E3 Journal of Biotechnology and Pharmaceutical Research Vol. 3(1), pp. 1-9, March 2012
Available online at http://www.e3journals.org/JBPR
ISSN 2141-7474 © 2012 E3 Journals

Full Length Research Paper

Determination of chemical and physical properties of *Hyptis pectinata* essential oil and their redox active profile

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Accepted 20 December, 2011

The aim of this study was therefore, to evaluate the redox properties of essential oil from *H. pectinata* (OEHP). TRAP/TAR assay indicated that OEHP is an antioxidant at 1 mg.mL^{-1}. However, OEHP was not effective in preventing lipoperoxidation in vitro. Furthermore, OEHP reduced deoxyribose oxidative damage by hydroxyl radicals at 1 mg.mL^{-1}, but enhanced hydroxyl production from 1 ng.mL^{-1} to 10 µg.mL^{-1}. Nitric oxide radical production was inhibited by OEHP at 1 µg.mL^{-1} to 1mg.mL^{-1}. OEHP did not show any ability to scavenge superoxide radicals and hydrogen peroxide. The results of this study show that OEHP may act as an antioxidant or pro-oxidant depending on its concentration and which species they interact with. Thus, OEHP use for medicinal purposes must be taken with restrictions, and more assays to better determine its safety and biological effects are necessary.

Key words: *H. pectinata*, redox profile, sesquiterpenes.

INTRODUCTION

Medicinal plants play an important role from both commercial and healthy-lifestyle perspectives. Towards the end of the twentieth century, the World Health Organization (WHO) estimated that an impressive 80% of the world's population probably rely mainly on natural medicines, with plant-originated medicines as the main component of this trend (in developed countries) or tradition (in developing countries) (Aquino et al., 2010).

The species *Hyptis pectinata* (L.) Poit, belonging to the Lamiaceae family and known in Brazil as "sambacaitá" or "canudinho," is used as a medicinal tea (infusion or decoction) for treating skin diseases, gastric disorders, nasopharyngitis, nasal congestion, fever and other infections caused by bacteria and fungi (Aquino et al., 2010; Nascimento et al., 2008). In Brazil, *Hyptis pectinata* is extensively used by the local population for the treatment of inflammation, bacterial infections, pain, and cancer (Bispo et al., 2001).

Volatile oils from a broad spectrum of plant species have shown antinociceptive, anti-inflammatory, antimicrobial, antiviral, antitumoral and antioxidant activities (Rufino et al., 2010). Some constituents from oils can act as pro-oxidants (such as free fatty acids and hydroperoxides) or as antioxidants, including tocopherols, phenols, and possibly phospholipids together with other components.

Free radicals were a major interest for early physicists and radiologists and much later were found to be a product of normal metabolism (Tepe et al., 2007). Today,

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we have well known that radicals cause molecular transformations and gene mutations in many types of organisms. Oxidative stress is well-known to cause many diseases, and scientists, in many different disciplines, have become more interested in natural sources, which could provide active components to prevent or reduce its impact on cells (Yan et al., 2002).

Given the lack of experimental data on the pharmacological and medicinal properties of *H. pectinata,* the objective of the present work was therefore, to evaluate the chemical composition and redox active properties of the volatile oils from *H. pectinata*.

MATERIALS AND METHODS

Cultivation of *H. pectinata* was undertaken at the Federal University of Sergipe (UFS) Experimental Farm, São Cristóvão, Sergipe, Brazil [10º18' 20.7" (S); 36º 39' 7.2" (W) and 120 m above sea level]. A reference specimen was deposited at the UFS herbarium (registry number 11126).

Essential oil extraction

The essential oil of dried leaves from *H. pectinata* (OEHP) was obtained by hydrodistillation on a Clevenger-type apparatus for approximately 3 h, until no more condensing oil could be seen. The essential oil was separated by extraction with ethyl acetate from the aqueous solution (hydrolate), dried with anhydrous Na_2SO_4 (yield 0.5% v/w), transferred into an amber glass flask, and kept at a temperature of 10°C until used.

Chemical and physical properties

Gas chromatography-mass spectrometry (GC-MS) and gas chromatography (GC-FID)

Oil sample was analyzed in a Shimadzu GC-MS QP 5050A (Shimadzu Corporation, Kyoto, Japan) gas chromatograph mass spectrometer, system equipped with an AOC-20i autosampler under the following conditions: J&W Scientific DB-5MS fused silica capillary column (30 m x 0.25 mm i.d., x 0.25 μm film thickness, composed of 5%-phenyl-95%-methylpolysiloxane). Helium (99.999%) was used as the carrier gas at a constant flow of 1.2 mL/min. The injection volume was 0.5 µL in ethyl acetate (split ratio of 1:100), the injector temperature 250°C and the ion-source temperature 280°C.min. The oven temperature was programmed from 50°C (isothermal for 1.5 min), with an increase of 4°C/min to 200°C, then 10°C/min to 300°C, ending with a 10 min isothermal period at 300°C. Mass spectra were taken at 70 eV with a scan interval of 0.5 s and fragments from 40 to 500 Da.

Quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (FID), using a Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan) instrument, under the following operational conditions: capillary ZB-5M5 column (5%-phenyl -95%- methylpolysiloxane fused silica capillary column 30 m x 0.25 mm i.d. x 0.25 μm film thickness), under the same conditions reported for the CG-MS. Concentrations of the respective constituents were calculated using the area of the signal in the GC spectra according to the order of elution.

Identification of individual components of the essential oils was performed by computerized matching of the acquired MS with those stored in NIST21 and NIST107 spectral library of the GC/MS data system and authentic standards. Relative retention indices (RRI) for all compounds were determined according to literature (Dool and Kratz, 1963) for each constituent, as previously described (Adams, 2007).

Thermal analysis

DSC curves were obtained in a DSC-50 cell (Shimadzu) using aluminium crucibles with about 2 mg of samples, under dynamic N_2 atmosphere (50 $mL.min^{-1}$) and heating rate of 10ºC.min^{-1} in the temperature range from 25 to 600ºC. The DSC cell was calibrated with indium (m.p. 156.6ºC; $\Delta H_{fus.}$ = 28.54 $J.g^{-1}$) and zinc (m.p. 419.6ºC). TG/DTG curves were obtained with a thermobalance model TGA 50 (Shimadzu) in the temperature range 25-900ºC, using platinum crucibles with ~3 mg of samples, under dynamic N_2 atmosphere (50 $mL.min^{-1}$) and heating rate of 10ºC.min^{-1}.

Redox active profile

Total antioxidant potential (TRAP) and total antioxidant reactivity (TAR)

TRAP/TAR was determined by measuring the chemiluminescence (CL) intensity of luminol induced by 2,2′-azobis(2-amidinopropan) dihydrochloride (AAPH) (Lissi et al., 1992). The background CL was measured by adding of AAPH and luminol. Then, the samples (OEHP from 1 $ng.mL^{-1}$ to 1 $mg.mL^{-1}$) were added, and the CL was measured in a liquid scintillator counter. The last count before the addition samples was considered as 100%. Graphs were obtained by plotting percentage of counts per minute (% cpm) versus time (s). The AUC (TRAP assay) was calculated using GraphPad Prism software. The TAR was calculated as the ratio of light intensity in absence of samples (I_0)/light intensity right after OEHP addition (I).

TBARS (thiobarbituric acid reactive species)

TBARS assay was employed to quantify lipid peroxidation (Draper and Hadley, 1990) and an adapted TBARS method was used to measure the antioxidant capacity of OEHP using egg yolk homogenate as lipid rich substrate (Silva et al., 2007) Briefly, egg yolk was homogenized (1% w/v) in phosphate buffer (pH 7.4), 1 mL of homogenate was sonicated and then homogenized with 0.1 mL of OEHP at different concentrations. Lipid peroxidation was induced by addition of 0.1 mL of AAPH solution (0.12 M). Samples were centrifuged with trichloroacetic acid at 1200 g for 10 min. An aliquot of 0.5 mL from the supernatant was mixed with 0.5 mL TBA and heated at 95ºC for 30 min. After cooling, sample's absorbance was measured using a spectrophotometer at 532 nm. The results were expressed as a percentage of TBARS formed by AAPH alone (induced control).

Hydroxyl radical (˙OH) scavenging assay

Hydroxyl radicals were generated by a Fenton system ($FeSO_4$-H_2O_2). When exposed to hydroxyl radicals, the sugar deoxyribose is degraded to malonaldehyde (MDA), which generates a pink chromogen on heating with TBA at low pH. The method for determining the scavenging on hydroxyl radicals was performed according to a previously described procedure (Payá et al., 1992).

Nitric oxide ($NO^{\cdot}$) scavenging assay

Nitric oxide was generated from spontaneous decomposition of

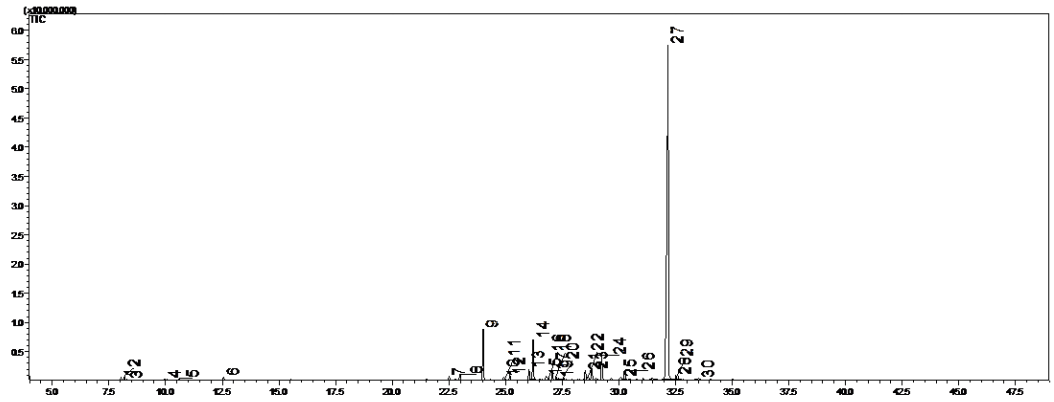

Figure 1. Total ion current (TIC) chromatogram of essential oil from *H. pectinata*. Numbers refer to compounds listed in Table 1.

sodium nitroprusside (SNP) in the phosphate buffer (pH 7.4). Once generated NO interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction (Basu and Hazra, 2006). The reaction mixture containing SNP in phosphate buffer and OEHP at different concentrations were incubated at 37ºC for 1 h. An aliquot was taken and homogenized with Griess reagent. The absorbance of chromophore was measured at 540 nm. Percent inhibition of nitric oxide generated was measured by comparing the absorbance values of negative controls (SNP and vehicle) and assay preparations.

Superoxide and hydrogen peroxide-scavenging activities (SOD/CAT-like activities)

The ability of OEHP to mimic the activity of superoxide dismutase (SOD-like activity) or to mimic the activity of catalase (CAT-like activity) was measured. CAT-like activity was assayed by measuring the rate of decrease in hydrogen peroxide (H_2O_2) absorbance at 240 nm (Aebi, 1984) and SOD-like activity was assayed by measuring spectrophotometrically by the inhibition of adrenaline auto oxidation at 480 nm, as previously described (Bannister and Calabrese, 1987).

Statistical analysis

Data are expressed as mean ± S.E.M. The obtained data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's test. All tests were performed in triplicate. Data analyses were performed using the GraphPad Prism 5.0 software. Differences were considered significant if $p < 0.05$.

RESULTS

Chemical and physical properties

Compounds from *H. pectinata* were previously identified (Figure 1), and only the major constituents are listed in Table 1. The identified compounds were sesquiterpenoids with high contents of calamusenone (62.61%), β-caryophyllene (8.45%), cis-β-guainene (5.67%), and caryophyllene oxide (3.63%). The chemical profiles of the essential oils of *H. pectinata* of São Cristóvão, Sergipe, differ from those published for other places (Arrigoni-Blank et al., 2008; Santos et al., 2008; Rohr et al., 1979).

The DSC and TG/DTG curves of OEHP are shown in Figure 2. By their data analysis, it can be seen that the major fraction (94.56%) of the compound evaporates up to 220°C. The DSC curve corroborates with TG analysis, showing an endothermic peak at 164.5°C attributed to the OEHP volatilization. The second mass loss step observed in the TG curve occurs from 220 to 450°C, presenting an exothermal DSC peak due to the burnout of the residual carbonaceous material of the previous steps.

Redox active profile

The TRAP and TAR assays indicate that OEHP presents a significant antioxidant activity at the highest dose tested (Figure 3A). At 1 $ng.mL^{-1}$ to 100 $\mu g.mL^{-1}$, OEHP showed the pro-oxidant profile (TRAP assay- Figure 3B). On the other hand, all doses of OEHP were not able to prevent lipoperoxidation induced by AAPH *in vitro* in a lipid-enriched system (Figure 4).

The OEHP at 1 $ng.mL^{-1}$ to 10 $\mu g.mL^{-1}$ enhanced hydroxyl radical production. On the other hand, at 1 $mg.mL^{-1}$ OEHP showed antioxidant activity against hydroxyl radicals (Figure 5). To determine the ability of OEHP to act as a reactive nitrogen species (RNS) scavenger, we evaluated the NO-scavenging activity by the Griess method and OEHP at 1 $\mu g.mL^{-1}$ until 1 mg.mL-1 showed a significant ($p<0.05$) NO-scavenging activity (Figure 6).

We also evaluated the ability of OEHP to act as the enzymes superoxide dismutase (SOD) and catalase (CAT), which act on superoxide radicals and hydrogen peroxide, respectively. Our results show that OEHP is not able to exert SOD- or CAT-like activity (Figure 7 and 8) at

Table 1. Chemical compounds present in *H. pectinata* essential oil.

Peak	RT (mim)	Compound	GC-FID (%)	RRI exp	Methods of identification
1	8.033	Sabinene	0.35	970	a, b, c
2	8.192	β-Pinene	0.45	976	a, b, c, d
3	8.283	1-Octen-3-ol	0.10	978	a, b, c
4	9.967	Limonene	0.18	1028	a, b, c, d
5	10.600	β-(E)-Ocimene	0.24	1045	a, b, c
6	12.567	Linalool	0.43	1099	a, b, c, d
7	22.517	α-Copaene	0.58	1374	a, b, c
8	22.992	β-Elemene	0.78	1388	a, b, c
9	24.008	β-Caryophyllene	7.03	1418	a, b, c, d
10	24.925	Aromadendrene	0.53	1446	a, b, c
11	24.983	*cis*-Muurola-3,5-diene	0.24	1448	a, b, c
12	25.200	α-Humulene	0.74	1454	a, b, c
13	26.033	Germacrene D	1.43	1480	a, b, c, d
14	26.208	*cis*-β-Guaiene	5.14	1485	a, b, c, d
15	26.758	γ-Patchoulene	0.92	1502	a, b, c
16	26.900	Germacrene A	0.16	1506	a, b, c
17	27.075	γ-Cadinene	1.29	1512	a, b, c
18	27.233	δ-Cadinene	0.42	1517	a, b, c
19	27.317	trans-Calamenene	0.14	1520	a, b, c
20	28.517	Germacrene B	1.58	1558	a, b, c
21	29.242	Caryophyllene oxide	3.58	1581	a, b, c, d
23	30.258	1,10-di-epi-Cubenol	1.30	1614	a, b, c
24	32.142	Calamusenone	67.84	1678	a, b, c, d
25	32.508	Cyperotundone	0.50	1690	a, b, c

RRI: Relative retention index calculated against *n*-alkanes (C8-C18) applying the Van den Dool and Kratz equation (X). Methods of Identifications: a- MS data compared to spectral database (NIST 21 and NIST107), b- published mass spectra (Adarns, 2007), c- relative retention index (RRI), d- Co-GC with authentic standard.

any concentration tested ($p<0.05$).

DISCUSSION

According to Stephenson (1956), all drugs exert a dose-dependent effect on their targets. However, this process depends on the amount of available receptors and the physical and chemical properties of the substances. This theory is applied to pharmacology. On the other hand, phytotherapy is not fully understood. It is possible that, at some concentrations, certain compounds of a plant extract, or oil, could act in synergism and cause a significant effect. At lower or higher concentrations, these compounds could be antagonized by different ones (Melo et al., 2005).

Consumption of foods and plants rich in natural antioxidants has been reported as being protective of certain types of cancer and may also reduce the risk of cardiovascular and cerebrovascular events. These actions of antioxidants have been attributed to their ability to scavenge free radicals, thereby reducing oxidative damage of cellular biomolecules such as lipids, proteins, and nucleic acids (Miraliakbari and Shahidi, 2008; Aruoma, 1998). Prevention of cardiac diseases, cancer, and neurodegenerative conditions observed in epidemiologic studies is ascribed to the presence of secondary metabolites in such diets and/or supplementations (Quintans-Jr et al., 2011).

The hydroxyl radical was inhibited by OEHP at 1

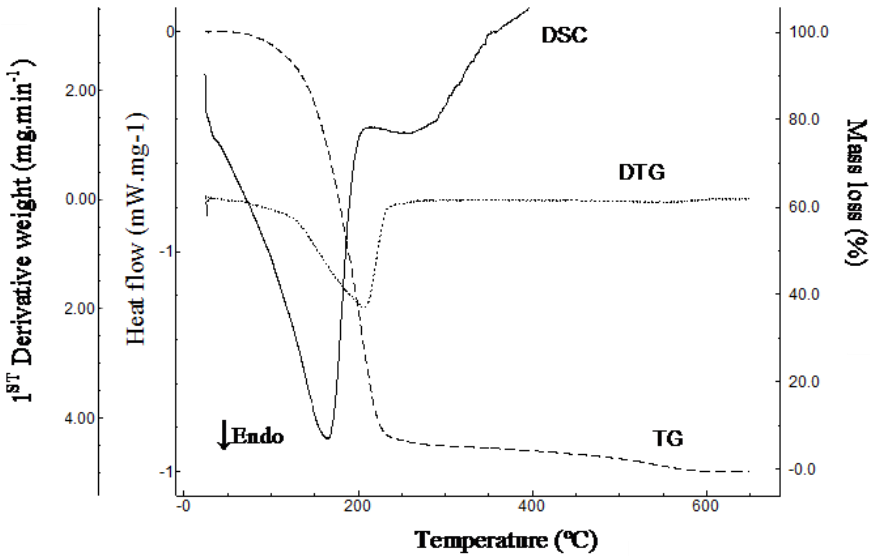

Figure 2. DSC and TG/DTG curves of OEHP in dynamic nitrogen atmosphere (50 mL.min^{-1}) and heating rate 10ºC.min^{-1}from *H. fruticosa* essential oil.

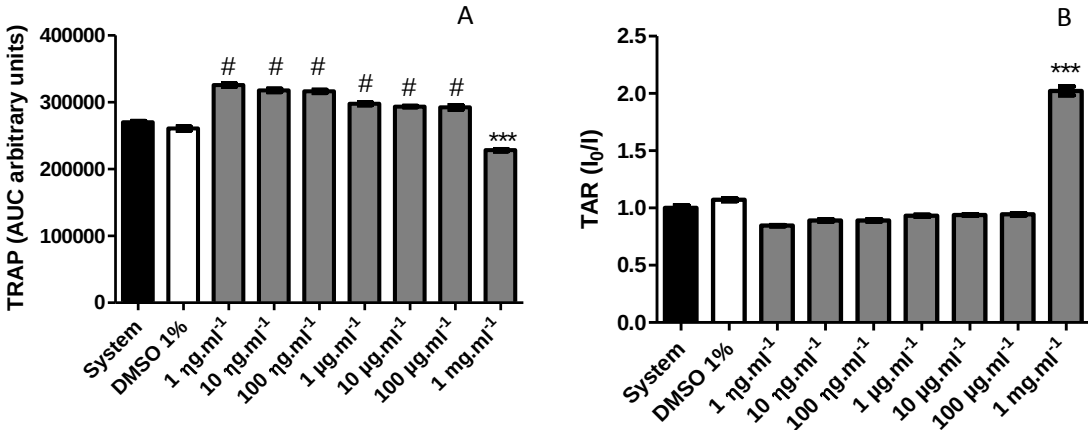

Figure 3. A) Total reactive antioxidant potential (TRAP) at different concentrations. B) Total antioxidant reactivity (TAR) was calculated as the ratio of light intensity in absence of samples expressed as percent of inhibition (I_0/I).Values represent mean ± S.E.D., experiments in triplicate, ANOVA followed by Tukey # p<0.001 (pro-oxidant) and ***p<0.001 (anti-oxidant) different from system (AAPH).

mg.mL^{-1}. This radical is an extremely reactive species formed in biological systems and has been implicated as a highly damaging species in free radical pathology. It has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis, and cytotoxicity (Serafini et al., 2011). The OEHP was, yet, able to scavenge reactive nitrogen species. Nitric oxide plays an important role in various types of inflammatory processes. It is produced by macrophages during the inflammatory response. This fact can explain the common use of *H. pectinata* in popular medicine.

It is probable that the active principle responsible by the redox active profile described in this work, is the sesquiterpenes (majority compost). In many cases, the antioxidant activity of essential oils cannot be attributed to the major compounds as minor compounds are likely to play a significant role in the activity, and synergistic effects being also reported (Candan et al., 2003; Mukazayire et al., 2011; Berger, 2007). However, the sesquiterpenes were related by other researchers (Barla et al., 2007; McBrien et al., 1995; Hibasami et al., 2003) to be responsible for the observed antitumor activity, showing strong growth inhibitory effect against human promyelotic leukemia (HL-60) cells and apoptosis.

Six known sesquiterpene lactones were found to be

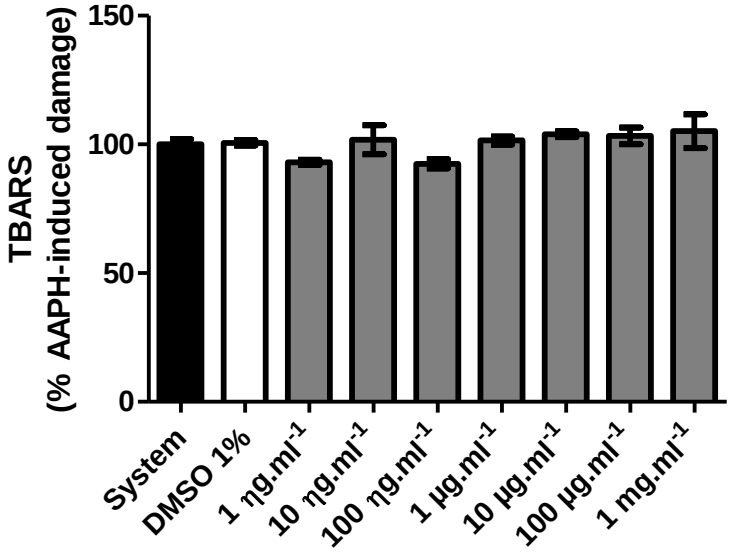

Figure 4. Thiobarbituric Acid Reactive Species (TBARS) was evaluated from *H. fruticosa* essential oil (1ηg.ml^{-1} – 1 mg.ml^{-1}) Values represent mean ± S.E.D., experiments in triplicate, ANOVA followed by Tukey.

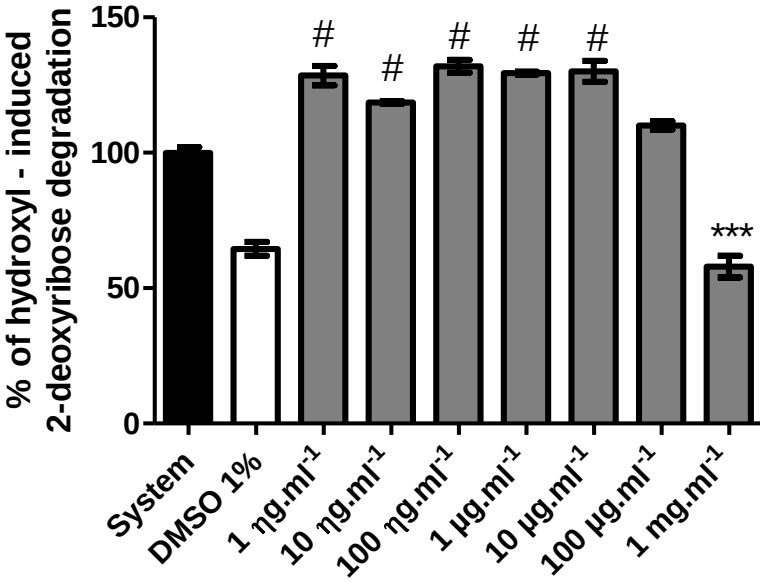

Figure 5. Hydroxyl radical-scavenging activity from *H. fruticosa* essential oil (1ηg.ml^{-1} – 1 mg.ml^{-1}). Values represent mean ± S.E.D., experiments in triplicate, ANOVA followed by Tukey # p<0.001 (pro-oxidant) and ***p<0.001 (anti-oxidant) different from system.

highly cytotoxic against the A2780 ovarian cancer cell line (Barla et al., 2007). It has been known that sesquiterpenes are essential for cytotoxicity against cancer cell line and antitumor activity. Our results also

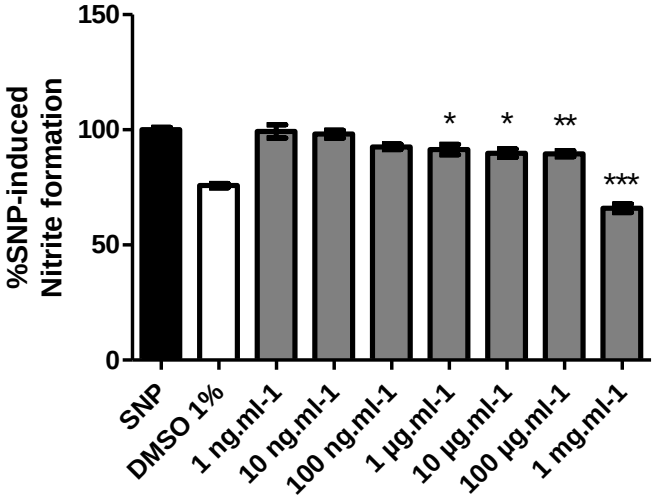

Figure 6. Nitric oxide (NO) scavenging activity from *H. fruticosa* essential oil (1ηg.ml^{-1} – 1 mg.ml^{-1}). Values represent mean ± S.E.D., experiments in triplicate, ANOVA followed by Tukey *p<0.05; **p<0.01 and ***p<0.001 different from system (SNP).

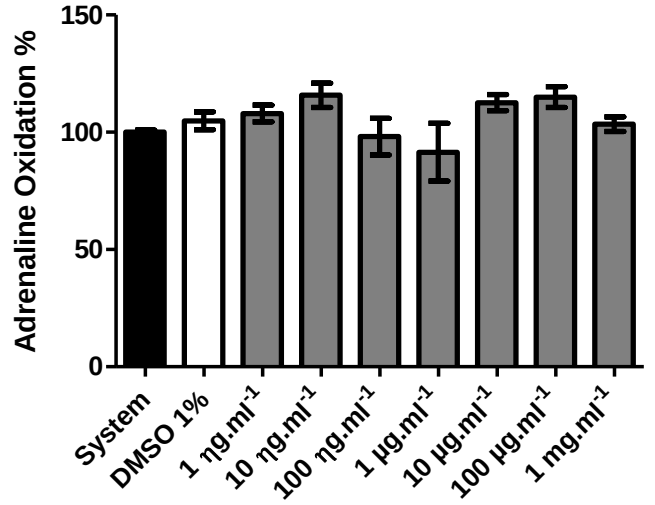

Figure 7. Determination of superoxide dismutase-like activity (SOD) from *H. fruticosa* essential oil (1ηg.ml^{-1} – 1 mg.ml^{-1}). Values represent mean ± S.E.D., experiments in triplicate, ANOVA followed by Tukey.

support this finding. Thus, sesquiterpene-containing plants might be an important source for the development of new therapeutics.

The antioxidant properties *H. pectinata* essential oils and documented use records of the herbs make them candidates for natural antioxidants that could be used as food supplements and for potential pharmaceutical applications. The investigated essential oils, *per se* or as sources of selected active constituents, may be an interesting alternative to synthetic antioxidants as

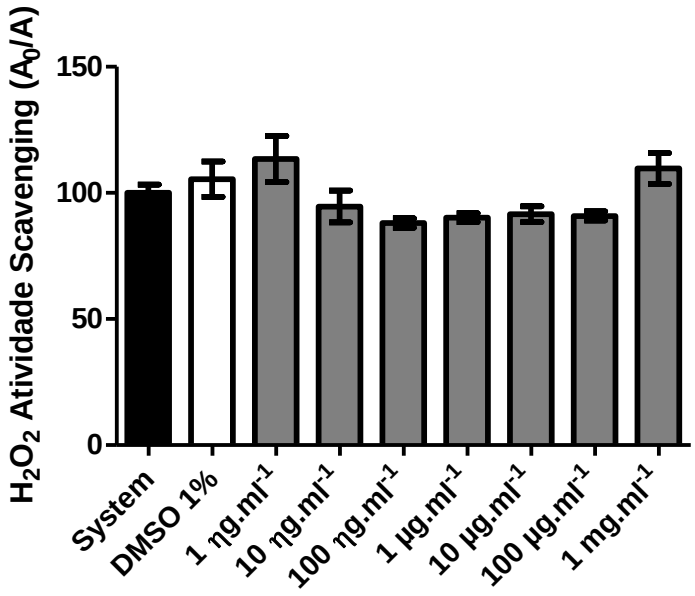

Figure 8. Determination of catalase-like activity (CAT) from *H. fruticosa* essential oil (1ηg.ml^{-1} – 1 mg.ml^{-1}). Values represent mean ± S.E.D., experiments in triplicate, ANOVA followed by Tukey.

additives for food, pharmaceutics and cosmetics preservation.

Conclusion

The results of this study show that OEHP can be of use as a source of natural antioxidants and this effect is probable attributed to the sesquiterpenes (majority compost of this oil). However, results clearly show that the oxidative properties of OEHP are greatly affected by type of assays used. Thus, it is suggested that further work could be done to elucidate this chemical mechanism.

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