

Preliminary study on the anti-inflammatory and antioxidant activities of the leave extract of *Hyptis fruticosa* Salzm. ex Benth., Lamiaceae

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RESUMO: “Avaliação das atividades anti-inflamatória e antioxidante do extrato etanólico das folhas da *Hyptis fruticosa* Salzm. ex Benth., Lamiaceae”. Várias espécies do gênero *Hyptis* são utilizadas na medicina popular para tratar processos inflamatórios. Para avaliar as ações anti-inflamatória e antioxidante da *Hyptis fruticosa* Salzm. ex Benth., Lamiaceae, utilizou-se extrato etanólico (EE) das folhas desta planta e suas partições *n*-C₆H₁₄, CHCl₃, AcOEt e MeOH/H₂O. O tratamento oral com o EE das folhas da *H. fruticosa* (100, 200 e 400 mg/kg) ou suas partições *n*-C₆H₁₄, AcOEt e MeOH/H₂O (50, 100 e 200 mg/kg) apresentou atividade inibitória sobre a formação de edema e migração leucocitária para a cavidade peritoneal induzidas pela carragenina em ratos (*p*<0,05). Entretanto, a partição CHCl₃ não causou nenhum efeito sobre a formação de edema e migração de células peritoneais. O EE bruto e a partição AcOEt apresentaram alto potencial antioxidante (IC₅₀ = 35,00±1,01 e 36,67±2,65 µg/mL DPPH, respectivamente), similar ao composto referência (IC₅₀ = 16,67±1,21 µg/mL). Em conclusão, demonstrou-se que a *H. fruticosa* apresenta atividades anti-inflamatória e antioxidante.

Unitermos: Anti-inflamatória, antioxidante, edema, *Hyptis fruticosa*, Lamiaceae, migração celular.

ABSTRACT: Various species of *Hyptis* are used in folk medicine as anti-inflammatory. In order to evaluate the actions of *Hyptis fruticosa* Salzm. ex Benth., Lamiaceae, studies were performed on anti-inflammatory and antioxidant activities. The ethanol extract (EE) of *H. fruticosa* leaves and its *n*-C₆H₁₄, CHCl₃, EtOAc, and MeOH/H₂O partitions were used in the following experiments. Oral treatment with the EE of *H. fruticosa* leaves (100, 200, and 400 mg/kg) or its *n*-C₆H₁₄, EtOAc, and MeOH/H₂O partitions (50, 100, and 200 mg/kg) elicited inhibitory activity (*p*<0.05) on carrageenan-induced oedema formation and leukocyte migration into the peritoneal cavity in rats. However, the CHCl₃ partition did not show any inhibitory effect on paw oedema and peritonitis experimental models. The EE and EtOAc partition present the highest antioxidant potential (IC₅₀ = 35.00±1.01 and 36.67±2.65 µg/mL DPPH, respectively), similar to the reference compound (IC₅₀ = 16.67±1.21 µg/mL). In conclusion, *H. fruticosa* shows anti-inflammatory and antioxidant activities.

Keywords: Anti-inflammatory, antioxidant, cellular migration, *Hyptis fruticosa*, Lamiaceae, oedema.

INTRODUCTION

The use of natural products with therapeutic properties is as ancient as human civilization (Esquenazi

et al., 2002). Most people living in developing countries are almost completely dependent on traditional medical practices for their primary health care needs and higher plants are known to be the main source for drug therapy

in traditional medicine (Calixto, 2005).

The genus *Hyptis* contains about 400 species distributed mainly at the central states of Brazil (Harley, 1988). Various species are used in folk medicine as anti-inflammatory, antinociceptive, anticonvulsant, and antiulcerogenic (Barbosa & Ramos, 1992; Akah & Nwambie, 1993; Kuhnt et al., 1995; Bispo et al., 2001). The aqueous extract and volatile oil of *Hyptis pectinata* showed antiedematogenic and antinociceptive activities in animal models (Bispo et al., 2001; Arrigoni-Blank et al., 2008).

Hyptis fruticosa Salzm. ex Benth., Lamiaceae, popularly known as "alecrim-de-tabuleiro" is an aromatic sub-bush plant which grows up to 1.5 m found on the Brazilian northeast coast. Previous studies have demonstrated that the essential oil from *H. fruticosa* presents antinociceptive peripheral and hypotensive activities (Menezes et al., 2007; Santos et al., 2007). Silva et al. (2006) have demonstrated that the aqueous extract from *H. fruticosa* presents antinociceptive activity.

Although *H. fruticosa* is popularly described as an anti-inflammatory, the potential *in vivo* anti-inflammatory activity of the extracts from *H. fruticosa* leaves has not been investigated. The goal of the present study was to evaluate the anti-inflammatory and antioxidant effects of the crude ethanol extract (EE) and various solvent partitions from *Hyptis fruticosa* leaves.

MATERIALS AND METHODS

Plant material, extraction of *Hyptis fruticosa* leaves and partitioning

Aerial parts of *Hyptis fruticosa* Salzm. ex Benth., Lamiaceae, were collected in the municipality of São Cristóvão, Sergipe State, Brazil, in January 2007 (11°01'47"S, 37°20'64"W). The plant was authenticated by Professor Clovis R. Franco, Department of Biology, Federal University of Sergipe, and a voucher specimen deposited in the Federal University of Sergipe Herbarium (voucher number ASE 10.922). The dried leaves (1300 g) of *H. fruticosa* at 40 °C in a forced air oven for 48 h were powdered and submitted to extraction at ambient temperature (25-28 °C) with 90% ethanol (8 L) for five days. After filtration, the solvent was removed with rotary evaporator under reduced pressure (45 °C). The percentage of yield of the leaves EE was 15.0% (195.0 g). The components of the crude EE were partitioned in a Soxhlet apparatus sequentially with solvents of increasing polarity. The crude EE (150.0 g) was suspended in a mixture of water:methanol (3:2, v/v) and extracted with hexane ($n\text{-C}_6\text{H}_{14}$, 4x 250 mL), chloroform (CHCl_3 , 4x 250 mL), and ethyl acetate (EtOAc, 4x 250 mL), successively. After the solvent removal under reduced pressure on a rotatory evaporator, hexane (9.4 g), chloroform (26.2 g), ethyl acetate (38.3 g), and hydromethanolic (MeOH/H₂O,

45.3 g) partitions were obtained.

Phytochemical screening

The methods of Harbone (1984) were used to screen the EE of *H. fruticosa* leaves used in this study for its chemical constituents.

Chemicals and drugs

The following chemicals and drugs were used: acetylsalicylic acid (ASA), carrageenan, dexamethasone, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and (-)-epigallocatechin from Sigma Chemical Co. (St. Louis, MO, USA). Solvents from Vetec (Rio de Janeiro, RJ, Brazil). All substances used were dissolved in 0.2% Tween 80 in 0.9% NaCl solution, with the exceptions of DPPH and (-)-epigallocatechin that were dissolved in methanol. The final concentration of Tween 80 did not exceed 0.2% and did not cause any effect *per se*.

Animals

Wistar rats (120-180 g) of both sexes were obtained from the Central Biotery of the Federal University of Sergipe (São Cristóvão, Brazil). Animals were maintained in plastic boxes at controlled room temperature (21±2 °C) with free access to food and water, under a 12:12 h light/dark cycle. All the experimental procedures were carried out during the light period of the day (8 a.m. to 5 p.m.) and complied with the guidelines on animal care of the Federal University of Sergipe Ethics Committee for Animal Use in Research (CEPA/UFS 25/07).

Measurement of paw oedema

The anti-inflammatory activity was evaluated using the paw oedema model induced by 1% carrageenan, administrated at volume of 0.1 mL/animal into the subplantar region of the rat right hindpaw (Winter et al., 1962). Paw oedema was measured plethysmographically (model 7150, Ugo Basile, Varese, Italy), at the time 0 and the intervals of 1, 2, 3, and 4 h immediately after the subplantar injection of carrageenan.

The EE of *H. fruticosa* leaves (100-400 mg/kg), its partitions (50-200 mg/kg), acetylsalicylic acid (ASA, 300 mg/kg), or vehicle (5 mL/kg) were administrated orally (*p.o.*) 1 h before the oedematogenic agent ($n = 6$ /group). Data obtained for the various groups were reported as means±s.e.m. and expressed in mL. The percentage inhibition in oedema experiment was calculated based on the area under the time-course curves ($\text{AUC}_{0-4\text{h}}$) using trapezoidal rule.

Leukocyte migration into the peritoneal cavity

The leukocyte migration was induced by intraperitoneal (*i.p.*) injection of carrageenan (500 µg/cavity, 500 µL) into the peritoneal cavity of rats 1 h after administration of the EE of *H. fruticosa* leaves (100-400 mg/kg, *p.o.*, n=6), its partitions (50-200 mg/kg, *p.o.*, n=6), dexamethasone (2 mg/kg, *s.c.*, n=6), or vehicle as previously described by Thomazzi et al. (2010). The animals were anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*) and were euthanized by cervical dislocation 4 h after carrageenan injection. Shortly after, saline containing EDTA (1 mM, *i.p.*, 10 mL) was injected into the peritoneal cavity. The total cells were counted in a Neubauer chamber, under optic microscopy. The results were expressed as the number of leukocytes/mL. The inhibition percentage of the leukocyte migration was calculated according the equation: $(1 - T/C) \times 100$, where *T* and *C* represent the treated and control group leukocyte counts, respectively.

Quantitative assay of antioxidant activity

The quantitative analysis of antioxidant activity was based on the method described by Brand-Williams et al. (1995), with minor modifications. The scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was followed by monitoring the decrease in absorbance at 515 nm, which occurred due to reduction by the antioxidant.

The calibration curve was established by preparing dilutions of a DPPH radical stock solution (40 µg/mL) to obtain final concentrations of 1, 5, 10, 15, 20, 25, 30, and 35 µg/mL. The absorbance of each standard concentration was then monitored in a spectrophotometer (UV BEL Photonics 1105) at 515 nm. The measures were carried out in triplicate with intervals of 1 min. The equation of the concentration x absorbance calibration curve for the DPPH radical was $C=110.547-0.02804A$, where *C* is the concentration of the DPPH radical in medium, *A* is the absorbance at 515 nm. The correlation coefficient was $r=0.9983$.

Solutions containing 500 µg/mL of *Hyptis fruticosa* leaves EE and its partitions were prepared in methanol, and diluted in concentrations of 1, 5, 10, 15, 20, 25, 30, and 35 µg/mL. The disappearance of DPPH radical was monitored by the decrease in absorbance at 515 nm, which was recorded after 0, 1, 5, and 10 min, and subsequently every 10 min up to 1 h. The negative control was pure methanol used for dissolving the samples, while the positive control was the (-)-epigallocatechin dissolved in methanol in concentrations of 1, 5, 10, 15, 20, 25, 30, and 35 µg/mL. The mixture of methanol and sample was used as blank.

The concentration of the DPPH radical in the reaction mixture was calculated based on calibration curve, where [DPPH] is expressed in µg/mL. The percentage of

remaining DPPH (%DPPH_{REM}) was calculated as follows: $\%DPPH_{REM} = [DPPH]_T/[DPPH]_{T_0} \times 100$, where *T* is the time when absorbance was determined (1-60 min) and *T*₀ is the time zero. The amount of antioxidant necessary to decrease the initial concentration of DPPH radical by 50% (IC50) was calculated by plotting the %DPPH_{REM} at time of 50 min (25 µg/mL). The results were expressed as µg antioxidant/mL DPPH±standard deviation.

Statistical analysis

The results of anti-inflammatory activity are presented as the mean±s.e.m. of *n* animals per group. The values of antioxidant effect are demonstrated as the mean±standard deviation (triplicate). Statistical evaluation of the data was performed using one-way analysis of variance (ANOVA) followed by Tukey's test. *p* values less than 0.05 were considered significant.

RESULTS

Phytochemical screening

Phytochemical screening of the EE of *H. fruticosa* leaves showed that the crude EE contain large amounts of chalcones, flavones, flavonols, phenols, saponins, steroids, tannins, triterpenes, and xanthenes. The *n*-C₆H₁₄ partition exhibited large amounts of steroids and triterpenes, and the CHCl₃ partition exhibited large amounts of chalcones and triterpenes. Furthermore, the EtOAc and MeOH/H₂O partitions presented significant quantities of flavones, flavonols, phenols, saponins, tannins, and xanthenes.

Carrageenan-induced paw oedema in rats

The anti-inflammatory effect of the EE of *H. fruticosa* leaves (100-400 mg/kg) was evaluated by the paw oedema model (n=6/group). As observed in Figure 1, the single oral treatment of rats with the EE of *H. fruticosa* leaves at 100, 200, and 400 mg/kg was capable of reducing ($p<0.05$) the oedema formation induced by carrageenan (1%, 100 µL/paw), an effect observed at 3 h after the administration of this phlogistic agent. Additionally, the EE at 100 and 400 mg/kg (*p.o.*, 1 h beforehand) reduced ($p<0.05$) the oedema formation induced by carrageenan at 2 and 4 h. Likewise, ASA (300 mg/kg, *p.o.*, 1 h beforehand, n=6) significantly inhibited ($p<0.001$) the oedematogenic response evoked by carrageenan in rats, at 2, 3, and 4 h (Figure 1).

In the assay with the EE the mean AUC found in carrageenan-treated rats was 2.97 ± 0.20 mL x h (n=6). Based on AUC values, the EE at 100, 200, and 400 mg/kg caused 33.3, 27.6, and 30.6% ($p<0.05$) of inhibition on oedema response, respectively (n=6/group). ASA at 300 mg/kg (n=6) caused an inhibition of 43.1% ($p<0.001$).

Table 1 shows that the $n\text{-C}_6\text{H}_{14}$ and MeOH/H₂O partitions of *H. fruticosa* EE at 50, 100, and 200 mg/kg (*p.o.*, 1 h beforehand) were capable of reducing ($p<0.01$) the oedema formation induced by carrageenan (1%, 100 $\mu\text{L}/\text{paw}$). The EtOAc partition of *H. fruticosa* EE was capable of reducing ($p<0.001$) the paw oedema formation at 50 mg/kg (*p.o.*, 1 h beforehand). However, the CHCl₃ partition (50, 100, and 200 mg/kg, *p.o.*, 1 h beforehand) presented no activity on oedema formation induced by carrageenan. The partitions of the EE reduced the oedema formation induced by carrageenan at 2 and 4 h (data not show).

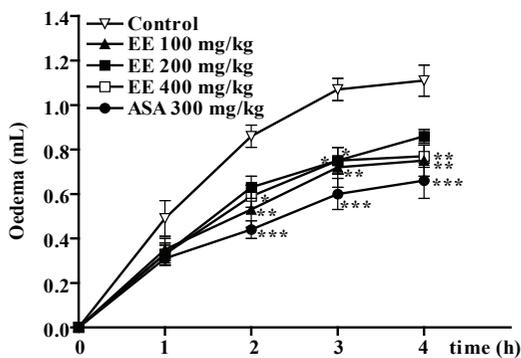


Figure 1. Effect of *Hyptis fruticosa* leaves EE on paw oedema. Rats were pre-treated (*p.o.*, $n=6/\text{group}$) with vehicle (control), acetylsalicylic acid (ASA, 300 mg/kg), or EE (100-400 mg/kg) 1 h before carrageenan (1%, 100 μL). Each value represents the mean \pm s.e.m. * $p<0.05$, ** $p<0.01$, and *** $p<0.001$, in relation to control group. ANOVA followed by Tukey's test.

Table 1. Inhibitory effects of the partitions of *Hyptis fruticosa* leaves EE on rat paw oedema induced by carrageenan based on the area under the curves (AUC) after 4h.

Dose (mg/kg)	MeOH/H ₂ O		EtOAc		$n\text{-C}_6\text{H}_{14}$		CHCl ₃	
	Oedema* (mL x h)	Inhibition (%)						
0	2.81 \pm 0.15	-	2.65 \pm 0.03	-	2.68 \pm 0.05	-	2.45 \pm 0.16	-
50	1.89 \pm 0.13 ^b	32.7	2.13 \pm 0.08 ^b	19.6	2.18 \pm 0.06 ^b	18.7	2.84 \pm 0.08	-
100	1.76 \pm 0.19 ^b	37.4	2.52 \pm 0.10	4.9	2.16 \pm 0.07 ^b	19.4	3.11 \pm 0.14	-
200	1.93 \pm 0.08 ^b	31.3	2.51 \pm 0.07	5.3	2.34 \pm 0.08 ^a	12.7	2.93 \pm 0.11	-
ASA	1.12 \pm 0.08 ^b	60.1	1.77 \pm 0.08 ^b	33.2	1.71 \pm 0.05 ^b	36.2	1.54 \pm 0.11 ^b	37.1

$n=6$. Small letters denote statistical significance, ^a $p<0.01$ and ^b $p<0.001$ in relation to control group. * mean \pm s.e.m.

Table 2. Inhibitory effects of the partitions of *Hyptis fruticosa* leaves EE on carrageenan-induced leukocyte migration into the peritoneal cavity after 4 h.

Dose (mg/kg)	MeOH/H ₂ O		EtOAc		$n\text{-C}_6\text{H}_{14}$		CHCl ₃	
	Cell x 10 ⁶ /mL*	Inhibition (%)	Cell x 10 ⁶ /mL*	Inhibition (%)	Cell x 10 ⁶ /mL*	Inhibition (%)	Cell x 10 ⁶ /mL*	Inhibition (%)
0	16.3 \pm 1.0	-	13.4 \pm 0.9	-	16.3 \pm 1.0	-	15.8 \pm 0.7	-
50	10.1 \pm 0.4 ^b	38.4	5.8 \pm 1.1 ^b	56.9	11.6 \pm 0.8 ^a	28.6	18.0 \pm 1.6	-
100	7.5 \pm 0.8 ^b	54.4	5.5 \pm 0.5 ^b	58.9	7.4 \pm 0.9 ^b	54.7	11.8 \pm 1.4	24.9
200	7.2 \pm 0.8 ^b	56.0	5.9 \pm 1.0 ^b	55.9	5.6 \pm 0.5 ^b	65.4	14.4 \pm 0.8	8.6
Dexa	5.3 \pm 0.3 ^b	67.6	4.5 \pm 0.2 ^b	66.1	5.7 \pm 0.3 ^b	64.8	6.2 \pm 0.3 ^b	61.0

$n=6$. Small letters denote statistical significance, ^a $p<0.01$ and ^b $p<0.001$ in relation to control group. *mean \pm s.e.m.

Carrageenan-induced peritonitis in rats

Carrageenan (500 $\mu\text{g}/\text{cavity}$) induced leukocyte migration into the peritoneal cavity 4 h after stimulus. Figure 2 shows the inhibitory effect of the EE of *H. fruticosa* leaves (46.7, 33.1, and 47.8% at 100, 200, and 400 mg/kg, respectively, $p<0.05$) on the carrageenan-induced response. The control drug dexamethasone (2 mg/kg, *s.c.*, 1 h beforehand) inhibited (62.5%, $p<0.001$) the carrageenan-induced leukocyte migration into the peritoneal cavity.

Table 2 shows that the $n\text{-C}_6\text{H}_{14}$, EtOAc, and MeOH/H₂O partitions of *H. fruticosa* EE at 50, 100, and 200 mg/kg (*p.o.*, 1 h beforehand) were capable of reducing ($p<0.01$) the carrageenan-induced leukocyte migration into the peritoneal cavity (500 $\mu\text{g}/\text{cavity}$, 4 h after). However, the CHCl₃ partition (50, 100, and 200 mg/kg, *p.o.*, 1 h beforehand) presented no activity on leukocyte migration into the peritoneal cavity induced by carrageenan.

Antioxidant activity

According to the IC₅₀ values, the antioxidant concentration needed to decrease by 50% the initial concentration of DPPH radical is the highest for the $n\text{-C}_6\text{H}_{14}$, CHCl₃, and MeOH/H₂O partitions, as compared with the reference compound (-)-epigallocatechin (triplicate, $p<0.05$, Table 3). The IC₅₀ values for EE and EtOAc partition (triplicate) were similar to IC₅₀ value of the reference compound.

Table 3. IC50 values of *H. fruticosa* leaves EE and its partitions determined by DPPH method.

Samples	IC50 ($\mu\text{g/mL}$ DPPH)*
EE of leaves	35.00 \pm 1.01 ^{ab}
<i>n</i> -C ₆ H ₁₄ partition	78.67 \pm 2.90 ^c
CHCl ₃ partition	91.33 \pm 2.18 ^c
EtOAc partition	36.67 \pm 2.65 ^{ab}
MeOH/H ₂ O partition	50.67 \pm 2.29 ^b
(-)-Epigallocatechin	16.67 \pm 1.21 ^a

Different small letters means significantly different at $p < 0.05$ (triplicate). * IC50 values of extracts (25 $\mu\text{g/mL}$) were calculated at 50 min.

DISCUSSION

This study demonstrated that *Hyptis fruticosa* EE and its *n*-C₆H₁₄, EtOAc, and MeOH/H₂O partitions present inhibitory activities in experimental models of paw oedema and cell migration. In addition, the data presented herein show that the EE and its EtOAc and MeOH/H₂O partitions present antioxidant potential.

Initially, we observed that the previous oral treatment with the EE of *H. fruticosa* leaves or its *n*-C₆H₁₄, EtOAc, and MeOH/H₂O partitions was effective in reducing the oedematogenic response evoked by carrageenan in rats between the second and fourth hours after the injection. Paw oedema formation is a result of a synergism between inflammatory mediators that increase blood flow and microvascular permeability (Ialenti et al., 1992). The carrageenan-induced rat paw oedema is characterized by an early phase (1 h) caused by the release of histamine, 5-hydroxytryptamine, and bradykinin followed by a late phase (2 h) mainly sustained by nitric oxide and prostaglandin release which causes oedema dependent on mobilization of neutrophils (Di Rosa et al., 1971; Salvemini et al., 1996).

To gain further insight into the anti-inflammatory effect induced by EE of *H. fruticosa*, we evaluated its effect in other experimental model of inflammation. Cell recruitment during inflammation depends on the orchestrated release of local mediators which are responsible for local vascular and tissue changes as well as for the recruitment of host defense cells (Luster et al., 2005). The leukocyte migration induced by *i.p.* injection of carrageenan involves many mediators, including eicosanoids, cytokines, and chemokines (Ogino et al., 1996; Fröde et al., 2001). The EE and its *n*-C₆H₁₄, EtOAc, and MeOH/H₂O partitions inhibited leukocyte migration induced by *i.p.* injection of carrageenan (in peritonitis model).

Previous study has demonstrated that the aqueous leaves extract of *H. pectinata* and several extracts of *Hyptis verticillata* shown anti-inflammatory activity (Kuhnt et al., 1995; Bispo et al., 2001). In

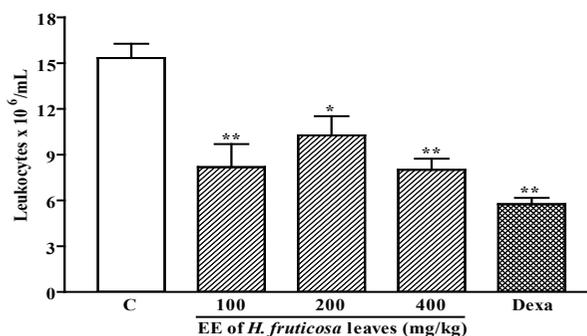


Figure 2. Effect of *Hyptis fruticosa* leaves EE on leukocyte migration. Rats were pre-treated with vehicle (C, *p.o.*), dexamethasone (Dexa, 2 mg/kg, *s.c.*), or EE (100-400 mg/kg, *p.o.*) 1 h before carrageenan (500 μg , *i.p.*). Cell counts were performed at the time 4 h after the carrageenan. Each value represents the mean \pm s.e.m. * $p < 0.05$ and ** $p < 0.001$, in relation to control group. ANOVA followed by Tukey's test ($n = 6/\text{group}$).

fact, various species of the family Lamiaceae has been reported to contain anti-inflammatory activity, *e.g.* *Plectranthus amboinicus*, *Clinopodium vulgare*, and *Lamium garganicum* (Akkol et al., 2008; Burk et al., 2009; Gurgel et al., 2009). The present study shown that *H. fruticosa* markedly inhibited the local and acute manifestations in experimental models of inflammation. A putative mechanism associated with this activity may be inhibition of the synthesis of many inflammatory mediators whose involvement in the oedema and cell migration is well-established. However, these possibilities remains to be tested in future studies.

The antioxidant capacity of many substances confers a therapeutic potential with anti-inflammatory property (Li et al., 2002; Librowski et al., 2005). In our work demonstrated that the EE and EtOAc partition have highest antioxidant potential, and the MeOH/H₂O partition has moderate antioxidant potential. However, the *n*-C₆H₁₄ and CHCl₃ partitions are poor sources of antioxidants. Some studies have demonstrated that the extracts of *Hyptis fasciculata* shown to be active as DPPH radical scavengers (Silva et al., 2005, 2009) and others Lamiaceae plants (*e.g.* some *Salvia* species, *Gontscharovia popovii*) represent good potential sources of natural antioxidants useful for either prevention or treatment of oxidative stress-related diseases (Firuzi et al., 2010).

The phytochemical study of *H. fruticosa* leaves detected large amounts of phenolic compounds in the EE, as well as, EtOAc and MeOH/H₂O partitions. Xanthenes, a widespread polyphenolic compound in plants, have extensive pharmacological activities such as antioxidant, antihypertensive, inhibition of platelet aggregation, antiulcer, and anti-inflammatory (Peres et al., 2000; Librowski et al., 2005). Flavonoids are the major secondary metabolites class with several descriptions of antioxidant property which confers a therapeutic potential with anti-inflammatory effect (Rajendran et al., 2000; Li et al., 2002; Matsuda et al., 2003). Flavonoids have been

shown to inhibit the inducible isoforms of cyclo-oxygenase (COX-2) and nitric oxide synthase (iNOS), lipooxygenase, microsomal monooxygenase, glutathione S-transferase, and nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, all involved in reactive oxygen species generation (Odontuya et al., 2005; Soobrattee et al., 2005). Others anti-inflammatory properties of flavonoids are their suggested ability to inhibit neutrophil degranulation and reduce complement activation, thereby decreasing the adhesion of inflammatory cells to the endothelium (Nijveldt et al., 2001). It has been demonstrated that tannins are also able to inhibit COX-2 and/or iNOS enzymes, as well as other mediators of the inflammatory process (Carvalho, 2004). A number of saponins have been previously reported to have anti-inflammatory effects. For example, soybean saponins were reported to suppress the release of proinflammatory mediators by peritoneal macrophages, saponins from *Platycodon grandiflorum* were reported to inhibit expression of iNOS and COX-2, and ginsenosides were reported to inhibit COX-2 expression (Ahn et al., 2005; Kang et al., 2005; Lee et al., 2005). These previous studies confirm our dates in this work that demonstrated the presence of flavonoids, saponins, tannins, and xanthenes in the EE, and EtOAc and MeOH/H₂O partitions with anti-inflammatory and antioxidant effects.

In addition, steroids have been reported as potent anti-inflammatory agents (Mencarelli et al., 2009). In our work demonstrated that the *n*-C₆H₁₄ partition contains steroids and its shows anti-inflammatory activity and poor antioxidant action. The CHCl₃ partition did not show anti-inflammatory and antioxidant activities, which is destitute of flavonoids, saponins, tannins, xanthenes, and steroids.

In conclusion, *H. fruticosa* shows anti-inflammatory and antioxidant activities. The identification and isolation of such bioactive components are in progress, which could elucidate the anti-inflammatory property of this plant.

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