S, MS (88.4/90.8)	93/121	1.93 (0.02)
20	*cis*-linalool oxide	1177	S, MS (80.3/86.2)	59/68/94	2.95 (0.06)
21	linalool	1191	S, MS (84.0/84.0)	136	46.93 (1.6)
22	epoxylinalol	1266	MS (83.5/84.2)	68/94	3.06 (0.29)
total of monoterpenes					98.89
			Sesquiterpenes		
23	α-guaiene	1375	MS (81.7/83.6)	95/147	1.17 (0.07)
24	α-bourbonene	1388	MS (80.1/80.7)	81/123/161	61.31 (1.13)
25	*β*-caryophyllene	1408	S, MS (87.5/87.6)	161/189/204	45.46 (3.22)
26	*trans*-α-bergamotene	1420	MS (72.2/72.6)	93/119/204	38.54 (0.46)
27	α-caryophyllene	1446	MS (75.9/76.4)	80/93/121	57.57 (1.74)
28	τ-muurolene	1467	MS (77.7/78.6)	105/161/204	203.87 (1.01)
29	germacrene D	1469	MS (84.5/88.8)	105/119/161	2698.50 (94.05)
30	cadinene	1507	S, MS (74.0/74.3)	105/119/161	281.85 (11.82)
31	α-calacorene	1532	MS (81.7/94.5)	142/157/200	7.37 (0.48)
total of sesquiterpenes					3395.64
			Miscellaneous Compounds		
32	methyl salicylate	1284	S, MS (86.4/90.4)	92/120/152	27.51 (0.31)
33	quinoline	1317	S, MS (91.6/93.0)	102/129	7.89 (0.23)
34	psoralene	1847	S, MS (88.0/93.8)	156/188	11.17 (0.92)

[a] RI = retention index. [b] ID = Identification method (fit/retrofit values, %). S = identified by comparison with reference compounds, MS = tentatively identified by NIST05. [c] QI = quantification ions. [d] Area expressed as arbitrary units.

From the identified compounds, heptanal (**3**), benzaldehyde (**4**), 1-hexanol (**9**), phenylpropyl alcohol (**12**), 6-methyl-5-hepten-2-one (**13**), α-thujene (**14**), α-pinene (**15**), *β*-pinene (**16**), limonene (**17**), terpinolen (**19**), *cis*-linalool oxide (**20**), linalool (**21**), α-bourbonene (**24**), *β*-caryophyllene (**25**), *trans*-α-bergamotene (**26**), α-caryophyllene (**27**), germacrene D (**29**), cadinene (**30**), methyl salicylate (**32**) and psoralene (**34**) were already reported in fruits and leaves of twenty *Ficus* species, including *F. carica* (*19*−*21*). According to the work of Grison-Pigé et al. (*21*), which described the occurrence of 99 volatile compounds in fruits of *Ficus* species, the most abundant ones were terpenoids (monoterpenes and sesquiterpenes), aliphatic compounds, like 1-hexanol and heptanal, and products from the shikimic acid pathway, as benzaldehyde.

The major class of identified compounds in *F. carica* latex was that of sesquiterpenes (ca. 91% of identified compounds), followed by alcohols (ca. 4%). Ketones represented the minor components (< 0.1%) (**Table 1**).

The short-chain aldehydes and alcohols are produced by plants in response to wounding and play an important role in the plant's defense strategies, pest resistance and protective effect against microbial proliferation (*7*). The presence of these types of

compounds in latex is not surprising considering that this material is produced for plant defense (*2*).

Among the five aldehydes detected, benzaldehyde was the major one in the sample (**Table 1**). This compound is formed from benzyl alcohol, by oxidation catalyzed by dehydrogenases (*8*). This is an important intermediate for perfumery, pharmaceutical, dyestuff and agrochemical industries (*23*). In addition, benzaldehyde has been successfully used to treat terminal human carcinomas and as antimicrobial agent (*24*).

Regarding alcohols, 1-hexanol (**9**) and 1-heptanol (**10**) were the ones in highest amounts (**Table 1**). Compounds derived from leucine, such as 3-methylbutanol (**6**), as well as phenylethyl alcohol (**11**) formed from phenylalanine, are abundant in various fruits, like strawberry, tomato and grape varieties (*22*). Although phenylethyl alcohol is present in low amounts in latex, it has long been known to possess antimicrobial properties and to act as a potent insect attractant (*18*).

6-Methyl-5-hepten-2-one (**13**) was the only ketone identified in latex (**Table 1**). This compound is odor-active and known to be an oxidative byproduct or derived from carotenoids degradation (*25*). Carotenoids were previously reported in fruit of *F. carica* (*26*) and are known to provide important visual cues associated with fruit ripeness (*25*).

As referred to above, sesquiterpenes constituted the main class of compounds identified in *F. carica* latex (**Table 1**). Among sesquiterpenes, germacrene D (**29**) was the more abundant one, followed by cadinene (**30**) and τ-muurolene (**28**). Germacrene D has been reported in fig leaves, and it can be important to insect behavior (*19*). This compound is considered to be a key intermediate in the biosynthesis of many sesquiterpenes and is known as an antimicrobial agent (*27*). τ-Muurolene and cadinene have antifungal properties (*28*).

Linalool (**21**) was the main identified monoterpene in *F. carica* latex. Grison et al. (*20*) reported that with respect to volatiles in *F. carica* fruits, the synthetic mixture of linalool, benzyl alcohol and linalool oxides was essential for the attraction of the pollinating wasp. Linalool is used as a scent in perfumed hygiene products, being also applied for its known antimicrobial properties (*29*). Other important monoterpenes, α-pinene (**15**), β-pinene (**16**) and limonene (**17**), exhibit strong antimicrobial activity too (*29*). Previous studies demonstrated that limonene can be utilized in the prevention of several types of cancer (*29*).

Among other miscellaneous compounds occurring in *F. carica* latex, methyl salicylate (**33**) (**Table 1**) is known to be essential in protecting local infections of plant against pathogens (*30*). *S*-Adenosyl-L-methionine-salicylic acid carboxyl methyltransferase catalyzes the formation of methyl salicylate from salicylic acid, a known anti-inflammatory and analgesic compound (*22*, *25*). Methyl salicylate is a common component of floral scent and is believed to be an important attractant of insect pollinators (*8*, *22*).

Quinoline (**33**), also determined in the analyzed sample (**Table 1**), is an alkaloid biosynthetically derived from anthranilic (2-aminobenzoic) acid (*31*). This compound is used as antimicrobial agent (*3*).

Psoralene (**34**) is the precursor of all types of furanocoumarins formed by dealkylation of (*S*)-(+)-marmesine by cytochrome P450-type enzymes (*32*). This compound has already been reported in *F. carica* leaves and fruits (*10*). Psoralene is a well-known photodynamic active drug that is capable of absorbing radiant energy and responsible for contact dermatitis (*33*).

Organic Acids. Organic acids profile of *F. carica* latex revealed to be composed by six organic acids: oxalic, citric, malic, quinic, shikimic and fumaric acids (**Table 2**). These compounds were already reported in *F. carica* aqueous lyophilized extracts of leaves, pulps and peels (*10*), but it is the first time that they are described in its latex. Malic and shikimic were the most abundant acids, each representing ca. 26% of total organic acids content, followed by quinic acid (ca. 24%), while fumaric acid was a minor compound (ca. 3%) (**Table 2**).

Table 2. Organic Acids Composition of *F. carica* Latex (mg/kg)[a]

organic acids	retention time (min)	latex
oxalic	20.16	379.6 ± 8.4
citric	30.14	309.7 ± 5.6
malic	36.63	808.4 ± 144.3
quinic	38.38	751.1 ± 111.4
shikimic	47.31	817.5 ± 21.8
fumaric	60.74	106.1 ± 36.0
total		3172.3

[a] Values are expressed as mean ± standard deviation of three assays.

Organic acids influence the organoleptic characteristics of fruits and vegetables, namely, flavor, and contribute to their acidity (*9*). In fact, the analyzed latex exhibited a pH of 5, which can be related, at least partially, to the presence of organic acids.

Antioxidant Capacity. In the present work, the antioxidant ability of *F. carica* latex was screened by DPPH assay, which allowed observation of a concentration-dependent potential (IC_{25} = 1049 μg/mL) (**Figure 1A**).

Superoxide radical is one of the most effective free radicals, implicated in cell damage as precursor of important reactive oxygen species, like hydroxyl radical and peroxynitrite, contributing to the pathological process of many diseases (*34*). The analyzed sample presented a protective effect against superoxide radical, in a concentration-dependent way, with an IC_{25} at 291 μg/mL (**Figure 1B**).

Nitric oxide is involved in several physiological processes, like blood pressure control, neural signal transduction, platelet function and antimicrobial defense (*34*). Despite the beneficial effects, an overproduction of this reactive species is associated with several types of biological damage (*35*). In addition, it reacts rapidly with superoxide radical to form peroxynitrite, a major damaging oxidant produced *in vivo* (*35*). *F. carica* latex displayed nitric oxide scavenging capacity, which was concentration-dependent (IC_{25} = 1768 μg/mL) (**Figure 1C**).

Under the assay conditions, when comparing with aqueous lyophilized extracts of other *F. carica* materials, namely, leaves, pulps and peels (*10*), it is possible to see that latex possesses a stronger antioxidant capacity than fruits, although leaves are the most effective material. As far as we know, this is the first study assessing the antioxidant activity of this latex.

Overall, the results obtained in the three assays revealed latex good ability to scavenge free radicals, which can be partially related to the presence of organic acids (*10*). In addition, this antioxidant activity may also be attributed to the presence of several volatile compounds known for their antioxidant properties, such as limonene, α-pinene, β-pinene, terpinolene and sesquiterpenes (*36*). Based on the scavenging capacity observed for both superoxide radical and nitric oxide, latex may also prevent the formation of other biologically important oxidative species resultant from the reaction of those two, like peroxynitrite and hydroxyl radical. However, the existence in latex of other nondetermined compounds, with antioxidant capacity, cannot be ignored.

Acetylcholinesterase Inhibitory Activity. Several studies indicate a considerable increase in the prevalence of Alzheimer disease over the next two decades. Most treatment strategies have been based on the cholinergic hypothesis, which postulates that memory impairments in patients suffering from this disease result from a deficit of cholinergic function in brain. One of the most

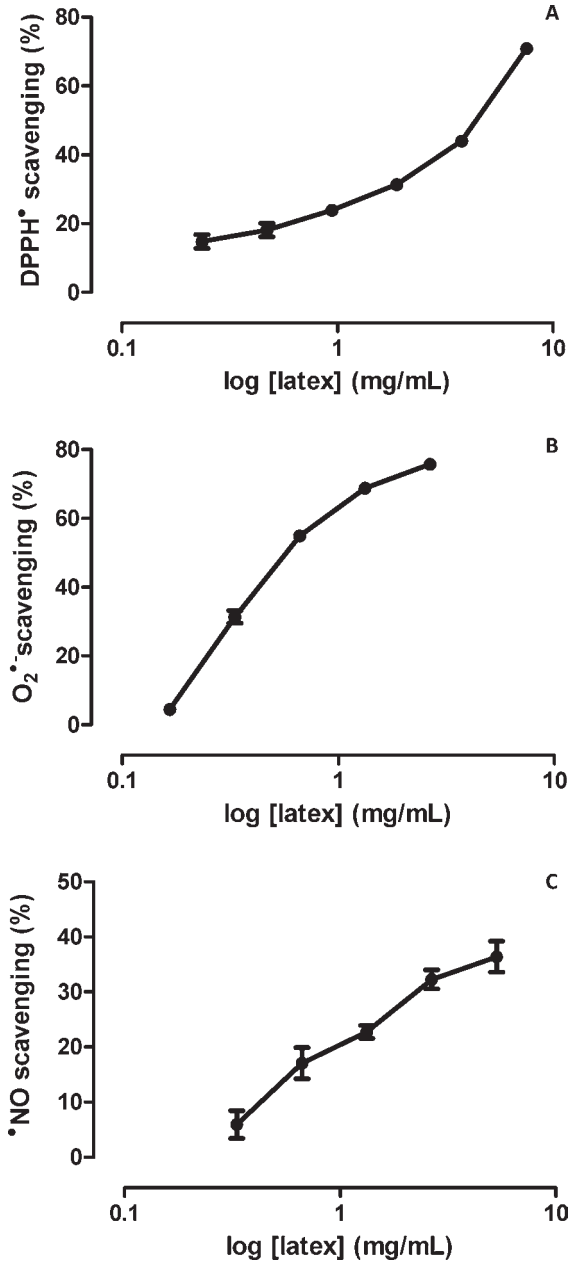

Figure 1. Effect of *F. carica* latex against (**A**) DPPH•, (**B**) superoxide radical ($O_2^{\bullet -}$) and (**C**) nitric oxide (•NO). Values show mean ± SE of three experiments performed in triplicate.

promising treatment approaches is to enhance the acetylcholine level in the brain using acetylcholinesterase inhibitors (*37*).

As far as we know, the effects of *F. carica* latex on enzyme activity were assessed for the first time. Latex exhibited low acetylcholinesterase inhibitory capacity: under the assay conditions, and for the highest tested concentration (5317 μg/mL), the effect corresponded to less than 10%. However, latex has more acethylcholinesterase inhibitory capacity comparing to leaves, pulps and peels, since these materials had no effect against this enzyme (*10*).

In conclusion, to our knowledge *F. carica* latex volatile profile was achieved for the first time and thirty-four volatile compounds were determined. As far as we know, this is also the first study describing the organic acids composition of this matrix and its antioxidant activity. The volatile and organic acids qualitative profiles of *F. carica* latex are similar to those of other *F. carica* materials, namely, leaves, peels and pulps, although some differences at quantitative levels were noticed (*10*, *21*). In addition, and as discussed above, the biological capacity of all materials is different (*10*, *21*). One should have in mind that latex also contains other components besides those determined herein. All these facts suggest that the biological activity of a given matrix is clearly conditioned by its metabolic profile, as observed before with other natural products (*34*, *38*, *39*).

The results obtained are very promising, constituting a base for the possible application of this matrix in food, cosmetic and pharmaceutical industries, due to its antioxidant capacity. However, precautions regarding the direct application of latex are needed, because, as mentioned above, this material presents keratolytic and corrosive properties.

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