# Anti-inflammatory, anti-oxidant, and apoptotic activities of four plant species used in folk medicine in the Mediterranean basin

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**Abstract**: The aim of this research was to study the potential anti-inflammatory activity of myrtle (*Myrtus communis*), sarsaparilla (*Smilax aspera*), Arabian or French lavender (*Lavandula stoechas*), and calamint (*Calamintha nepeta*) along with their apoptotic effects on the pro-inflammatory cells, and the correlation of these effects with the plants' potential anti-oxidant activity. Myrtle extract exhibited the highest inhibitory activity in the paw oedema induced by carrageenan (60% at 3 h), whereas calamint, lavender, and sarsaparilla produced inhibitions of 49%, 38%, and 47%, respectively. None of them had an effect on the TPA-induced ear oedema. Moreover, all the extracts except sarsaparilla showed different degrees of anti-oxidant activity. Lavender and myrtle at 200 µg/mL decreased cell viability by 63% and 59%, respectively, after 3 h of incubation. Neutrophil elimination through apoptosis could be implicated in the resolution of acute inflammation in the case of lavender, whereas the reduction of reactive oxygen species produced by neutrophils, such as the superoxide anion and the hydroxyl radical, could be implicated in the overall reduction of inflammation. These results may support the traditional use of these plants.

**Keywords**: *Myrtus communis*, *Smilax aspera*, *Calamintha nepeta*, *Lavandula stoechas*, anti-inflammatory activity, apoptotic activity, anti-oxidant effects.

## INTRODUCTION

Plants develop different kind of antioxidants that aid in antioxidant defense system, protecting plants against damage caused by active $O_2$ formed due to exposure to ultraviolet radiation. For that, plants have different groups of compounds, principally preventive anti-oxidants (enzymes such as catalases or peroxidases), radical scavenging compounds (ascorbic acid or carotenoids), and enzymes that repair DNA and genetic material. In the group of anti-oxidant and free radical scavenging agents, plants synthetize different compounds, principally phenolic derivatives, such as flavonoids, phenyl-propanoids, stilbenes and other. These same compounds are the potential anti-oxidant agents of interest for human, preventing the damage caused by reactive oxygen species (ROS), such as LDL-cholesterol oxidation or cartilage degeneration in rheumatic diseases (Misra *et al*., 2007).

Inflammation is a physiopathological event characterized by redness, heat, pain, swelling, and decreased function. It involves different mechanisms attributable to a large variety of mediators, and occurs in three phases. In the first phase a local vasodilation and increased vascular permeability of vasoactive amines, kinins, and arachidonic acid metabolites occur. The second phase is characterized by the infiltration of leukocytes and phagocytic cells and entails the recruitment of inflammatory cells, which involves the release of chemotactic mediators and cytokines, especially interleukin-1 (IL-1) and tumour necrosis factor (TNF) (Ríos *et al*., 2000).

Spontaneous apoptosis of neutrophils is fundamental for maintaining a normal level of circulation and ensuring a rapid resolution of inflammatory responses (Smith, 1994). Because mature human neutrophils have the shortest life span of all leukocytes, a reduction in neutrophil apoptosis has already been linked to several inflammatory conditions, including rheumatoid arthritis and acute respiratory distress syndrome (Chilvers *et al*., 2000).

At the site of inflammation, the stimulated polynuclear cells are capable of producing reactive oxygen species such as the cytotoxic superoxide anion ($^{\bullet}O_2^-$), which can react with other molecules to produce hydroxyl radical ($^{\bullet}OH$), an extremely reactive which is could initiate the lipid peroxidation. These events produce tissue damage and could be the cause for developing a chronic process, for that the treatment or prevention of acute inflammation in the early phases is of high interest for preventing chronic processes. In this context, the use of medicinal plants with anti-inflammatory and anti-oxidant activity is a classic remedy for preventing and treating inflammations so that they do not become chronic (Ríos *et al*., 2000; Schinella *et al*., 2002).

The plants selected for this study are all utilized in the Mediterranean region as remedies for various diseases especially in which infection, inflammation and pain are

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relevant components. Of them, myrtle (*Myrtus communis* L.) has long been used in folk medicine as an orally administered treatment for infectious and inflammatory process, such as prostatitis, bronchitis, sinusitis, and colds (Gruenwald 2000). Moreover, it has been reported to have antimicrobial and anti-inflammatory activities (Khare 2007). For its part, sarsaparilla (*Smilax aspera* L.) is used as an anti-inflammatory and antipruritus agent, as well as an antiseptic (Khare 2007). Preparations from the roots of this plant are used to treat inflammatory skin processes, including psoriasis, along with rheumatic complaints and inflammation of the urinary tract (Gruenwald 2000). Flowers from Arabian or French lavender (*Lavandula stoechas* L.) have been shown to exert different effects on the nervous and digestive systems, but also has remarkable properties in neuralgia, and rheumatic afflictions (Khare 2007). Another plant, namely calamint (*Calamintha nepeta* (L.) Savi), is used to treat febrile colds and respiratory diseases (Gruenwald 2000). Other uses include labour induction (Leonti 2009) and as a vulnerary against insects and venomous animals bites (Passalacqua *et al*., 2007; Scherrer *et al*., 2005).

Although these are all common medicinal plants used widely in the entire Mediterranean area, specific studies on their anti-inflammatory and anti-oxidant activities are scarce. Only in the case of myrtle the compounds myrtucommulone and semimyrtucommulone, two oligomeric, nonprenylated acylphloroglucinols found in the leaves of the plant, have been isolated and studied specifically for their anti-inflammatory activity (Feisst *et al*., 2005; Rossi *et al*., 2009). In contrast, no specific studies on French lavender, calamint, or sarsaparilla as anti-inflammatories or anti-oxidants have been published to date. For that, the aim of this work is to establish the anti-inflammatory activity of the four medicinal plants, as well as the effects on apoptosis on pro-inflammatory cells in the resolution of the inflammation and the correlation with their potential anti-oxidant activity.

## MATERIALS AND METHODS

### *Plant material*

The plants were collected by Dr S. Amira in Jijel (Algeria) at 300 m above sea level and identified by Dr. S. Belagoun, a botanist in the Department of Biology at the University of Setif (Algeria), and by Professor G.G. Franchi, a phytochemist in the Department of Pharmacology at the University of Siena (Italy). *Calamintha nepeta* (L.) Savi (Lamiaceae), *Myrtus communis* L. (Myrtaceae), and *Smilax aspera* L. (Smilacaceae) were all collected in October 2007, while *Lavandula stoechas* L. (Lamiaceae) was collected in May of the same year. For future reference, voucher specimens have been deposited at the Department of Biology, University of Setif (Algeria), numbered M69, S83, C26, and L42, respectively.

### *Extraction*

Dried aerial parts of *Calamintha nepeta* and flowers of *Lavandula stoechas* (20 g of each) were extracted with cold methanol (250 mL ×3) at room temperature for 24 h. The reunified extractive liquids were evaporated at vacuum and two extracts were obtained: *C. nepeta* (4.62 g, 23.1%) and *L. stoechas* (2.58 g, 12.9%). In the case of *Myrtus communis* and *Smilax aspera*, fruits (200 g) from each plant were extracted with cold distilled water (100 mL ×3) at room temperature for 24 h. The filtered liquids were then lyophilized to obtain the following dry plant extracts: *M. communis* (7.68 g, 3.8%) and *S. aspera* (5.46 g, 2.7%).

### *Anti-inflammatory activity*

#### Carrageenan-induced hind-paw oedema in mice

Oedema was induced in the right hind-paws of the subject mice through subplantar injection of a suspension of λ-carrageenan (3% w/v in saline, 25 μL). Plant extracts (200 mg/kg) dissolved in ethanol/Tween 80/$H_2O$ (1:1:10, v/v) and indomethacin (10 mg/kg) in saline buffer (NaCl 0.9%, $NaHCO_3$ 0.1%, pH 7.4) was administered per os 1 h before the carrageenan injections. The oedema was measured 1, 3, and 5 h after challenge and expressed as the difference between the inflamed and non-inflamed paws (plethysmometer Ugo Basile, Comerio, Italy). Oedema inhibition was expressed as the percentage of volume reduction of treated groups (problem and positive control) respect to the control group (vehicle only).

#### 12-O-Tetradecanoylphorbol 13-acetate (TPA)-induced oedema in mouse ears

Oedema were induced by topical application of 2.5 μg of TPA (Sigma-Aldrich) to the right ears (20 μL, 10 μL on each side) of the mice. Calamint and lavender (1 mg/ear) and indomethacin (Sigma-Aldrich, 0.5 mg/ear) dissolved in acetone and myrtle and sarsaparilla (distilled water) were applied topically simultaneously with TPA. After 4 h, the mice were sacrificed and two ear punches were taken from each animal and differences between treated and non-treated ears were measured. The swelling inhibition was expressed as the mean weight reduction of treated groups in comparison with the group treated only with TPA (control).

### *Total phenol and total flavonoid analysis of the extracts*

The total phenolic content of the extracts was determined with the aid of the Folin–Ciocalteu reagent and expressed as μmol equivalents of quercetin per mg of dry extract (Singleton *et al*., 1998). The total flavonoid present in plant extract was evaluated by means of a colorimetric method based on the formation of a flavonoid-aluminium complex (at 510 nm) and the final results were expressed as μmol equivalents of rutin per mg of dried extract (Sakanaka *et al*., 2005).

***In vitro antioxidant activity***

The antioxidant activity was established by different methods. All of them were performed in triplicate. The extracts were assayed at different concentrations (1 to 100 µg dry extract/mL). The entire reagents were purchased to Sigma-Aldrich.

**DPPH$^{\cdot}$ radical scavenging activity**

Reduction of this radical was determined according to Cavin (1998), using 10 µL of plant extract in 990 µL of a DPPH$^{\cdot}$ solution in methanol (0.04 mg/mL). The mixtures were measured in triplicate at 517 nm after incubation for 20 min in the dark. Reduction of DPPH$^{\cdot}$ was expressed as µmol of Trolox equivalents per mg dried extract assayed.

**2,2′-azino-bis(3-ethylbenzothiazoline)-6 sulfonic acid radical ($ABTS^{\cdot+}$) scavenging activity** (Re *et al*., 1999). $ABTS^{\cdot+}$ radical was generated by reacting 7 mM of ABTS solution in water with 2.45 mM of potassium persulfate in the dark for 12-16 h. The radical scavenging reaction was started by adding 10 µL of either diluted extracts or vehicle to 990 µL of 7 mM of $ABTS^{\cdot+}$ and the absorbance recorded at 734 nm 25 min after addition of the sample. Results were expressed as µmol of Trolox equivalent per mg of dried extract.

**Galvinoxyl radical scavenging activity** (Shi *et al*., 2001). An aliquot of 900 µL of galvinoxyl methanol working solution (5 mM) was added to 90 µL of a sample (at different concentrations) and the mixture was allowed to react at 37 ºC and the absorbance measured at 428 nm after 20 min. Results were expressed as µmol of Trolox equivalent per mg of dried extract.

**Superoxide radical scavenging activity** (Schinella *et al*., 2000). Superoxide radical was generated through enzymatic oxidation of hypoxanthine with xanthine oxidase grade I (0.06 U) and was detected by the reduction of nitroblue tetrazolium (NBT), monitored spectrophotometrically at 560 nm. The effect on enzyme activity was evaluated by measuring the uric acid formation from xanthine (15 min incubation at 25 °C); absorbance was measured at 295 nm. Results were expressed as µmol of Trolox equivalent per mg of dried extract.

**Scavenging of peroxynitrite anion**. Peroxynitrite was synthesized according to protocol established by Balavoine and Geletii (1999) and the peroxynitrite anion scavenging activity of the extracts was determined with pyrogallol red as the detecting molecule. Results were expressed as µmol of ascorbic acid equivalents per mg of dried weight of the extract.

**Ferric reducing activity (FRAP assay)** (Benzie and Strain, 1996). The assay was performed using 990 µL of FRAP reagent (acetate buffer, 2,4,6-tripyridyl-s-triazine (TPTZ) solution and $FeCl_3 \cdot 6H_2O$) and 10 µL of appropriately diluted extracts or vehicle. Absorbance readings at 593 nm were recorded 20 min after the start of the reaction. Results were expressed as µmol of ascorbic acid equivalents per mg of dried weight of the extract.

**Human plasma lipid peroxidation** (Schinella *et al*., 2007). Heparin plasma (100 µL with 200 ± 20 µg total cholesterol) was diluted with 350 µL of PBS and oxidated by addition of 50 µL $CuSO_4$ 10 mM) and reaction stopped with EDTA after 180 min at 37°C the reaction. The incubations and controls were performed in the presence of the extracts (10-100 µg/mL). Thiobarbituric acid reactive substance (TBARS) production was used as an indicator of lipid peroxidation (Pompella *et al*., 1987). The results were expressed as the percentage of inhibition respect to the negative control. Butylated hydroxytoluene (BHT) was used as a positive control.

***Apoptosis in human cells***

Neutrophils from human anti-coagulated peripheral blood from healthy donors were isolated by means of sequential sedimentation in dextran 2% in saline and subsequent centrifugation in Histopaque-1077. The residual erythrocytes were removed with cold water and neutrophils washed (PBS pH 7.4, twice) and resuspended in the same buffer (1 mg/mL glucose, 0.4 mM $Mg^{2+}$, and 1.20 mM $Ca^{2+}$). Cell viability was determined with the aid of the trypan blue dye exclusion method.

The cytotoxicity of compounds (200 µg/mL) in human polymorphonuclear ($2.5\times10^6$) cells was determined by the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide], after incubation (37 °C for 3 h in PBS pH 7.4). For the control negative (100% viability) was used PBS only. The formazan produced by the cells was dissolved in sodium dodecyl sulfate (at 10% in 0.01 M HCl) and incubated overnight. Cytotoxicity was evaluated at 570 nm with a 630 nm-reference filter and chlorpromazine was used as a positive control.

**Analysis of apoptotic (hypodiploid) nuclei**. Nicoletti (1991). Cells ($2.5 \times 10^6$ per tube) were suspended in ice-cold ethanol (70%) and stored at –20 ºC for at least 30 min, then were washed (PBS at 4 ºC), resuspended in 500 µL of DNA staining solution (20 µg/mL propidium iodide plus 0.2 mg/mL RNAse A in PBS) and incubated at room temperature (in the dark for 30 min). Fluorescence of individual nuclei was measured (minimum of 20,000 events were counted per sample) with a Becton Dickinson FACS Scan flow cytometer and the percentage of apoptotic cell nuclei (hypodiploid DNA peak) calculated.

**Annexin V-fluorescein isothiocyanate (FITC) in apoptotic cells** (Homburg *et al*., 1995). The cells ($2.5\times10^6$) were incubated in presence of extracts (200

**Table 1**: Effects of the plant extracts on carrageenan-induced mouse paw edema. Extracts (200 mg/kg) were orally administered. Footpad edema was induced 1 h later by subplantar injection of carrageenan (3% w/v in saline). Footpad volume was measured 1, 3 and 5 h after the challenge with the irritant.

| | Δ Paw Volume (μL ± s.e.m.) [a] | | | I% [b] | | |
|---|---|---|---|---|---|---|
| Time [c] | 1 | 3 | 5 | 1 | 3 | 5 |
| Control | 3.8 ± 0.5 | 11.0 ± 0.5 | 9.2 ± 0.8 | – | – | – |
| *C. nepeta* | 2.4 ± 0.5 [ns] | 5.6 ± 1.3** | 9.0 ± 0.3 [ns] | 37 | 49 | 2 |
| *L. stoechas* | 2.8 ± 0.6 [ns] | 6.8 ± 0.5* | 6.2 ± 0.6 [ns] | 26 | 38 | 33 |
| *M. communis* | 2.8 ± 0.3 [ns] | 4.4 ± 0.7** | 6.0 ± 0.9* | 26 | 60 | 35 |
| *S. aspera* | 2.4 ± 0.4 [ns] | 5.8 ± 1.1** | 7.6 ± 1.0 [ns] | 37 | 47 | 17 |
| Indomethacin | 2.2 ± 0.3* | 3.9 ± 0.4** | 5.8 ± 0.3* | 42 | 65 | 37 |

[a] Paw volume expressed as the mean of the difference between right and left paw volume ± S.E.M. $n = 5$.
[b] %I = Percentage of inhibition with respect to the control treated with the vehicle. [c] Time in hours after treatment.
[*] $P < 0.05$ with respect to the control group (Dunnett's *t*-test). [**] $P < 0.01$ with respect to the control group (Dunnett's *t*-test).
[ns] Not significant

μg/mL) and then washed twice with cold PBS and resuspended ($1\times10^6$ cells/mL) in a binding buffer solution (10 mM Hepes/NaOH pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$). Finally cells ($1\times10^5$) were transferred to a culture tube (5 mL) to which were added annexin V-FITC (5 μL) and propidium iodide solution (10 μL, 50 μg/mL in PBS). The tubes were then incubated. After incubation (15 min at room temperature in the dark), 400 μL of binding buffer were added to each tube and flow cytometry analyses were carried out.

## STATISTICAL ANALYSIS

Values are expressed as mean ± S.E.M. Percentages of inhibition (%I) were calculated from the differences between animals from positive control or extract-treated groups and animals treated only with carrageenan or TPA (negative control). One-way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons was used and values of *P* less than 0.05 were considered significant.

## RESULTS

### *Anti-inflammatory properties*

*Myrthus communis* extract exhibited the higher activity in the carrageenan-induced paw oedema, with an inhibition of 60% at 3 h. Moreover, it was the only extract which showed significant activity at 5 h, with 35% inhibition (table 1). However, none of the extracts had any effect on the TPA-induced ear oedema (data not show).

**Table 2**: Total phenolics are expressed as μg equivalents of quercetin per mg of dry extract (Folin-Ciocalteau method) and total flavonoids expressed as μg equivalents of rutin per mg dried (colorimetric method, based on the formation of a complex flavonoid-aluminium).

| Plant extract | Total phenols | Total flavonoids |
|---|---|---|
| *Calamintha nepeta* | 789 | 65.9 |
| *Lavandula stoechas* | 472 | 41.3 |
| *Myrtus communis* | 117 | 8.0 |
| *Smilax aspera* | 79 | 2.8 |

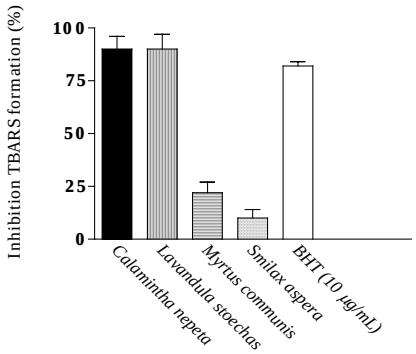
### *Anti-oxidant effects*

Total content in flavonoids and phenols of each plant extracts are compiled in table 2. The scavenging properties of the extracts was measured in terms of their ability to bleach the stable radicals $DPPH^{\bullet}$, $ABTS^{\bullet+}$, and galvinoxyl, with the activity values expressed as Trolox equivalents in the extract. The results are given in table 3. The FRAP assay, which measures the antioxidant effect of a given substance in the reaction medium as reducing ability, was also performed. Activity values for this assay were expressed as ascorbic acid equivalents in the extract (table 3). The scavenger properties of the extracts against the superoxide anion and peroxynitrite were also determined (table 3). Finally, the lipid peroxidation of human plasma by the extracts was evaluated with the aid of non-enzymatic generation systems. The extracts were found to inhibit lipid peroxidation in human plasma; the results are summarized in fig. 1.

The anti-oxidant capacity of plant extracts largely depends on their composition and the conditions of the testing system used. Because many factors play a role, the effects of the extracts cannot be wholly described with one single method. It is thus necessary to perform more than one type of antioxidant capacity measurement to get a full understanding of the various mechanisms of

antioxidant action. In this study, the antioxidant activity of the extracts was evaluated on the basis of the following methods: the analysis of their scavenging effects with regard to the $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and superoxide radicals, as well as those of peroxinitrite; the testing of their ability to reduce ferric (III) iron to ferrous (II) iron in the FRAP reagent; and their capacity to inhibit lipid peroxidation using copper-induced human plasma oxidation as the biological system. The results for the antioxidant activity of the various extracts (table 3) indicate that all the species studied exhibit free radical scavenging activity in a wide range, with *Calamintha nepeta* and *Lavandula stoechas* extracts showing similar antioxidant activity and *Smilax aspera* exerting a somewhat lower activity.

**A)**




**B)**

| **Plant extract** | $IC_{50}$ values (µg/mL) |
|---|---|
| *Calamintha nepeta* | 28 |
| *Lavandula stoechas* | 33 |
| *Myrtus communis* | >100 |
| *Smilax aspera* | >100 |
| Butylated hydroxytoluene (BHT) | 5.5 |

**Fig. 1**: Effects of plant extracts on lipid peroxidation of human plasma. A) Inhibition of TBARS formation produced in presence of 100 µg/mL of the extracts and 10 µg/mL of butylated hydroxytoluene (BHT) in the condition assays. B) $IC_{50}$ values (µg/mL) of the tested extracts o TBARS formation. For details see methods.

***Assay of cytotoxicity***

Our research included an assessment of the cytotoxicity of the extracts on human PMN cells. The MTT assay revealed that the ability of cells to reduce the MTT salt to formazan decreased significantly in the presence of *Lavandula stoechas* (63.4 ± 4.4%) and *Myrthus communis* (59.4 ± 5.3%) extracts. The results were expressed as a percentage of the control (cells treated with 0.5% DMSO). No cytotoxic activity was detected in selected concentrations of *Calamintha nepeta* and *Smilax aspera* extracts. Chlorpromazine at 100 µM and 10 µM reduced the viability in 15.1 ± 1.5% and 33.3 ± 5.3% respectively.

The nature of the cytotoxicity was evaluated with different flow cytometry methods, including the exposure of membrane phosphatidylserine through Annexin V-FITC binding and the development of hypodiploid nuclei by propidium iodide (PI). While only a small proportion of PMN cells in the untreated population underwent apoptosis [annexin V-FITC positive, propidium iodide negative ($A^+/IP^-$)], the proportion was significantly greater for the cells treated with *Lavandula stoechas* (20.4% vs 5.2% of the control, $P<0.01$) (fig. 2b), as was the increase in the development of apoptotic nuclei (25.4% vs 5.8% of the control, $P<0.01$) (fig. 2a). The population of PMN cells treated with *Myrthus communis* produced the highest percentage (9.2% vs 4.2% of the control, $P<0.05$) of necrotic cells ($A^+/IP^+$) with a typical distribution indicative of the process (fig. 2b).

## DISCUSSION

Inflammation is a complex physiopathological response to different stimuli. It can be treated and resolved by acting on the different mediators, enzymes, and pathways implicated in the process. This can include influencing the known arachidonate metabolism, inhibiting either certain transcription factors or the production and/or scavenging of the free radicals produced during the process, and by acting on the cells implicated in the process, such as macrophages and lymphocytes. For this reason, the study of the anti-oxidant capacity of plant extracts and their potential effects on pro-inflammatory cells to induce apoptosis could provide useful insight into the mechanisms of action of their anti-inflammatory activity. To this end, we selected four species, *Calamintha nepeta*, *Lavandula stoechas*, *Myrthus communis*, and *Smilax aspera*, which are used in folk medicine in the Mediterranean region to treat several inflammatory diseases. For example, calamint has been used as an analgesic and anti-inflammatory agent to treat the symptoms of febrile colds and respiratory diseases (Gruenwald 2000) while French lavender is used to treat rheumatic affections (Khare 2007). Myrtle serves as an anti-inflammatory in prostatitis, bronchitis, and sinusitis (Gruenwald 2000; Khare 2007) and sarsaparilla is utilized as an anti-inflammatory agent for skin diseases, psoriasis, rheumatic complaints, and inflammation of the urinary tract (Gruenwald 2000; Khare 2007). In the present study, all the extracts showed anti-inflammatory activity in the carrageenan test, but had no effect on the acute TPA-induced ear oedema in mice. These negative effects may be partly due to the high polarity of the extracts assayed since their major compounds are all polyphenols: flavonoids and anthocyanins in berry extracts from *Myrtus communis* (Montoro *et al*., 2006), saponins and phenolic compounds (resveratrol) in *Smilax aspera* (Belhouchet *et al*., 2008), flavonoids in *Lavandula stoechas* (Upson *et al*, 2000) and flavonoids (acacetin-glycosides) in calamint (Marín *et al*., 2001). This lack of

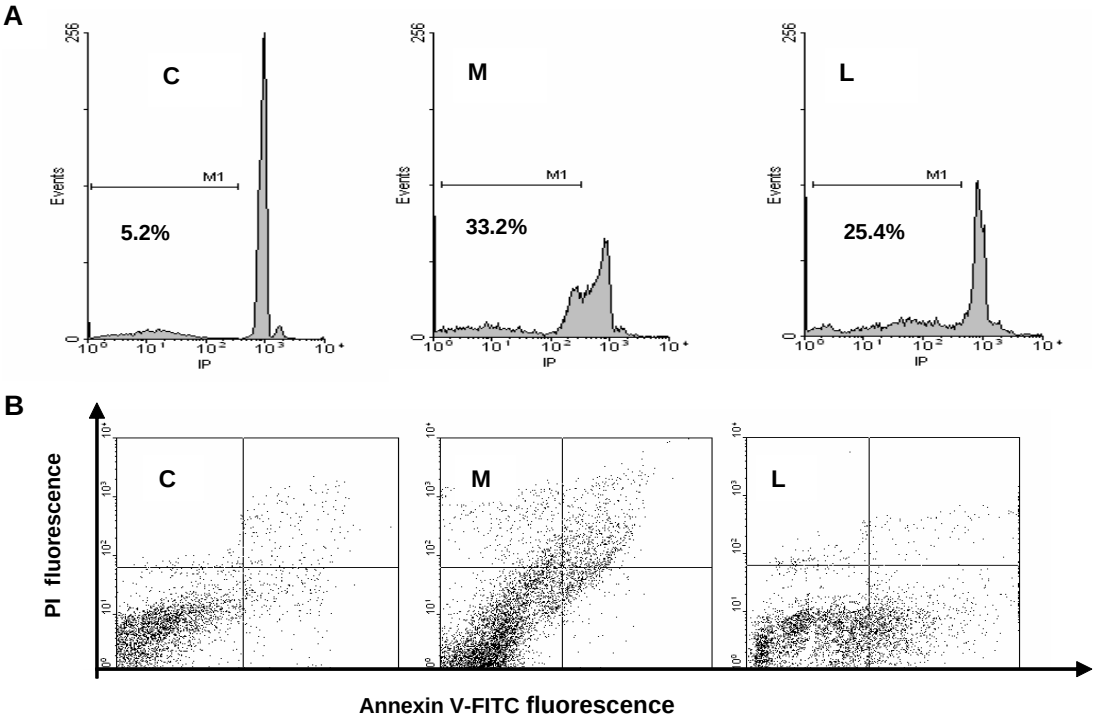



**Fig. 2**: Promotion of neutrophils apoptosis by the extracts. A) Typical histograms showing the percentage (M1) of nuclei with hypodiploid DNA content. Graph correspond to experiment of the extracts at 200 µg/mL. B) Representative dot plots of annexin V/propidium iodide (PI) staining are shown. The lower left quadrant contains the vital (double negative) population. The lower right quadrant contains the apoptotic (annexin $V^+/PI^-$) population. Finally, cells in the top right quadrant (annexin $V^+/PI^+$) are in later stages of apoptosis and necrosis. C = Control, M = *Myrtus communis*, L = *Lavandula stoechas*.

activity after topical application has been repeatedly described for polar compounds. In addition to the polar compounds cited, other non-polar fractions are also present in these plants, such as the essential oils in *M. communis*, *L. stoechas*, and *C. nepeta*, but in these cases only antimicrobial activity has been previously reported. While polar compounds are not easily absorbed through the skin, they can be absorbed orally. We thus used oral administration for testing the possible systemic anti-inflammatory effect and found that all the extracts exerted activity at 3 h. However, only the *M. communis* extract was active 5 h after administration. Nevertheless, because all the extracts used in these experiments were polar fractions obtained with methanol, we also decided to study their antioxidant effects.

Several studies have evaluated the relationships between the anti-oxidant activities of plant products and their phenolic contents. However, these relationships are difficult to explain on the basis of quantitative analyses alone. In our work, the extract of *Myrthus communis* showed a high scavenging activity for DPPH, $ABTS^{\bullet+}$, galvinoxyl, and a high ability to reduce the FRAP reagent with relatively low concentrations of total phenols and flavonoids. This relatively high total antioxidant activity for an extract with low phenolic content suggests that the type of phenolics may be more of a determinant for these activities than their amounts, and the flavonoids and anthocyanins described by Montoro *et al*. (2006) have higher anti-oxidant capacity than the phenolics described for the other species. Our results are in good agreement with those obtained by Shahidi (2003), who reported that differences in the anti-oxidant activities of plant extracts could be due to different qualitative and quantitative compositions of their phenolic constituents.

Proteins, nucleic acids, and lipids are all significant targets of cellular injuries. Lipid peroxidation, for example, is an oxidative alteration of polyunsaturated fatty acid components of different cellular structures. Metal ions at µM concentrations are known to play a role in the oxidation of human plasma lipids. Since it has been suggested that low density lipoprotein (LDL) oxidation induced *in vitro* in whole plasma probably reflects oxidation *in vivo* more adequately than in vitro oxidation of the isolated lipoprotein (Spranger *et al*. 1998), we decided to assess the antioxidant capacity of the extracts with regard to lipoperoxidation protection using whole

**Table 3**: Antioxidant activity of plant extracts. Scavenging activity of DPPH, $ABTS^{\bullet+}$ and galvinoxyl radicals, superoxide radical and peroxinitrite, and reducing activity of FRAP.

| Plant extract | DPPH [(a)] | $ABTS^{\bullet+}$ [(a)] | Galvinoxyl[(a)] | Superoxide anion [(a)] | Peroxinitrite [(b)] | FRAP [(b)] |
|---|---|---|---|---|---|---|
| *C. nepeta* | 140 | 537 | 313 | 6893 | 12.5 | 1227 |
| *L. stoechas* | 130 | 457 | 227 | 4700 | 9.1 | 1175 |
| *M. communis* | 163 | 726 | 229 | 720 | ND | 1351 |
| *S. aspera* | 3 | 124 | 5 | 484 | ND | 93 |

[(a)] Results are expressed as Trolox equivalent (µg)/mg dry weight of extract
[(b)] Results are expressed as ascorbic acid equivalent (µg)/mg dry weight of extract
n.d.: no detected activity at final concentration of 100 µg/mL

human plasma. All the extracts were tested at a final concentration of 100 µg/mL and the inhibition of human plasma lipid peroxidation was assayed with the TBARS test (fig. 1). In these assays, *Calamintha nepeta* and *Lavandula stoechas* were the most active extracts, with inhibition value percentages greater than 80% while *Myrthus communis* and *Smilax aspera* showed less activity (< 25% of inhibition). In this case, the most active plants have higher concentration of essential oils, whereas the phenolic contents is minor than in the myrtle or sarsaparilla extracts.

Neutrophils are constitutively programmed to die by apoptosis, leading to phagocytic clearance of intact senescent cells by macrophages. For this reason, neutrophil elimination through apoptosis is of considerable interest as a mechanism for promoting the resolution of acute inflammation and avoiding a persistent inflammatory response. Moreover, reactive oxygen species produced by neutrophils, such as the superoxide anion, peroxynitrite anion and the hydroxyl radical, are responsible for tissue injury in many cases (Savill and Haslett, 1999). The role of free radicals in inflammation has been widely and clearly demonstrated and some of the possible inflammatory mechanisms proposed for phenolic compounds are linked to radical scavenging, inhibition of ROS production or inhibition of enzymes pro-oxidants (García-Lafuente *et al*., 2009). Recent evidences suggest that ROS may act as important regulators of apoptosis, because different studies demonstrated that oxidants or pro-oxidants can induce apoptosis, while anti-oxidants can block or delay apoptosis (Schinella *et al*., 2002; Maraldi *et al*., 2009). The resolution of inflammation thus requires the effective down-regulation of key neutrophils, which renders any increase in the apoptosis of these inflammatory cells quite useful for controlling the resolution of inflammation (Hallett *et al*., 2008).

These results are interesting in the context of our present research, in which we have observed a correlation between anti-inflammatory, anti-oxidant and apoptotic activities in the lavender extract. It has not the higher activity but has the best correlation between the anti-oxidant and apoptotic effects. In the case of myrtle, it had the higher anti-inflammatory effect and the high toxicity against PMN cells. These properties could indicate a potential interest of this species against inflammatory process. Further studies should be focused on the fractionation and possible isolation of active fractions and compounds and studying the possible mechanism of action of the active fractions and an examination of the major compounds that contribute to neutrophil apoptosis and their role in the signal-transduction pathways. For that we will centred in the isolation and study of the anti-apoptotic properties of compounds from lavender and the anti-inflammatory properties of principles from myrtle.

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