Chemical Composition, Antioxidant and Anti-Inflammatory Properties of Salvia Officinalis Extract from Algeria

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ABSTRACT

Background: Due to its flavoring and seasoning properties, Salvia officinalis has been widely used in the preparation of many foods. In folk medicine in Asia and Latin America, it has been used for the treatment of various types of disorders, including seizures, ulcers, rheumatism, inflammation, dizziness, and high blood sugar. Objective: The purpose of this study is to determine the chemical composition by HPLC/UV, antioxidant activity and lipid peroxidation; thus, the, the anti-inflammatory effect of the ethanolic extract of Salvia officinalis (EES) on certain homeostatic parameters, inflammatory biomarkers and antioxidant status in Wistar rats subjected to inflammation induced by carrageenan. Method: Male rats (n = 24) were exposed to inflammation of the peritoneal by carrageenan (200 µL: 2%) and treated for 5 days with ethanolic extract of Salvia officinalis (EES) in order to repair the damage caused by inflammation on homeostasis, TNF-α and PGE2. Results: The results of scavenging of DPPH and lipoperoxidation of the extract, showed an IC50 of 29.69 ± 1.32 and 46.17 ± 1.51 µg/mL, respectively. The identification of EEC by HPLC shows the presence of polyphenolic acids (salvianolic acid, rosmarinic acid, caffeic acid, ferulic acid) and many flavonoids (Cirsimaritin, Catechin, Acacetin, kaempferol, pinocembrine, quercetin). Salvia extract contains 221.08 ± 2.36 mg EAG/g and 80.54 ± 1.3 mg EQ/g dry extract. Compared to the control group, carrageenan induced a substantial decrease (P<0.05) in antioxidant enzymes and a highly significant increase (P<0.05) in homeostatic parameters (blood sugar, CRP and fibrinogen), biomarkers of inflammation (TNF-α and PGE2) and malondialdehyde levels. Conclusion: The administration of Salvia extract corrects this perturbation where there is an improvement in antioxidant enzymes and a decrease in biomarkers of inflammation. Salvia officinalis has been able to repair carrageenan-induced perturbations in homeostasis and inflammation markers in Wistar rats.

Key words: Salvia officinalis, HPLC/UV, Lipoperoxidation, Biomarkers of inflammation, Oxidative stress.

INTRODUCTION

Medicinal plants are generally used for the prevention of several diseases or for the curative treatment. Antioxidant activity holds a prominent position among the properties underlying these virtues. Many medicinal plants contain many different types of phytochemicals which are the sources of natural antioxidants such as α-tocopherols, phenolic acids, flavonoids, and tannins. In addition to their antioxidant functions, these compounds have other biological properties, antimicrobial, anticancer and anti-inflammatory effects. On the other hand, oxidative stress refers to the excessive production of reactive oxygen species (ROS) in cells and tissues, which may not be neutralized by the antioxidant method. The disorder in this protective mechanism may result in damage to cellular molecules such as DNA, proteins and lipids by increasing the chances of mutagenesis.

Reactive oxygen species are normally produced in a limited quantity in the body and are important compounds involved in the regulation of processes involving the maintenance of cell homeostasis and functions such as signal transduction, gene expression and receptor activation.

Hydroxyl radicals are the most destructive EROs of oxidative stress due to their intense reactivity resulting in velocity constants between 108 and 1010 mol-1.L.s-1.

Over production of ROS, especially over a prolonged period of time, can damage cell structure and functions and induce somatic mutations and preneoplastic and neoplastic transformations. Indeed, excessive production of ROS can cause irreversible damage to cells resulting in cell death through necrotic and apoptotic processes (Wang et al., 2004).

Excessive production of ROS can cause tissue damage that can lead to inflammatory process. Various inflammatory stimuli such as natural or artificial chemicals have been reported to initiate the inflammatory process leading to the synthesis and secretion of pro-inflammatory cytokines.

The activation of nuclear-orkappa B factor/active protein-1 (NF-kB/AP-1) and the production of tumor necrosis factor alpha (TNF-κ) playing a
critical role in the inflammatory process leading to several chronic
diseases. 18

In addition, Elinav14 showed that the inflammatory response is associated
with the release of various inflammatory mediators, cytokines and with
an oxidative stress-induced nitroso-redox imbalance. Among these
generations of COX-2, IL-6, TNF-α and NO induce the expression of
adhesion molecules and the sequestration of leukocytes from the
bloodstream to the site of inflammation causing tissue damage.

However, phytochemicals, such as polyphenols, can modulate
inflammatory processes. 19 Polyphenols are the abundant antioxidants
of many food materials. 20 Polyphenols are secondary plant metabolites
involved in the defensive system, including protection from ultraviolet
rays and pathogenic agents. 21 Polyphenols have anti-inflammatory and
antibiotic properties, and can activate the Nrf² transcription factor in
addition. Nrf² plays a key role in cell protection against oxidative stress
and inflammation. 14

Among these plants, officinal sage (Salvia officinalis L.), belonging to
the labiate family according to Maatoug 15 , is made up of small shrubs
with thin windy leaves, with a characteristic camphoric smell. It is an
aromatic and medicinal plant that is generally used either naturally
or as an extract or as an essential oil. In addition to a traditional use
(family food and popular medicine), this plant is used by the perfumery
and cosmetology industries, by the food industry and finally by the
pharmaceutical industry.

It is necessary to consume this plant in moderation. For example,
regulations in some countries limit the possibilities of using sage
due to the existence of chemical components that can cause
accidents when too high doses of this product are ingested. But the
existence of interesting properties makes that, despite the existence of
toxic components, sage and its extracts are attributed to many medicinal
virtues according to Stary and Jirasek, 22, Catione, 17, Biere, 24: antiseptic,
antispasmodic, ca1lmante, cephalique, digestive, febrifuge. The Latin
name clearly demonstrates the importance of sage in the traditional
pharmacopoeia.

The purpose of this study was to assess the antioxidant activity and anti-
inflammatory power of sage.

MATERIALS AND METHODS

Plant materials

The leaves of Salvia officinalis were the subject of this study. They were
collected in February 2017, in the commune of Mohamadia (W. Mascara),
located in the west of Algeria, 80 km south-east of Oran, 35 km
north of Mascara, 40 km from Mostaganem and 57 km from
Relizane.

Chemicals and reagents

The TFA, EtOH, acetonalitrile, formic acid, AlCl₃, Folin-Ciocaltene,
2,2-diphenyl-1-picrylhydrazyle (DPPH), gallic acid, ascorbic acid,
quercetin, Na₂CO₃ were obtained from Sigma-Aldrich (St Louis, MO,
USA).

Preparation of Salvia officinalis extract

10 g of Salvia officinalis leaves were cleaned, cut and then homogenized
using a mixer (Moulinex). They were then extracted with 100 mL of
80% ethanol in a hermetically sealed glass container for 72 hours at
room temperature in the dark. Filtration is performed on Whatman
N°1 filter paper, and the solvent has been recovered from the filtrate by
evaporation in a HANVAPOR type rotavapor, at a temperature of 40 °C.
The extract obtained is called extract ethanolic Salvia officinalis (EES)
and it was stored at +4 °C in a dark glass bottle until use. 19

HPLC/UV identification and quantification of phenolic compounds in Salvia officinalis extract

High performance liquid chromatography (Agilent 1100) was carried out
on the ethanolic extract of Salvia officinalis (EES) in order to identify
its different constituents by separating them according to their elution
rates on an Agilent 1200EC poroshell column (100 mm x 2.1 mm, 2.7
µm), using mobile phases: water/TFA/formic acid (99: 0.25: 0.75) (A)
and acetonitrile (B). Elution occurred at a flow rate of 0.6 mL/min with
an aliquot of 10 µL and a temperature of 55°C. Using a gradient process
(t/min, percentage B) as follows: (0, 0), (1, 10), (2, 12.5), (3, 15), (9, 80),
(10, 100), (11, 100), (14, 0) with 5 min. He recorded chromatograms
at 270 and 320 nm. They reported chromatograms at 270 and 320 nm.
The sample was prepared by diluting the EES with 1: 100 (v/v) methanol.
The components of Salvia officinalis were identified by comparing their
retention times and UV spectra with different phenolic standards
(trans-cinnamic acid, gallic acid, benzoic acid, ferulic acid, m-coumaric
acid, caffeic acid, rosmarinic acid and ellagic acid), flavonoids (catechin,
hesperidin, thymol, galangin, tectochrysin, pinocembrin, acacetin,
rutin, chrysín, apigenin, kaempferol and quercetin) and other organic
compounds (ascorbic acid and menthol). The standards were dissolved
in methanol to obtain stock solutions at a rate of 1 mg/mL. These
phenolic compounds were identified using calibration curves of the
different standards expressed in mg per 1 g of salvia.

Antioxidant activity in vitro of Salvia officinalis

Determination of total phenols

Total polyphenols were quantified according to the analytical method
of Singleton 23 using Folin Ciocalteu’s reagent. Mix 0.5 mL of the ethanolic
extract of Salvia officinalis with 0.5 mL of distilled water and 0.5 mL of
folin ciocaltel. After 03 min, 0.5 mL of 10% sodium carbonate (Na₂CO₃)
is added. Leave the reaction medium to react for 1 hour at room
temperature, and then read the absorbance at 760 nm. The calibration
curve is made with gallic acid, using the same dosage measurement.

Determination of flavonoids

The content of flavonoids in the EES extract was measured according
to the experimental protocol of Woisky and Salatino. 21 1 mL of salvia
extract is mixed with 1 mL of 2% aluminum trichloride. The absorbance
is measured at 430 nm after 30 min incubation at room temperature.
The calibration curve was plotted using quercetin.

DPPH Radical Scavenging Assay

The antioxidant activity of the EES extract was calculated using the
stable 2,2-diphenyl 1-pycrilhydrazile (DPPH) radical as defined by
Arnous. 22 The preparation of the samples consists of mixing 0.025 mL
of ethanolic extract of Salvia officinalis at different concentrations (0.5,
1, 5, 10 and 50 µg/mL) with 0.975 mL of DPPH (60 µM), incubating for
30 min under light protection and reading the absorbance at 517 nm.
The absorbance results obtained were converted into the rate of anti-
free radical power (% RSA or Radical Scavenging Activity) of DPPH
according to the equation:

%RSA= [(Abscontrol – Abs)/ Abscontrol] x 100

Lipid peroxidation

Mixed 100 µL of EES (5–100 µg / mL) with 100 µL tris-HCl buffer (10
mM, pH 7.4). After 1 hour of incubation at 37 ° C, added 100 µL of
thiobarbituric acid, then incubate the reaction mixture at 100 °C for
1 h and read the absorbance using spectrophotometry at 532 nm. The
malondialdehyde curve was used to analyze the results expressed as
nmol MDA/mg protein. 23
Anti-inflammatory activity in vitro

Anti-Hyaluronidase Activity

With a few modifications, the inhibition rate of hyaluronidase was calculated according to the method defined by Silva. Mixed 50 µL of ethanolic extract of *Salvia officinalis* EES (5, 10, 20, 50 and 100 mg/mL) with 50 µL hyaluronidase enzyme (350 units) and incubated at 37°C for 20 minutes. Then, to activate the enzyme, 1.25 µL of calcium chloride was added. 0.5 mL of hyaluronic acid sodium salt was added after incubating the reaction medium at 37°C for 20 minutes. 0.1 mL of potassium tetraborate was added after incubation at 37°C for 40 minutes and the mixture was incubated for 3 minutes in a boiling water bath. In order to avoid the reaction, the mixture was put at 6°C and then 3 mL of p-dimethylaminobenzaldehyde was added. The incubation was conducted for 20 min at 37°C.

Finally, at 585 nm, the absorbance was measured. They did all the tests three times.

Antioxidant and anti-inflammatory activity in vivo

Animals

Twenty-four male Wistar rats weighing between 100 and 150 g were used in this experiment. The rats were provided by the Algerian Pasteur Institute. The Protocol is in conformity with the recommendations of the National Institute of Health (NIH-USA).

Upon receipt, the rats were randomly placed into 4 experimental groups in metabolic cages for an adaptation period of 2 weeks at room temperature and a photoperiod of 12/12 h. Rats have free access to food (kibble from the animal feed production company, Bouzaréa, Algiers) and water. All rats have access to water and food.

Acute carrageenan-induced inflammatory reaction in the peritoneal cavity of rats

After the adaptation period, first and second group rats (G1 and G2) received 1 mL of physiological saline daily orally, while group 3 and 4 (G2 and G3) animals received 1 mL of 250 mg/kg/day ethanol extract of *Salvia officinalis* (EES) orally.

On the 5th day, thirty minutes after treatment, the rats in the groups (G2 and G3) received an injection of 200 µL of carrageenan (2%) intraperitoneally (i.p) in order to induce inflammation.

Two hours after induction of inflammation; the rats were kept under mild chloroform anesthesia before being sacrificed.

Blood is collected by cardiac puncture in dry tubes and heparin or EDTA tubes. A dissection has been made on the rats; their peritoines are carefully separated, inspected, rinsed with physiological water and then preserved in PBS for prostaglandin determination.

Biochemical studies

The albumin and blood sugar assay are performed by the colorimetric method (Kit Biosystems). Fibrinogen is measured by performing the functional chromonometric method using titrated calcium thrombin (100 NIH units/mL) containing a heparin inhibitor (fibrinprest Automate).

Erythrocytes antioxidant enzymes activities

Lipid peroxidation scavenging (malondialdehdyde MDA) was performed on erythrocytes according to the protocol of Yagi. The enzymatic activity of catalase is determined in the erythrocyte according to the method of Lück and Aebi which consists of a spectrophotometric analysis of the rate of decomposition of hydrogen peroxide. Superoxide dismutase (SOD) activity is measured according to the method of Elstner. The principle is based on the chemical reaction that generates the superoxide ion (O2-·) from molecular oxygen in the presence of EDTA, MnCl2, and mercaptoethanol. The activity of glutathione peroxidase (GSH-Px) of erythrocytes was determined according to the method described by Paglia and Valentine. The principle of the test is based on the conversion of NADPH + H+ to NADP+ as a result of a series of reactions.

Biomarkers of inflammation

The ELISA kit (Thermo Fisher Scientific, USA) was used to assess the amount of TNF-α present in peritoneal fluid. Another ELISA Prostaglandin E2 ELISA package (Abcam Explore More, UK) was used to test PGE2 in peritoneal fluid.

Statistics

Analysis was performed by SigmaStat software (SPSS, 3.0, SPSS, Inc., Chicago, IL). Data were presented as mean ± standard deviation and were assessed by one-way ANOVA, with Dunnett’s post hoc test. Row ANOVA analysis with Dunns post hoc test was used where appropriate.

RESULTS

EES chemical composition

Table 1 and Figure 1 displays the phenolic acid and flavonoid content of *Salvia officinalis*. Salvianolic acid (6.27 mg/g at 4.58 min) and Rosmarinic acid (7.85 mg/g at 2.38 min) are the main phenolics acids contained, while catechin is the predominant flavonoids (5.96 mg/g at 0.42 min) and quercetin (4.75 mg/g at 3.89 min).

In vitro antioxidant activity

Total polyphenols and flavonoids

The determination of total polyphenols and flavonoids shows that the Ethanol *Salvia officinalis* Extract (EES) contains 221.08 ± 2.36 mg EAG/g and 80.54 ± 1.3 mg EQ/g respectively, as shown in Table 2.

Antioxidant activity (DPPH) assay and lipoperoxydation

The anti-free radical activity of EES (ethanolic extract of *Salvia officinalis*) is 86% for the concentration 100 µg/mL, with an IC50 of 29.69 ± 1.32 µg/mL (Table 2). The inhibitory concentration IC50 of antioxidant capacity via the TBARS test is 46.17 ± 1.51 µg/mL (Table 2).

Hyaluronidase inhibition

From our results, it is observed that the inhibitory activity of hyaluronidase increases with the increase in the concentrations of *Salvia officinalis* extract (EES) (IC50 value of 21.86 ± 0.29 mg/mL). The percent inhibition was 92% at a salvia concentration of 100 mg/mL (Figure 2).

In vivo anti-inflammatory effects of *Salvia officinalis*

Biochemical findings

In the light of our results (Table 3), it is observed that carrageenan caused a significant increase (P <0.05) in glycemia of +70% compared to the rats of the negative control group (G1). On the other hand, the rats of G4 (which were administered with 250 mg/kg of EEC and then injected with 200 µL of carrageenan), showed a significant decrease in blood sugar levels by 47% compared to G2. In the G3 group (which received only the extract ethanolic of *Salvia officinalis*), administration of EES to rats for 15 days resulted in no change in blood sugar levels compared to the control group.

Carrageenan-induced inflammation is followed by a very large (P < 0.05) increase in CRP of +10 mg/L in the G2 group of animals. This
Table 1: Composition of extract ethanolic of *Salvia officinalis* (EES) by HPLC/UV (mg/g).

| Peak number | Compounds                  | Amount (mg/g EES) | Retention time (min) |
|-------------|----------------------------|-------------------|----------------------|
| 1           | Catechin                   | 5.96              | 0.42                 |
| 2           | Cirsimaritin               | 4.38              | 2.38                 |
| 3           | Luteolin                   | 3.4               | 3.7                  |
| 4           | Quercetin                  | 4.75              | 3.89                 |
| 5           | hesperidin                 | 2.71              | 4.02                 |
| 6           | Acacetin                   | 4.09              | 4.58                 |
| 7           | Rutin                      | 3.52              | 4.71                 |
| 8           | Kaempferol                 | 4.72              | 5.25                 |
| 9           | Pinocembrin                | 3.12              | 5.73                 |
| 10          | Apigenin                   | 1.9               | 6.73                 |
| 11          | Apigenin acetylglucoside   | 1.82              | 6.89                 |
| 12          | Chrysin                    | 2.68              | 7.26                 |
| 13          | Thymol                     | 2.38              | 7.37                 |
| 14          | Hispidulin                 | 3.84              | 7.85                 |
| 15          | Gallic acid                | 1.41              | 8.42                 |
| 16          | Carnosic acid              | 2.49              | 2.13                 |
| 17          | Rosmarinic acid            | 7.85              | 2.38                 |
| 18          | Ferrulic acid              | 3.58              | 3.75                 |
| 19          | ascorbic acid              | 1.8               | 3.89                 |
| 20          | Salvianolic acid           | 6.27              | 4.58                 |
| 21          | caffeic acid               | 3.62              | 5.25                 |
| 22          | Trans cinnamic             | 1.36              | 5.66                 |
| 23          | Sagerinic acid             | 1.29              | 7.85                 |

**Figure 1:** Chromatograms by HPLC/UV analysis of EES at a wavelength of 270 (a) and 320 nm (b): 1. Catechin; 2. Cirsimaritin; 3. Luteolin; 4. Quercetin; 5. hesperidin; 6. Acacetin; 7. Rutin; 8. Kaempferol; 9. Pinocembrin; 10. Apigenin; 11. Apigenin acetylglucoside; 12. Chrysin; 13. Thymol; 14. Hispidulin; 15. Gallic acid; 16. Carnosic acid; 17. Rosmarinic acid; 18. Ferrulic acid; 19. Ascorbic acid; 20. Salvianolic acid; 21. Caffeic acid; 22. Trans cinnamic; 23. Sagerinic acid.
amount was effectively reduced by 250 mg / kg EES (G3) + 7.33 mg/L compared to the group G1 (table 3). The administration of EES in combination with carrageenan in the G4 group showed a very significant decrease (P < 0.05) in CRP (+ 7.33 mg / L) compared to the group G2. The albuminemia is reduced by - 29 and - 16.1 g/L in groups G2 (received an injection of carrageenan) and G4 (received 250 mg/Kg of EES and 200 µL of carrageenan) compared to the control (G1); whereas it is not significantly (p> 0.01) different in the control rats (47.16 g/L) and those having received only salvia (48.7 g/L) (Table 3).

Oxidative stress status

From our results, it is observed that the rats of group G2 (which received an injection of 200 µL of carrageenan) show a high plasma concentration of MDA of up to 7.31 mM / L compared to the control group where we noted a rate up to 2.44 mM / L (Table 4). In the G3 group, administration of salvia alone resulted in a significant decrease in this rate (-52%) compared to the control group (G1). In the G4 group (rats treated with salvia and carrageenan at the same time), the level of plasma MDA (4.21 mM / L) was reduced by 2 times the value of that of the control group (G1). The enzymatic activity of catalase in rats in the G2 group was significantly decreased compared to rats in the control group (-105.36 U / mg Hb). This reduction in catalase activity indicates oxidative stress caused by carrageenan exposure of rats. Compared with the rats in the control group (G1), salvia increased catalase activity in the rats in group 3 (+15.77 U / mg Hb). A significant increase (P <0.05) in catalase activity was noted in the rats of the group G4 which received EES in combination with carrageenan (+ 79%) compared to the rats of the G2 group. Compared to the enzyme activity of rats in the control group, the decreased SOD and GPx enzyme activity of rats in the G2 group was 22.22 U/cg Hb and -96.33 U/g Hb, respectively (Table 4).

Inflammation markers

According to the results reported in Table 5, the inflammation of the peritoneum induced by carrageenan is accompanied by a highly significant increase (+1121 pg / mL) (P <0.05) in the level of prostaglandin E2 (PGE2) in animals of the group (G2) compared with the control group (G1).

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**Table 2: Content of total phenolic and flavonoid compounds in ethanolic extract of *Salvia officinalis*.

| Phenolic compounds | Antioxidant activity |
|--------------------|---------------------|
| Ethanolic extract of *Salvia officinalis* | Polyphenols (mg EAG/g of salvia) | Flavonoids (mg EQ/g of salvia) | IC_{50} DPPH (µg/mL) | IC_{50} TBARS (µg/mL) |
| 221.08 ± 2.36 | 80.54 ± 1.3 | 29.69 ±1.32 | 46.17 ± 1.51 |

The values are expressed as mean ± SD (n=3).

**Table 3: Biological parameters of the experimental groups of rats.

| Parameter | Experimental groups |
|-----------|---------------------|
| Blood sugar | G1  | G2  | G3  | G4  |
| (g/L) | 1.9 ± 0.15 | 4.36 ± 0.18 | 1.87 ± 0.12** | 2.28 ± 0.11* |
| Albumin (g/L) | 47.16 ± 0.95* | 18.16 ± 2.55* | 48.7 ± 1.11* | 31.06 ± 2.01** |
| Fibrinogen (g/L) | 2.43 ± 0.27** | 7.68 ± 0.69** | 2.62 ± 0.21* | 3.47 ± 0.42* |
| CRP (mg/L) | 2.66 ± 0.68 | 12.66 ± 2.01* | 5.33 ± 0.25* | 7.66 ± 0.68** |

The values are expressed as mean ± SD (n=5).

*Significant difference from the control group (p < 0.05). **Significant difference from the carrageenan inflammation group (p < 0.05).

**Figure 2: Inhibition of hyaluronidase (%) activity by ethanol extract of *Salvia officinalis* (EES) for each concentration. The values are expressed as mean ± SD (n=3).
The results of their work, Durling 36 concur with previous studies which showed that the gradient of the HPLC analysis has been required to separate as many flavonoids and phenolic acids as possible in a short time.

The gradient of the HPLC analysis has been required to separate as many flavonoids and phenolic acids as possible in a short time. Zimmermanna29 identified by HPLC/MS/MS, that besides rosmarinic acid and luteolin-3-glucoside, sage contains other phenolic acids such as Salvianolic acid, Methydihydrojasmonic acid, Chlorogenic acid, and luteolin-O-glucoside, as well as other flavone glycosides, in sage was also confirmed by Cvetkovich.30

Some other phenolic compounds have also been found in sage extract, such as chlorogenic acid, isorhamnetine-luteolin, apigenin-7-O-glucoside, caffeic acid, homoplantaginin and apigenin-acetylglucoside, which accounted for 49.11% of the total peak area, according to the study by Yuan.28

In accordance with previous results, our results show that sage varies considerably depending on the composition of the solvent and the results are consistent with previous studies which have shown that the nature of the solvent exerts a great power on the phenolic extraction capacities in many species.32,33

Moreover, this result was consistent with previous reports suggesting that a binary solvent system (ethanol/water) is more efficient than a mono-solvent system (water or pure ethanol) in the extraction of phenolic compounds in terms of their relative polarity.42,43

The results of their work, Durling 46 concur with previous studies which observed that total polyphenols increased during a shorter extraction period, while increasing the extraction time potentially increases the loss of solvent by evaporation, while suggesting that an estimated extraction time does not exceed 3 h.

Our results are in agreement with those of Kianbakht77, who found a total flavonoid content that was 912.03 mg EAG/g while, the total phenolic content of the extract was 738.59 mg EAG/g. Matkowski85 showed that Salvia officinalis reveals a total polyphenol content of 62.2 mg EAG/g of salvia.

Whereas, Hamrouni87 reported that Salvia officinalis had very low phenolic compound and flavonoid levels than ours, respectively, 2.337 mg EAG/g and 0.923 mg EQ/g.

Differences in plant variety, time, temperature, solvent, equivalent phenolic acid and method of extraction can result from these differences.40,41

In fact, antioxidant activity may be due to different mechanisms, such as prevention of chain initiation, peroxide decomposition, prevention of continuous hydrogen abstraction, free radical trapping, reduction capacity and binding of transition metal ion catalysts.42

In various chronic pathologies, such as cancer and cardiovascular diseases, among others, free radicals involved in the lipid peroxidation process are known to play a major role.43 The DPPH+ is considered to be a model of a stable lipophilic radical. Lipid autoxidation initiated a chain reaction in lipophilic radicals. Antioxidants react with DPPH+, reducing a number of DPPH+ molecules equal to the number of their available hydroxyl groups.44 (Xu et al., 2005).

The antiradical scavenging (RSA) of the DPPH radical observed by Annamalai45 increases as the microwave power and infrared temperature increases, while it decreases when the oven drying temperature increases. The effect of carnosol entrapment of radicals is comparable to that of α-tocopherol.46,47 The superoxide trapping activity of rosmarinic acid derivatives is 15 to 20 times higher than that of trolox; a water-soluble synthetic vitamin E. Arici48 supported the hypothesis by proving that the radicals and molecules produced form as a result of exposure to radiation. These free radicals can react with O2 in the long term and cause the formation of hydroperoxides, which create alcohols, aldehydes, aldehyde esters and hydrocarbons.

Martins49 recorded an IC50 value for inhibiting anti-free radical activity RSA for methanol sage extract of 32.97 µg/mL. Otherwise, Albano50 report a more moderate percentage inhibition of RSA with an IC50 of 2.8 µg/mL in an aqueous extract of Salvia officinalis. This difference in value is due to the chemical composition of Salvia officinalis, the place of harvest and much more the climate.51

In addition, the presence of rosmarinic acid also contributes to the activity detected, hence Cuvelier52 provided a correlation between...

### Table 4: Anti-oxidant enzyme activity in the plasma of the experimental groups of rats.

| Parameter       | Experimental groups |
|-----------------|---------------------|
|                 | G1                  | G2                  | G3                  | G4                 |
| MDA (mmol/L)    | 2.44 ± 0.21         | 7.31 ± 0.5”         | 3.46 ±0.33”         | 4.21 ± 0.33”       |
| SOD (U/g Hb)    | 33.61 ± 1.87”       | 11.39 ± 1.32”       | 39.11± 2.33”        | 26.73 ± 0.91”      |
| CAT (U/mg Hb)   | 140.16 ± 8.2’       | 34.8 ± 3.07’        | 155.93 ± 6.72”      | 95.9 ± 3.54’       |
| GPs (U/g Hb)    | 160.46 ± 6.65’      | 64.16 ± 2.59’       | 178 ± 5.24’         | 105.7 ± 3.89’      |

The values are expressed as mean ± SD (n=5).

*Significant difference from the control group (p < 0.05). **Significant difference from the carrageenan inflammation group (p < 0.05).

### Table 5: Concentrations of prostaglandin E2 (pg/mL) and TNF-α (pg/mL) in peritoneal exudates of rats for the tested groups.

| Parameter         | Experimental groups |
|-------------------|---------------------|
|                   | G1                  | G2                  | G3                  | G4                  |
| PGE2              | 221 ±17.8           | 1342 ±22.8”         | 316 ±17.4”          | 467 ±23”           |
| TNF-α (pg/mL)     | 2375 ± 145”         | 7954 ±241”          | 2268 ±166”          | 4322 ± 255”        |

The values are expressed as mean ± SD (n=5).

*Significant difference from the control group (p < 0.05). **Significant difference from the carrageenan inflammation group (p < 0.05).
antioxidant efficacy and sage composition, indicating that carnosol, rosmarinic acid and carnosic acid had the greatest antioxidant activities among its constituents. Although, some flavonoids are potent antioxidants, the flavonoids identified made a rather low contribution to the total antioxidant capacity of the extracts due to their low abundance. In addition to rosmarinic acid, other flavonoids of S. officinalis, in particular quercetin and rutin, have strong antioxidant activities.53

Sadeghnia54 revealed that rutin reversed by hexachlorobutadiene induces an increase in lipid peroxidation and depletion of thiol content in the kidney. Zhang55 found that sage had excellent antioxidant capacity and that its addition to Chinese sausage effectively inhibited protein oxidation, as indicated by the TBARS value which was around 10 µg/mL lower.

A decrease in serum concentration of total proteins may be a sign of chronic hepatopathy but also of a nutritional deficiency in protein, anorexia, poor assimilation, kidney loss, effusion, hemorrhage, hyperhydration, or burns as indicated by Dietz and Wiesner.56 Our results showed that Salvia officinalis increases the level of total proteins, which confirms that it has an anti-inflammatory effect. Whereas fibrogen is a soluble protein synthesized by the liver, it is a marker of inflammation.57

Transferrin is the plasma protein that transports iron into the body, and is reduced in inflammatory states.58 In our work, the decrease in transferrin is an index of inflammation as it decreases in rats injected by carrageenan and increases in rats treated with EES.

In addition, Mansourabadi59 reported that flavonoids from S. officinalis extracts reduce inflammation in the mouse carrageenan model and induce an analgesic effect in a dose-dependent manner. This is due to flavonoids and terpenes, the molecules most likely to lead to anti-inflammatory activities.60,61

Although the anti-inflammatory action of ursolic and rosmarinic acid from Salvia officinalis is twice as powerful as that of indomethacin.62

Rathee63 noted that anti-inflammatory activity is attributed to flavonoids and phenolic acids. Our Salvia officinalis extract is rich in compounds such as caffeic acid, gallic acid and flavonoids such as salvigenin, terpenes and tannins.64,65

Flavonoids, as confirmed by Bahmani66, have effects on opioid receptors and alpha-adrenergic receptors that can inhibit enzymes involved in inflammation and pain. In addition, flavonoids work in inflamed tissue and alpha-adrenergic receptors that can inhibit enzymes involved in the maintenance of renal homeostasis and in the fever phenomenon.67

In acute inflammation, the C-reactive protein, also known as CRP, is a plasma protein as well as the migration of leukocytes to the blood capillary. In turn stimulates the increase in vascular permeability with leakage of plasma proteins as well as the migration of leukocytes to the blood peritoneal cavity.68

In acute inflammation, the C-reactive protein, also known as CRP, is a glucoprotein that increases very quickly in the blood.69 Furthermore, prostaglandins are one of the main mediators of inflammation and pain. Indeed, it is involved in acute inflammation, inflammatory pain and also in the development of chronic inflammation. On the other hand, PGE2 plays an important role in the protection of the gastric mucosa, in the maintenance of renal homeostasis and in the fever phenomenon.68

Increased levels of TNF-α were identified in the inflammatory groups. Salvia officinalis extract decreased this rate by more than 57%.69

A decrease in serum concentration of total proteins may be a sign of chronic hepatopathy but also of a nutritional deficiency in protein, anorexia, poor assimilation, kidney loss, effusion, hemorrhage, hyperhydration, or burns as indicated by Dietz and Wiesner.56

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CONFLICTS OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication.

ABBREVIATIONS

CAT: Catalase; CRP: C Reactive Protein; DPPH: 2,2-Diphenyl-1-Picrylhydrazyl; IC₅₀: Inhibitory Concentration 50; EAG: Gallic Acid Equivalent; GSH-Px: glutathione peroxidase; HPLC/UV: High Performance Liquid Chromatography/Ultraviolet; IL-1 β: Interleukin-1 β; IL-6: Interleukin-6; MDA: Malondialdehyde; PBS: Phosphate-buffered saline; PGE: Prostaglandin E2; EQ: Quercetin Equivalent; SOD: Superoxide dismutase; TBARS: Thiobarbituric Acid Reactive Substances; TNF-α: Tumor Necrosis Factor-α.

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GRAPHICAL ABSTRACT
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