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Chemical composition and biological activity of natural extracts obtained from a Brazilian aromatic plant (*Aloysia gratissima*) by supercritical CO_2 and hydrodistillation

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Abstract

Aloysia gratissima (Gillies & Hook.) Tronc. (Verbenaceae) is an aromatic shrub native to South America which is widely used in traditional medicine to treat several diseases. In this study we report on the chemical composition and biological activity (anti-inflammatory and antioxidant activity) of extracts obtained from the aerial parts of the Brazilian plant *Aloysia gratissima* using two extraction methods: hydrodistillation and extraction with supercritical carbon dioxide. Three major components were identified in the essential oil, namely *trans*-pinocamphone (13.5-16.3%), β-pinene (10.5-12.0%) and α-guaiol (6.6-8.7%), whereas the major compounds were guaiol (16.7%), β-caryophyllene (9.2%) and germacrene D (8.6%) in the supercritical fluid (SFE) extract. None of the two samples presented a significant inhibitory effect in vitro on soybean 5-lipoxygenase. The antioxidant activity using both the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ORAC assays showed poor activity.

Key words: Aloysia gratissima, antioxidant activity, anti-inflammatory activity, essential oil, supercritical fluid extraction

Introduction

Medicinal plants have been used in many countries to treat health problems. The extracts and essential oils of numerous plants have been isolated in order to be evaluated for their in vitro and in vivo biological activities, so that the therapeutic properties attributed to the folk medicine can be elucidated and validated. Most of the therapeutic properties are related to their secondary metabolism products, such as terpenoids. Brazil, Cuba, India, Jordan and Mexico are countries of rich flora and tradition in the use of medicinal plants [1]. Many plants of the Brazilian ecosystem, as those from the Cerrado (savannah), the Atlantic and the Amazon rain-forests, have been used in the treatment of several tropical diseases, including leishmaniasis, malaria, schistosomiasis, and those caused by bacterial and fungal infections [2].

The family Verbenaceae comprises about 175 genera and 2300 species, distributed in the tropical and subtropical areas, mainly in the temperate zone of the Southern hemisphere [3]. *Aloysia*, which belongs to this family, is a genus of sweet aromatic shrubs including approximately 40 species [4]. *Aloysia gratissima* (Gill. & Hook.) Tronc. (synonyms: *Aloysia lycioides* Cham., *Lippia lycioides* (Cham.) Steud., local common names: "Cedrón de Monte",

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"Niñarupá, "Reseda del campo", "Brazilian lavender") is a shrub growing up to 3 m which is native to South America, frequently found in Argentina, Brazil, Paraguay, Uruguay, and in the southern regions of Bolivia, Mexico and North America [5].

An infusion of the aerial parts is widely used in Argentina as a tonic to treat stomachic, digestive, diaphoretic disorders and also as an aphrodisiac [5]. In addition, it is used in Bolivia as a carminative, stomachic, sedative, and as an antispasmodic in Paraguay and Uruguay [6]. In Brazil the juice from the leaves is used to treat bronchial and digestive tract disorders [7].

Some chemical investigations were performed on the essential oils obtained from aerial parts of *A. gratissima*; most of them concern plants collected in Argentina and mention different major constituents: various monoterpenes (pinenes, limonene, 1,8-cineole) as well as sesquiterpenes [8-10]; pulegone (65.8%) was mentioned in the flowers oil of the species harvested in Córdoba [11], sesquiterpenic compounds dominated by cadinol (17-33%) and caryophyllene oxide (11-15.8%) were identified in the whole plant volatile extract [12-15].

More recently, Ricciardi et al [5], reported the chemical composition of oils obtained from plants collected during different seasonal stages in three localities of the province of Corrientes in Argentina. A significant variation in the oil composition was reported, the major constituents being α-thujone (6.8-17.5%), β-elemene (traces to 35.7%), viridiflorol (0.9-33.6%), β-caryophyllene (1.8-28.0%), germacrene D (1.9-10.1%), bicyclogermacrene (3.8-12.8%), (*E*)-nerolidol (traces to 11.6%) and 10-*epi*-cubebol (0.1-13.4%). Despite this variability, the oil yields did not show significant differences (0.7-1.3%) and the sesquiterpenic group was always predominant (57%-89.2%).

Analyses of essential oils obtained from flowers [16] and leaves [17] of *A. gratissima* harvested in Uruguay indicated a significant amount of sabinene in both samples; the major sesquiterpenes differed according to the plant part: β-bisabolene and copaenol in the leaves, β-caryophyllene, caryophyllene oxide and globulol in the flowers.

A. gratissima from Brazil is poorly studied. A literature survey indicates the presence of 1,8-cineole (3%), esters (9.7%) along with limonene and sesquiterpenic alcohols [18]; more recently pulegone, limonene, spathulenol, α-pinene, α-thujone and pinocamphone have been reported [19]. In the hexane extract of the aerial parts of the species collected in Goiânia (State of Goiás, Central west of Brazil) the sesquiterpenoids guaiol, bisabolol and spathulenol were reported [4].

Biological investigations previously performed on *A. gratissima* essential oils indicated antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* [12]. Significant fungicidal activity against *Ascosphaera apis* involved in mycosis development [13] as well as nematicidal effect against the root-knot nematode *Meloidogyne* [15] has been reported. Antiviral activities of the essential oil [14] and of the ethanolic and aqueous extracts of *A. gratissima* have been observed [20]. The crude methanol extract of *A. gratissima* gave good result according the DPPH method, and in most cases they performed better than the reference antioxidant BHA [21]. It is worth noting that all these samples presented similar chemical characteristics, dominated by sesquiterpenic structures; on another hand, no significant antimicrobial activity against *Candida albicans* was observed (MIC above 2.0 mg ml^{-1}) with a sample of *A. gratissima* essential oil collected in Brazil; unfortunately, the results were reported without any information on the oil chemical composition [1].

The biological properties of aromatic and medicinal plants are frequently related to the presence of volatile compounds, which explains the fact that most of the studies involving these plants are focused on their essential oils. However, it could be interesting to also study the extract in order to evaluate all the substances that might show biological activity. To obtain the extract, a variety of solvents can be used as water, ethanol or hexane. Another possibility is to use supercritical fluid extraction (SFE). This extraction process has been used to extract several classes of organic compounds (alkaloids [22], terpenoids [23], lipids [24], saponins [25]). In general, carbon dioxide is used as the solvent due to its low critical temperature (31.1°C) and moderate critical pressure (73.8 bar). The process makes use of the advantages of supercritical fluid such as gas-like viscosity and liquid-like density. Because of the nonpolar characteristics of carbon dioxide eventually a cosolvent or modifier is added such as for the extraction of glycosides [26]. In this way, supercritical extraction technology for the isolation of compounds that could possibly be used as phytotherapeutic substances should be considered. This technology using high pressure gases as solvent, presents several advantages for this application: this is a clean process and the extracts obtained are of high grade purity.

Due to the high costs of manufactured medicines when compared to the low cost related to the use of plants and their extracts in a more natural form, there is a growing interest in studying the Brazilian native plants with therapeutic activities already known by the folk and traditional medicines, that might possess biological activities.

The aim of the present work was to compare the chemical and biological characteristics (anti-inflammatory and antioxidant activities) of the extracts obtained from the aerial parts of the Brazilian species *A. gratissima* according to two extraction techniques: hydrodistillation and supercritical carbon dioxide extraction.

Materials and methods

Plant material and extraction procedures

Aloysia gratissima was cultivated in the experimental farm of the Chemical, Biological and Agricultural Pluridisciplinary Research Center (CPQBA-UNICAMP). The plants were cultivated using the organic system, under direct sun light, and with irrigation. The plant's density was 1.5 plant/m^2. The plants were harvested in November 2005 and May 2006. The species were deposited at the Herbarium of UNICAMP (voucher UEC 121.393). Leaves, flowers and

stems were dried at 40 °C and triturated. The particle size distribution was determined using a set of standard sieves of the Tyler Series (W.S Tyler), meshes 16, 24, 32 and 48. The sieves containing the raw material particles were subjected to mechanical agitation in a magnetic agitator (Bertel, Piracicaba, Brazil). The mass of the particles restrained in each sieve was determined in an analytical scale (Marte, series AS2000 ± 0.01g, Germany).

The essential oil of *Aloysia gratissima* (leaves, flowers and stems particles) were obtained by hydrodistillation, using a Clevenger-type apparatus. The water plus oil mixture was separated by decantation, dried with anhydrous sodium sulphate, and kept in sealed flasks until analyses. The sample mass was about 50 g for every essay and the operating time was equal to 2 h 30 min.

The operating conditions used for obtaining of SFE extract were 40°C and 200 bar. Twenty grams of *Aloysia gratissima* leaves, flowers and stems were used in the SFE experiment; the CO_2 flow rate was 4.14 g/min. and the assay continued for 3 h. The SFE unit used was built at the Technical University of Hamburg-Harburg (TUHH), Hamburg, Germany [27]. The CO_2 at the required conditions was pumped into the line, entering in contact with the triturated particles of *Aloysia gratissima* used to pack the 100 ml stainless steel extraction column. The mixture of CO_2 and extract had its pressure decreased in the outlet valve, so the extract could be collected in a flask cooled by an ethylene glycol thermostatic bath, and, then, the extract mass was weighted. A micrometering valve was used to control the CO_2 flow rate that was measured in a calibrated flowmeter. The extraction yield was calculated as the ratio between the mass of extract and the mass of dried raw material.

Chemical analysis

Gas chromatography

The volatile extracts were analyzed on a Varian CP-3380 gas chromatograph, equipped with flame ionization detectors fitted with two silica capillary columns : CP Sil 5 CB low bleed/MS (100% dimethyl polysiloxane, Chrompack/Varian, Palo Alto CA) capillary column (30 m x 0.25 mm i.d. x 0.25 µm film) and Supelcowax 10 (polyethylene glycol, Supelco Inc, Bellefonte PA) fused capillary column (30m x 0.25 mm i. d. x 0.25 µm film); temperature program was 50-200°C at 5°C/min, injector temperature 220°C, detector temperature 250°C, carrier gas N_2 0.8 ml/min. The linear retention indices of the components were determined relatively to the retention times of a series of n-alkanes and the percentage compositions were obtained from electronic integration measurements without taking into account relative response factors.

Gas chromatography/ mass spectrometry

GC/MS analyses were performed using two different mass spectrometers: (i) an Agilent 6890 GC equipped with a DB-Wax fused silica column (30 m × 0.25 mm i.d × 0.25 µm) and interfaced with an Agilent mass selective detector (Model 5973 Network. Palo Alto, CA); column temperature program was 40 to 220°C (3°C/min); injector temperature, 245°C; carrier gas, helium: flow rate, 1.1 ml/min, injection type, split (1µl of sample dissolved in 1ml of diethyl ether); ionization voltage, 70 eV; (ii) a Hewlett-Packard apparatus equipped with a HP1 fused silica column (30 m × 0.25 mm; film thickness 0.25 µm) and interfaced with a quadrupole detector (Model 5970); column temperature program was from 70 to 200 °C (10 °C/min), injector temperature, 220 °C, carrier gas, helium: flow rate, 0.6 ml/min, ionization voltage, 70 eV.

The identification of the constituents was assigned on the basis of comparison of their retention indices and their mass spectra with those given in the literature [28-30] and with the stored laboratory mass spectral library.

Anti-inflammatory activity

Reagents

Soybean lipoxygenase (EC. 1.13.11.12) was purchased from Fluka whereas nordihydroguaiaretic acid (NDGA) (lot 500.38.9) and linoleic acid sodium salt (lot 822-17-3) were obtained from Sigma Aldrich Chemical Co; potassium phosphate buffer 0.1 M, pH 9 was prepared with analytical grade reagents purchased from standard commercial sources. Deionized water was used for the preparation of all solutions.

Determination of lipoxygenase inhibitory activity

Lipoxygenase is known to catalyze the oxidation of unsaturated fatty acids containing 1-4 diene structures. The conversion of linoleic acid to 13-hydroperoxy linoleic acid was followed spectrophotometrically by the appearance of a conjugated diene at 234 nm. NDGA, a known inhibitor of soybean lipoxygenase, was used as a reference drug. The experimental conditions were adapted from those described in literature [31-34].

The reaction was initiated by the addition of aliquots (50 µl) of a soybean lipoxygenase solution (prepared daily in potassium phosphate buffer 0.1 M pH 9.0 in a sufficient concentration to give a measurable initial rate of reaction) to 2.0 ml of a solution of 100 µM sodium linoleate dissolved in phosphate buffer. The enzymatic reactions were performed in absence or in presence of the inhibitor and their kinetics were compared. The inhibitors were dissolved in ethanol in such a manner that an aliquot of each (10 µl) yielded a final concentration of maximum 100 ppm in each assay (the solubility of the essential oil was checked in this range of concentrations by the determination of its specific extinction coefficient).

The initial reaction rate was determined from the slope of the straight line portion of the curve. The calculation of the percentage inhibition of the enzyme activity was based on the comparison between the samples being analyzed and the control sample (which was prepared using 10 µl of ethanol instead of 10 µl of the inhibitor ethanolic solution) using the following equation: I % = 100 x [Vi(c) – Vi(s)] / Vi(c)]. Each inhibitor concentration was tested in triplicate and the results averaged; the concentration that gave 50% inhibition (IC_{50}) was calculated from the outline of the inhibition percentages reported as a function of the inhibitor concentration.

Antioxidant activity - DPPH method

Reagents

1,1-diphenyl-2-picrylhydrazyl [lot 1898-66-4] and rutin hydrate [153-18-4] were purchased from Sigma-Aldrich

Chemical Co; the solutions were prepared with analytical grade solvents purchased from standard commercial sources.

Determination of antioxidant activity

Antioxidant activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to the method described by Mellors and Tappel [35], adapted to essential oil screening [36].

DPPH, a free stable radical scavenger, was dissolved in ethanol at a concentration of 100 µM. To 2.0 ml of the ethanolic solution of DPPH, 100 µL of a methanolic solution of the natural antioxidant reference rutin at different concentrations were added. Rutin was selected as the reference compound according to Gil et al. [37]. The essential oil was tested through the same method. The control, without antioxidant, was prepared by adding 100 µl of pure methanol to the DPPH ethanolic solution. The decrease in absorption was measured at 517 nm after 30 min, at room temperature. The actual decrease in absorption induced by the tested compound was calculated by subtracting that of the control.

Measurements were performed in triplicate and the concentration required for 50 % reduction (50% scavenging concentration SC_{50}) was determined graphically.

All the spectrophotometric measures were performed with a SAFAS UV mc2 spectrophotometer, equipped with a multicells/multikinetics measurement system and with a thermostated cells-case.

Antioxidant activity - ORAC method

Reagents

Randomly methylated β-cyclodextrin (RMCD) was purchased from Cyclolab R & D Ltd (Budapest, Hungary). FL (fluorescein sodium salt), Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-2-carboxylic acid), and rutin hydrate [153-18-4] were purchased from Sigma-Aldrich Chemical Co.APPH (2,2'-Azibis (2-amidino-propane) dihydrochloride was obtained from Wako Chemical USA, Inc.

Sample Preparation

Approximately 20 mg of sample was dissolved in 100 µl of acetone. An aliquot of this solution was appropriately diluted with 0.7% RMCD solution (w/v) made in a 50% acetone-water mixture (v/v), and was shaken for 1 h at room temperature in a shaker adjusted at 400 rpm. The sample solution was ready for analysis after further dilution with 0.7 % RMCD.

Automatic ORAC assay

The procedure was based on previous reports using fluorescein as the fluorescent probe [38] and adapted to lipophilic antioxidants evaluation [39]. With the exception of samples and Trolox standards, which were prepared using 0.7% RMCD as a solvent, all other reagents were prepared in 75 mM phosphate buffer (pH 7.4). For the final assay mixture (2 ml total volume), FL (6.3×10^{-8} M) was used as a target for the free radical attack and APPH (1.28×10^{-2} M) was used as a peroxyl radical generator. 0.7% RMCD was used as the blank and Trolox (0.1-5 µM) was used as the control standard. The analyzer was programmed to record the fluorescence of FL every minute after the addition of APPH. All measurements were performed at 30°C and expressed relatively to the initial reading.

The inhibition effects were evaluated by using the differences between the areas under the FL decay curves (AUC) obtained for the blank and the samples [39].

For pure compounds (rutin), the final relative ORAC values are usually calculated with the following equation (1):

relative ORAC value = $[(AUC_{sample} - AUC_{blank})/ (AUC_{Trolox} - AUC_{blank})] \times$ (molarity of Trolox/molarity of sample) (1)

and expressed as Trolox equivalents (TE) [38].

For mixtures (essential oil and supercritical fluid extract), equation (2) is used instead [40]:

relative ORAC value = $[(AUC_{sample} - AUC_{blank})/ (AUC_{Trolox} - AUC_{blank})] \times$ (molarity of Trolox/concentration of sample) (2)

The ORAC unit is then expressed as micromoles of Trolox per gram of sample (µM TE/g).

For a better comparison purpose, the relative ORAC values of the reference antioxidant rutin and those of our samples (essential oil and SFE) were expressed in the same units: (µM TE/g).

Measurements were performed in duplicate with a fluorescence spectrophotometer Hitachi F-2500 equipped with a thermostated cell holder with stirrer. Excitement and emission wavelengths were 493 and 517 nm, respectively.

Results and discussion

Hydrodistillation of flowering tops of *Aloysia gratissima* collected at two different periods of the year gave essential oils with 1.1 % (November 2005) and 1.2% (May 2006) yields. The CO_2 supercritical extract yield of the same material (November 2005) was about 2%. The relative percentages of the volatile constituents of the three extracts are presented in Table 1.

The results indicate no significant variations of the essential oils compositions according to the harvest period. Both samples contain a majority of monoterpenic compounds with three major components: pinocamphone (13.5-16.3%), β-pinene (10.5-12.0%) and pinocarvyl acetate (7.3-8.3%). Guaiol (6.6-8.7%) and bulnesol (3.7-4.1%) are the main constituents of the sesquiterpenic fraction which represents only 34.0 to 44.3% of the total oil. On another hand, the sesquiterpenes represent 73% of the volatile fraction of the supercritical fluid extract, indicating probably a loss of the more volatile components during the extract process; the other hypothesis might be the necessity of a longer hydrodistillation process to get the totality of the less volatile constituents and a higher sesquiterpenic content in the essential oils.

The chemical compositions described in our work are very different from those previously published on *A. gratissima*. To the best of our knowledge, a high pinocamphone content was never before reported for this species; this monoterpenic ketone was mentioned only once in a Brazilian sample [19]; on another hand, guaiol was identified in an hexane extract of another sample of the

Table 1. Percentage composition of the volatile constituents of essential oils and supercritical CO_2 extract from *Aloysia gratissima* (Gill. & Hook.) Tronc. collected in Brazil.

compounds	RI CP Sil 5	RI CWX 20M	oil 1	oil 2	CO_2 extract
α-thujene	930	-	0.1	0.3	-
α-pinene	939	-	1.2	2.0	-
camphene	970	-	0.2	0.2	-
sabinene	974	-	0.4	0.5	-
β-pinene	982	1100	10.5	12.0	0.3
octen-3-ol	982	1429	0.2	0.2	-
myrcene	986	1145	1.8	2.1	-
p-cymene	1020	1250	0.2	0.2	-
limonene	1030	1180	1.8	2.0	-
1,8-cineole	1030	1200	0.2	0.3	-
(*E*)-β-ocimene	1042	1230	0.4	0.5	-
trans-sabinene hydrate	1064	1440	0.2	0.2	-
terpinolene	1087	1270	0.2	0.2	-
linalool	1090	-	0.8	0.9	0.3
cis-sabinene hydrate	1095	1522	0.4	0.4	-
cis-*p*-menth-2-en-1-ol	1116	-	0.3	0.5	-
α-campholenal	1125	1462	0.1	0.3	-
trans-pinocarveol	1139	1655	2.2	2.9	1.6
nopinone	1143	-	1.1	1.5	0.4
pinocamphone	1154	1482	13.5	16.3	6.3
pinocarvone	1158	-	0.5	0.5	0.4
isopinocamphone	1167	1509	4.4	5.1	2.0
terpinen-4-ol	1172	-	0.2	0.2	0.1
α-terpineol	1180	-	0.2	0.2	0.1
myrtenal	1186	1588	0.8	1.1	0.6
myrtenol	1194	1749	1.1	1.4	1.3
trans-carveol	1211	-	0.2	0.3	0.2
carvone	1231	-	0.2	0.2	0.4
myrtanol	1255	-	0.1	0.1	-
bornyl acetate	1281	1531	0.4	0.5	1.1
pinocarvyl acetate	1291	1608	7.3	8.3	5.7
α-ylangene	1388	-	0.2	0.1	0.3
β-elemene	1395	1522	0.5	0.5	0.9
β-bourbonene	1398	1531	0.4	0.5	0.7
β-caryophyllene	1436	1547	6.8	4.8	9.2
α-humulene	1469	1616	1.8	1.4	2.6
allo-aromadendrene	1477	1643	0.5	0.4	0.3
epi-cubebol	1485	1805	0.2	0.2	-
germacrene D	1494	1678	4.6	3.0	8.6
bicyclogermacrene	1511	1705	2.4	1.9	3.2
cubebol	1526	1882	0.8	0.7	0.9
δ-amorphene	1530	-	0.6	0.7	0.3
elemol	1576	1985	1.5	1.0	1.5
germacrene B	1590	1762	2.4	1.8	5.1
caryophyllene oxide	1598	1915	1.8	1.3	2.4
spathulenol	1600	2060	1.7	1.3	2.4
guaiol	1606	2027	8.7	6.6	16.7
rosifoliol	1621	2163	1.9	1.5	3.2

eudesm-3-en-7-ol	1647	-	1.4	1.1	2.1
thujopsanone-3	1652	-	0.5	0.4	1.1
α-eudesmol	1665	2200	1.1	0.9	3.4
bulnesol	1676	2144	4.1	3.7	6.9
eudesma-4(15),7-dien-1β-ol	1696	-	0.4	0.2	1.2
total identified			**95.2**	**95.0**	**93.8**

Percentages calculated using FID on the CP Sil 5 column
Oil: essential oil from plant harvested in November 2005; Oil 2: essential oil from plant harvested in May 2006; supercritical CO_2 extract of plant harvested in November 2005.

species growing in Brazil [4], along with spathulenol and bisabolol; this last sesquiterpenic alcohol was not found in our samples.

Owing to its high content of pinocamphone and isopinocamphone, the essential oil of this chemical variety of *A. gratissima* from Brazil may be compared to that of *Hyssopus officinalis*, which is much used in flavour work and in perfumery [41]. Nevertheless, these two components are cited as the toxic components of the hyssop oil, which was found to possess a convulsant action on rats [42]. Once more, the necessity of an accurate knowledge of the chemical variety of the plant before using it in aromatherapy is demonstrated.

The potential anti-inflammatory activities of the essential oil1 and the supercritical fluid extract obtained from the same plant material were evaluated comparatively to that of NDGA. No significant inhibitory effect on the soybean lipoxygenase could be observed in the range concentration explored (4-20 g/L)

The antioxidant activities of the same samples were compared to that of a natural reference compound, rutin, using two different methods (DPPH and ORAC). The corresponding results are given in Table 2; they indicate an efficiency of the same magnitude for the essential oil and the supercritical fluid extract: the fact that the SC_{50} of the essential oil could not even be measured in our experimental conditions (SC_{40} = 1.5 g/L) indicated that its antioxidant activity was weaker than that of the extract (SC_{50} = 1.3 g/L) according to the DPPH method; the same was observed with the second method, the supercritical fluid extract being slightly more active than the essential oil (respectively 225 and 203 µM TE/g extract).

In both cases, the activities of the *A. gratissima* extracts are weaker than those of rutin. The antioxidant activity of rutin as determined by the DPPH method was approximately 325 and 375 folds larger than the SFE extract and the essential oil, respectively. By the ORAC method it was determined that the antioxidant activity of rutin was approximately 2.6 and 3 folds larger than the SFE extract and the essential oil, respectively.

The same order of magnitude of reactivity had been obtained in previous studies with the two methods for another antioxidant taken usually as a reference compound, t-butyl hydroxy toluene (BHT): SC_{50} = 8.8 mg/L using the DPPH method [43] and 0.16 TE (µmoles Trolox equivalent) [40] which corresponds to 727 µM TE/g_{BHT} according the ORAC method.

In conclusion, this variability in the reactivity highlights the incidence of the tests used in vitro in the biological investigations and the need for remaining cautious in their interpretation. Furthermore, in this case, there is no evidence for selecting the extraction method in relation to anti-inflammatory or antioxidant applications.

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Table 2. Antioxidant activities of a reference compound (Rutin) and *Aloysia gratissima* extracts.

DPPH method	ORAC method
$SC_{50\ (rutin)}$ (g/L) 0.0040 ± 0.0003	$ORAC_{rutin}$ (µM TE/g_{rutin}) 587 ± 48
$SC_{50\ (SFE)}$ (g/L) 1.3 ± 0.1	$ORAC_{SFE}$ (µM TE/g_{SFE}) 225 ± 18
$SC_{40\ (oil\ 1)}$ (g/L) 1.5 ± 0.2	$ORAC_{oil\ 1}$ (µM TE/$g_{oil\ 1}$) 203 ± 15

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