Anti-inflammatory and Antioxidant Activity of Rosemary Essential Oil

Duaa Y. Khalil [1], Omar M. Hassan [1*]

Abstract

Background: Rosmarinus officinalis L. (rosemary) is a widely used perennial shrub with diverse culinary and therapeutic applications, particularly in traditional medicine. Its essential oil (EO) has been studied extensively for its pharmacological properties, including antioxidant and anti-inflammatory effects. Methods: This research extracted rosemary EO from plants collected in Rawa, Iraq, in March 2023, utilizing steam distillation. Gas chromatography-mass spectrometry (GC-MS) was employed to analyze the EO's phytochemical composition. Anti-inflammatory properties were assessed using a protein denaturation assay, while antioxidant activity was evaluated through a modified ferric reducing antioxidant power (FRAP) assay. Statistical analysis was conducted using ANOVA and Tukey tests. Results: The GC-MS study identified eucalyptol (34.25%), α-pinene (20.98%), and camphor (13.75%) as the essential oil's primary volatile components. GC-MS analysis revealed 24 components in rosemary EO, with monoterpenes dominating (94.88%). Major constituents included α-pinene, camphor, and eucalyptol. The protein denaturation assay demonstrated significant inhibition of protein breakdown by rosemary EO, indicating anti-inflammatory potential. Moreover, the EO exhibited considerable antioxidant activity, comparable to the positive control Trolox, attributed to its high concentration of α-pinene. The rosemary essential oil showed high antioxidant properties and the inhibition rate of free radicals was 87.45%. It also showed acceptable anti-inflammatory activity at 50 mg/mL compared with diclofenac sodium. Conclusion: Rosemary EO showed promising antioxidant and anti-inflammatory properties, likely attributable to its chemical composition. Our findings showed that rosemary essential oils were safe and have positive biological effects. As a result, they could function as health-promoting components in the medical sector.

Keywords: Rosemary essential oil, antioxidant, anti-inflammatory, phytochemical analysis, biological activities.

Significance | Rosemary essential oil, antioxidant, anti-inflammatory, phytochemical analysis, biological activities.

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Introduction

Rosmarinus officinalis L. is a perennial shrub that exhibits a sweet aroma and thrives in the Mediterranean Sea region and many Asian nations. It is extensively utilized in culinary and therapeutic practices worldwide (De Oliveira et al., 2019). Traditional medicine has employed essential oils for several purposes, including antispasmodic, moderate analgesic, hypnotic, antidepressant, emotional disturbance treatment, migraine management, alleviation of intercostal neuralgia, rheumatic pain, and memory enhancement (Rahbardar et al., 2020). Numerous studies conducted both in-vivo and in-vitro have demonstrated the diverse therapeutic properties of *R. officinalis* and its constituents. These properties encompass antidepressant, anti-hysteric, and ameliorative effects on memory and mental fatigue (Hosseinzadeh et al., 2004), (Sasaki et al., 2013) as well as neuroprotective, antinociceptive, antioxidant, and anti-inflammatory effects (Hou et

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al., 2012), (Benincá et al., 2011).

The leaves of Rosmarinus officinalis are widely cultivated. In ethnomedicine, they have been used for their stimulant, analgesic, anti-inflammatory, and anti-fatigue effects, providing relief from physical and mental fatigue, anxiety, and improving memory and learning. Confirmed pharmacological actions of rosemary include antibacterial and antioxidant activities, antiviral effects, anti-inflammatory properties, antifungal properties, antiproliferative activity against cancer cells, non-genotoxic activity, and reduction of thrombin (Bajalan et al., 2017; Al-Megrin et al., 2020; Altinier et al., 2007; Malvezzi de Macedo et al., 2020; Nieto et al., 2018; Lamponi et al., 2021).

Regarding biological models used to investigate its antidepressant properties (Pangcong Liu, 2021; Guo et al., 2018), rosemary infusion has shown anticholinesterase and antidepressant/anxiolytic properties (Ferlemi et al., 2015). One study on normal volunteers reported positive cognitive effects following ingestion of rosemary water, similar to those elicited after inhalation of rosemary essential oil aroma (Moss et al., 2018).

Essential oils (EOs) are normally obtained through steam distillation of the aerial and underground parts of aromatic plants and are a mixture of naturally occurring molecules (Anastasiou et al., 2020). These compounds have been used in traditional medicine and have demonstrated broad-spectrum antibacterial activity. Due to this characteristic, EOs are of particular interest as alternative drugs to antibiotics. The action of EOs on bacterial and fungal infections relies on their biocidal effect on the integrity of the cell wall and cell membrane through permeation into the polysaccharide matrix of biofilm and its disruption (Nut et al., 2021). Another research describes that EOs can interact with proteins on the bacterial surface and the first attachment protein of bacteria to an inert surface, suggesting that EOs also have anti-adhesion capacity (Lagha et al., 2019).

Since the 21st century, natural EOs have been studied for their efficacy against germs, antioxidants, and cancers, offering potential for treating and preventing various pathologies (Tian et al., 2022; Hong et al., 2022). The chemical composition of phytochemicals in EOs is influenced by several factors, such as plant species, stocking period, soil type, climate, composition and proportion of collected plants, variety, age, and the agrochemicals used for plant preparation (Borges et al., 2019). Studies using animals or humans often do not provide sufficient details about the biological characterisation of plant material and phytochemical analysis of EOs. This study aimed to evaluate the biological activities of rosemary essential oil, including its antioxidant and anti-inflammatory properties.

Materials and Methods

Essential Oil Extraction

Rosmarinus officinalis L. was collected from Rawa, Iraq, in March 2023. The leaves were dried for two days before being extracted at a temperature of 25°C. Steam distillation was used to extract the essential oil using a glassware distillation device. 100 g of dried rosemary leaves were distilled in 900 mL of water for three hours. Finally, the essential oil was preserved in airtight vials.

Gas chromatography analysis

The Rosemary essential oil was examined using an Agilent 7890 GC-MSD system equipped with a 5975-mass selective detector (MSD). The dimensions of the GC Capillary Column were 30 m in length, 0.25 mm in diameter, and 0.25 µm for film thickness. The study was performed utilizing nitrogen as the carrier gas, at a 1 mL/min flow rate. Next a three-minute period at a temperature of 60 °C, The GC oven was set to raise the temperature by 20 °C every minute to 280°C for a duration of 10 minutes, and ultimately to 300 °C. The sample was diluted in methanol, 1:100, v/v) and delivered at a constant temperature of 250 °C for one minute, using a split ratio of 1:20 (Rufino et al. 2015). The individual component's concentration and retention time were determined using the area percentage of the GC-MS chromatogram. The retention index (RI) of the series (n-C7-C40) of n-alkane homologous groups was determined using the same column and circumstances (Adams, 2012).

Protein denaturation assay

Anti-inflammatory properties were examined using Protein denaturation assay according to Becer et al. (Becer et al., 2023). The experiment involved the combination of various quantities of rosemary essential oil (800, 400, 200, 100, 50, and 25 mg/mL) with a 50 mL solution of BSA at a concentration of 1.5 mg/mL. Next, the groups were incubated at 37°C for 20 minutes. The mixes were incubated at 37 °C and then incubated at 55 °C for 5 minutes. Later a volume of 25 mL of the phosphate buffer solution (pH=6.3) was added to each mixture. Finally, the samples went through standardisation. They underwent treatment with the Folin-Ciocalteu reagent and alkaline copper reagent (1:1, v/v) at a concentration of 1 per cent. This was done by the repeated addition of the two mentioned reagents (three times). The samples were stirred well after each addition. Afterwards the samples were incubated at 55°C for 10 minutes and allowed to cool to ambient temperature. Then they were read at 650 nm using the BioTek Synergy H1 Multimode Microplate Reader. The experiment was conducted in triplicates.

The protein denaturation inhibition % was determined using Equation (1).

$$\text{inhibition }\% = \left(1 - \frac{\text{ODs}}{\text{ODc}}\right) \times 100$$

In this case, ODs stands for sample absorbance and ODc for control absorbance.

Antioxidant properties

At the same time, the antioxidAP) test, was determined. Based on (Berenice et al., 2019), a greatly modified method has been used to evaluate the reducing power of the samples. Every run of the experiment started with a fresh FRAP reagent produced right in the middle of the experiment. This reagent was prepared by mixing 300 mM of acetate buffer, at pH 3.6 with 20 mM of FeCl3.6H2O and 10 M of 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ), dissolved in 40 M of HCl, in a ratio of 5:1:1. The standards were prepared using FeSO4.7H2O solutions. For every sample, 60 µL of test samples (standard, rosemary EO, control and blank) were mixed with an equal volume of 55 µL of 300 mM acetate buffer (pH = 3.6) and 80 µL of the newly produced FRAP reagent and the reduction reaction might continue for extra 5 min after that point. Once the samples were transferred to a microplate, the response was measured using spectrophotometry at 594 nm.

Statistical Analysis

The data was shown as the mean and standard deviation of all replicates. The SPSS v23 program was used for statistical analysis. The differences among groups were analyzed using ANOVA and Tukey tests.

Results and Discussion

Phytochemical Components

The phytochemical composition of rosemary essential oil was analyzed using GC-MS technology, revealing 24 components that account for 99.95% of the oil (Table 1). The primary constituents were camphene (6.81%), borneol (5.07%), camphor (13.75%), α-pinene (20.98%), and eucalyptol (1,8-cineole) (34.25%). Monoterpenes dominated the essential oil compounds, constituting 94.88%, while sesquiterpenes comprised only 5.07%. These findings align with previous research on rosemary from Palestine (Al-Maharik et al., 2022).

Research indicates significant variability in the relative abundances of camphor, 1,8-cineole, and α-pinene, suggesting the existence of two intermediate species: the 1,8-cineole/camphor type and the camphor/1,8-cineole/borneol type (Lakusic et al., 2012). The sample in this study is clearly of the 1,8-cineole chemotype.

Socaci et al. (2008) identified the main constituents of fresh R. officinalis essential oil as 1,8-cineole (6.08%), octanone (7.46%), and α-pinene (72.45%). According to Pintore et al. (2002), essential oils from Corsica and Sardinia contain verbenone (24.9%, 4.4%), α-pinene (24.6%, 13.7%), bornyl acetate (17.0%, 11.3%), camphor (14.1%, 2.9%), and 1,8-cineole (11.3%, 3.4%). Additionally, Akrout et al. (2010) identified three separate R. officinalis chemotypes in essential oils from Algeria, Greece, Italy, Yugoslavia, Tunisia, Morocco, and France, containing approximately 20-30% of 1,8-cineole, camphor, and α-pinene. The relatively high concentration of myrcene in Argentinean and Portuguese oils provides another possible chemical composition (Akrout et al., 2010).

The observed diversity in the chemical composition of rosemary essential oil can be attributed to variations in plant cultivation, harvesting practices, bioclimate, and geographical location (Al-Maharik et al., 2022).

Anti-inflammatory activity:

Rosemary essential oil has demonstrated the ability to reduce inflammation, highlighting one of its significant biological activities. The anti-inflammatory properties of rosemary essential oil were evaluated using a denaturation assay of bovine serum albumin. The results revealed that rosemary essential oil effectively inhibits protein breakdown, suggesting its potential application as an anti-inflammatory agent in the pharmaceutical sector.

Table 2 summarizes the anti-inflammatory effects of rosemary essential oil. The data show that the prevention of protein (bovine serum albumin) denaturation varies with the concentration of rosemary essential oil. The essential oil was tested at concentrations ranging from 25 to 800 mg/mL, and the inhibition ratio increased from 19.75% to 81.20% as the concentration increased. Diclofenac sodium (25 mg/mL) was used as a positive control and inhibited protein denaturation by 25.84%. While rosemary essential oil inhibits protein breakdown, its inhibition of protein denaturation is lower than that of the positive control. The major components of rosemary essential oil, such as camphor, α-pinene, and 1,8-cineole, are believed to contribute to these anti-inflammatory properties (Borges et al., 2019; Rufino et al., 2015).

Antioxidant activity:

Compared to synthetic chemical antioxidants, plant-based antioxidants may be safer, offer more therapeutic benefits, and effectively scavenge free radicals. An accumulation of free radicals poses a significant threat to human health and is linked to various degenerative diseases, such as diabetes, Alzheimer's, cardiovascular diseases, neurodegenerative disorders, cancer, and chronic renal disorders. Natural antioxidants are increasingly used to treat a range of illnesses, as oxidative stress plays a crucial role in disease development. Several industries utilize R. officinalis essential oil (EO) as a bio-preservative due to its antibacterial and antioxidant properties. Research has also revealed numerous positive health effects of rosemary EO (Raškovic´ et al., 2014; Ojeda-Sana et al., 2013).

Both rosemary essential oil and the positive control, Trolox, were tested for antioxidant activity. The results showed an inhibition rate of 87.45% for rosemary essential oil, compared to 90.18% for Trolox. Figure 1 illustrates the inhibition percentages. The

Table 1. The phytochemical composition of the essential oil from rosemary leaves

Phytochemical Name	content %
1,8-Cineole	34.25
α-Pinene	20.98
Camphor	13.75
Camphene	6.81
Borneol	5.07
β-Pinene	4.58
α-Terpineol	2.77
Verbenone	2.61
p-Cymene	1.82
β-Caryophellene	1.26
Terpinene-4-ol	0.96
Linalool	0.71
Pinocarvone	0.69
γ-Terpinene	0.58
α-Terpinene	0.42
Myrcene	0.39
Terpinolene	0.36
Methyl eugenol	0.36
Isobornyl acetate	0.35
Caryophyllene oxide	0.34
α-Copaene	0.29
δ-Cadinene	0.24
Tricyclene	0.23
γ-Muurolene	0.13
Total	99.95

Table 2. Determination of the inhibition of BSA denaturation at different rosemary essential oil concentrations

REO concentration (mg/mL)	% Protein Denaturation Inhibition	P value
Diclofenac sodium (25 mg/mL)	25.84	-
25	19.75	n.s
50	24.37	n.s
100	31.90	n.s
200	42.65*	0.05
400	63.72**	0.01
800	81.20***	0.001

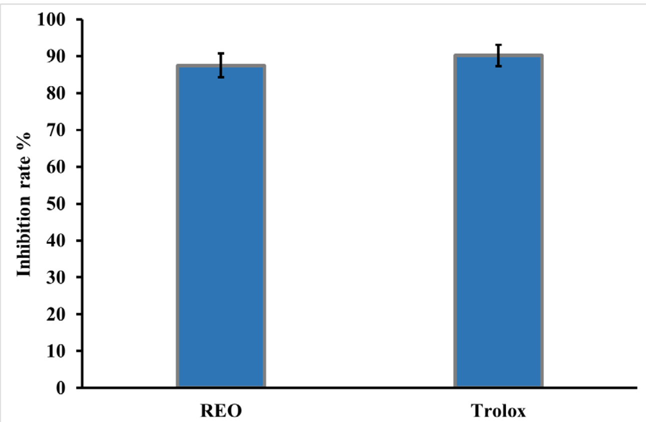

Figure 1. Antioxidant property for rosemary essential oil (REO) and Trolox

exceptional antioxidant activity of rosemary essential oil is likely due to its high concentration of α-pinene.

Variations in the quantities of key components in rosemary essential oils affect their radical scavenging abilities. Insawang et al. (2019) state that 1,8-cineole is the most effective monoterpene at reducing reactive oxygen species compared to other monoterpenes. Goze et al. (2016) demonstrated that these terpenes exhibit antioxidative properties similar to phenolic mixtures. Rosemary oil with a similar composition showed strong in vitro and in vivo antioxidant capacity (Raškovic et al., 2014).

Conclusion

In conclusion, this study demonstrated the presence of key constituents such as eucalyptol (1,8-cineole), α-pinene, camphor, camphene, and borneol. The antioxidant activity of rosemary essential oil can mitigate oxidative stress and subsequent cellular injury, while its anti-inflammatory properties can alleviate pain and inflammation. These attributes suggest that rosemary essential oil could be a valuable therapeutic natural ingredient for use in aromatherapy and pharmaceuticals. The findings underscored the potential of rosemary essential oil as an effective alternative to synthetic chemical antioxidants and anti-inflammatory agents, offering a natural remedy for various health conditions and reinforcing its historical use in traditional medicine. Further research and development could optimize its application in diverse therapeutic contexts.

Author contributions

D.Y.K. conducted the practical work and edited the manuscript. O.M.H. performed the statistical analysis, wrote the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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