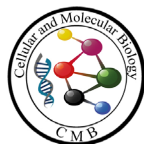
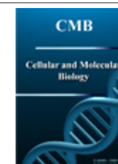
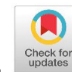
Cell. Mol. Biol. (ISSN: 1165-158X)



Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology





Original Article

Unveiling the therapeutic potential: anti-inflammatory and antioxidant properties of selective medicinal plants

Najeh Krayem [1], Farah Jribi [1], Mona Alonazi [2], Musarat Amina [3], Habib Horchani [4], Aida Karray [1], Slim Cherif [1], Abir Ben Bacha[2*]

[1] *Laboratory of Biochemistry and Enzymatic Engineering of Lipases, ENIS, University of Sfax, Soukra Road, BP1171, Sfax 3038, Tunisia*

[2] *Department of Biochemistry, College of Science, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia*

[3] *Department of Pharmacognosy, Pharmacy College, King Saud University, Riyadh 11495, Saudi Arabia*

[4] *Science Department, Environmental and Biotechnology Research Group, College of Rivière-Du-Loup, Rivière-Du-Loup, QC G5R 1R1, Canada*

Article Info

Article history:

Received: November 10, 2024
Accepted: January 31, 2025
Published: March 31, 2025

Use your device to scan and read the article online

Abstract

This study highlights the potential of plant extracts as sustainable and cost-effective alternatives to traditional anti-inflammatory drugs, owing to their rich bioactive compounds. The chemical composition and biological activities of ethanolic extracts from Artemisia campestris, Haloxylon articulatum, and Retama raetam were investigated. Extraction yields ranged from 2.94% to 6.84%, with A. campestris showing the highest phenolic content (85.59 ± 2.4 mg GAE/g) and R. raetam having the highest flavonoid concentration (34.77 ± 3.09 mg CE/g). HPLC analysis identified therapeutic phenolic and flavonoid compounds, including sinapic, quinic, and caffeic acids in A. campestris, p-coumaric acid in H. articulatum, and salicylic acid in R. raetam. Antimicrobial tests revealed that Gram-positive bacteria like Staphylococcus aureus and Bacillus cereus were sensitive to the extracts, though Gram-negative strains were unaffected. Antifungal activity was limited, with only H. articulatum showing inhibition of Rhizoctonia solani. Strong antioxidant activities were noted, particularly in H. articulatum and R. raetam extracts (IC50 = 130 µg/mL). In anti-inflammatory assays, all extracts exhibited dose-dependent inhibition of enzymes linked to inflammation, including COX-1, COX-2, 5-LOX, and sPLA2. A. campestris demonstrated the most potent inhibition, reaching 100% inhibition of sPLA2 at 200 µg/mL, while A. campestris and R. raetam provided significant protection in human red blood cell membrane stabilization assays. These results suggest that these plant extracts have considerable biological potential, especially in enzyme inhibition related to inflammation, making them promising candidates for future therapeutic use.

Keywords: Plant extracts, Anti-inflammatory, Phenolic, Flavonoid, Antimicrobial anti-oxidant.

1. Introduction

Inflammation is a common biological response initiated by disturbances in tissue balance induced by several factors such as infections, injuries, oxidative stress, or environmental toxins. This response involves the activation of both the innate and adaptive immune systems [1]. Oxidative stress, which results from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defenses, is closely associated with inflammation. Overproduction of ROS can damage cellular components and trigger inflammatory pathways, leading to a persistent cycle of oxidative stress and chronic inflammation. Consequently, addressing both oxidative stress and inflammation is crucial for effectively managing diseases related to inflammation [2].

Inflammatory mediators such as eicosanoids, leukotrienes, prostaglandins, and thromboxane produced by the cyclooxygenase (COX) and lipoxygenase (LOX) pathways contribute to the development of chronic inflammation [3]. COX and LOX enzymes are involved in arachidonic acid (AA) metabolism released by certain secretory phospholipases A_2 ($sPLA_2$), in particular group (G) IIA, IB, X, and exogenous GV-$sPLA_2$ isoforms [4]. Prostaglandin production is mediated through the two COX isozymes, COX-1 and COX-2 [4]. The isozyme COX-1 is constitutively expressed in most tissues while COX-2 is inducible [5]. Eicosanoids and leukotrienes are synthesized through a critical secondary biosynthetic pathway that is initiated by the activation of 5-LOX. LOX enzymes are classified into 5/8/12/15-LOX classes according to their positional specificity of AA oxygenation: the 5-, 12- and 15-LOX insert O2 at the C-5, -12 and -15 positions of AA, respectively, and produce the 5-, 12- and 15- hydroperoxy-eicosatetraenoic acid [4]. This pathway leads to the production of leukotriene B4, a strong inflammatory mediator associated with the onset of conditions like atherosclerosis, cancer,

* Corresponding author.
E-mail address: aalghanouchi@ksu.edu.sa (A. Ben Bacha).
Doi: http://dx.doi.org/10.14715/cmb/2025.71.3.11

and cardiovascular diseases [3].

Non-steroidal anti-inflammatory drugs (NSAIDs) are often the primary treatment choice for inflammatory diseases because they target COX-1 and COX-2 which are responsible for producing the key mediators of inflammation. By inhibiting these enzymes, NSAIDs reduce the production of pro-inflammatory eicosanoids [3]. However, long-term treatment with traditional NSAIDs such as ibuprofen, indomethacin, diclofenac, ketoprofen, naproxen, piroxicam and nabumetone is associated with gastrointestinal bleeding, ulceration and perforation [6].

The non-selective inhibition of the constitutive COX-1, or the simultaneous inhibition of both COX-1 and COX-2, is linked to the significant adverse effects of NSAIDs. The gastrointestinal complications associated with traditional NSAIDs, along with the withdrawal of selective COX-2 inhibitors like valdecoxib and rofecoxib from the market due to cardiovascular risks, have further complicated the treatment of inflammation [7]. In the search for new drugs that minimize side effects while maintaining strong anti-inflammatory efficiency, the innovative dual inhibition of COX-2 and 5-LOX has been proposed [7]. Several dual inhibitors have been explored to inhibit COX-2 and 5-LOX enzymes, aiming to prevent the production of prostaglandins and leukotrienes, which are involved in inflammatory processes. This multi-target approach is gaining popularity in drug discovery due to its enhanced safety profile and reduced risk of side effects on the gastrointestinal, renal, and cardiovascular systems [6]. Darbufelone, tepoxalin and licofelone were designed as anti-inflammatory drugs and tested as inhibitors of both COX and 5-LOX in humans in phase III clinical trials [3,7]. However, their commercialization did not proceed because of high kidney and liver toxicity and insufficient efficacy [7]. This highlights the need to find or design new drugs able to inhibit both COX and 5-LOX enzymes, as this approach offers a promising avenue for creating compounds with improved efficacy and fewer side effects. Such dual inhibitors could be beneficial not only for controlling inflammation but also for treating other conditions, such as cancer, where both COX-derived prostanoids and LOX-derived leukotrienes are involved [3]. Therefore, identifying new dual inhibitors of both COX-2 and 5-LOX could improve the anti-inflammatory efficacy of drug candidates. In line with this, medicinal plants are widely used in traditional herbal medicine and are well-regarded for their effectiveness in addressing various diseases, especially inflammation [8]. The use of phytotherapeutic remedies for treating chronic inflammatory conditions can offer an effective alternative to patient therapy [9]. Actually, the pharmacological effects of various used plants are primarily attributed to their content of polysaccharides (such as inulin), phenolic compounds, and polyphenols like flavonoids (quercetin). They also contain terpene compounds found in essential oils (such as anethole, apiol, and carvone), fatty acids (petroselinic acid), coumarins (coumarin, umbelliferone), phenolic acids (gallic acid, ellagic acid), hydrolysable tannins (gallotannin), as well as steroid and phenolic glycosides (sitosterin, urticin) [9]. It has been reported that the anti-inflammatory effect of several plant extracts is attributed to the polyphenol contents that are able to incorporate into AA metabolism by inhibiting various pro-inflammatory enzymes COX-1/2, LOX, and $sPLA_2$s [9].

A. campestris belonging to the Asteraceae family, is a polymorphic species with several subspecies and varieties, known for its mild aromatic properties. It is native to North Africa, particularly prevalent in central and southern Tunisia [10]. Research has highlighted the antivenom, anti-inflammatory, antirheumatic, and antimicrobial properties of several *A. campestris* extracts [10]. The phytochemical analysis of *A. campestris* identified the presence of tannins, polyphenols, flavonoids, and saponins, as major compounds as well as essential oils [11].

H. articulatum, belonging to the Chenopodiaceae family, is predominantly found in desert and semi-desert regions with high-salinity soils. It is commonly used in traditional medicine, particularly for treating diabetes, and is noted for its antiseptic and anti-inflammatory properties since its leaves and stems are particularly rich in alkaloids and phenolic compounds [12].

The genus *Retama* belongs to the Fabaceae family and comprises four species found in the Mediterranean region and North Africa. *Retama raetam* (*R. raetam*) is a native plant commonly found in the North and East Mediterranean regions. Pharmacological research has identified a range of medicinal properties for this plant, including antimicrobial, antioxidant, antiviral, diuretic and hypoglycemic. Additionally, Retama species are known to contain essentially flavonoids and alkaloids [13].

The biological effects observed in various plant extracts have been attributed to a complex mixture of several compounds among them monoterpenes, oxygenated monoterpenoids, sesquiterpenes, phenolic acids, flavonoids, and phenylpropanoids [4]. The extensive array of bioactive compounds found in these plants presents a promising opportunity for developing new therapies. This underscores the need for continued research to fully explore and use these natural molecules in the fight against several diseases notably inflammation. Although the described *in vitro* and especially in vivo anti-inflammatory effect of extracts from *A. campestris*, *H. articulatum* and *R. raetam*, the inhibitory effect of enzymes involved in inflammation pathways was not investigated so far. Accordingly, this study aimed to address the current gap in knowledge by assessing the anti-inflammatory activity of selected traditional medicinal plants, thereby contributing to a deeper understanding of their therapeutic potential. Therefore, in addition to analyzing and identifying the chemical compositions of the ethanolic extracts of three medicinal plants (*A. campestris*, *H. articulatum*, and *R. raetam*), the present study focused on evaluating substantial antioxidant activity and inhibiting most important inflammatory enzymes such as sPLA2, COX-1, COX-2, and 5-LOX. Through investigating the relationship between inflammation and oxidative stress, this study also established a basis for creating natural, plant-based treatments for inflammatory diseases.

2. Materials and Methods

2.1. Extraction method

The plants used in this study *A. campestris*, *H. articulatum*, and *R. raetam* were collected from the region of Sfax in Tunisia. The aerial parts of the three plants were first dried in the shade, weighed and macerated in an amber bottle containing ethanol solvent at a 1/5 ratio (weight/volume), with regular agitation in an orbital shaker set at 160 rpm at room temperature for 72 h. Then, the ethanol extracts were concentrated under vacuum using a rotary

evaporator at 60°C and subsequently stored at 4 °C until further use.

2.2. Determination of total phenols

The total phenolic content of the ethanolic extracts of *A. campestris*, *H. articulatum* and *R. raetam* was determined using the Folin-Ciocalteu reagent composed of acidic polyheterocycles containing phosphotungstic and phosphomolybdic acids. Following the oxidation of phenolates and the reduction of the polyheterocycles, a stable blue molybdenum-tungsten complex is formed, which strongly absorbs at 765 nm [14]. For the assay, 200 µL of each extract was mixed with 1 mL of 10-fold diluted Folin-Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate. After mixing, the mixtures were incubated in the dark for 30 minutes. The absorbance was then measured at 765 nm. A standard curve using different concentrations of GA was prepared simultaneously to interpret the results. The total phenolic content of the extracts is expressed as mg GAE/g DW.

2.3. Determination of total flavonoids

The total flavonoid content was measured using aluminum chloride and sodium hydroxide. A volume of 200 µL of the diluted extract was mixed with 60 µL of 5 % $NaNO_2$. After 5 minutes, 60 µL of 10% $AlCl_3$ was added and mixed using a vortex. After 5 minutes, 400 µL of 1 M NaOH was added. The absorbance was immediately measured at 510 nm against a blank containing only the reagents. The total flavonoid content is expressed as milligrams of catechin equivalent per gram of extract, based on a standard curve of GA [14].

2.4. High-performance liquid chromatography (HPLC) analysis

The ethanolic extracts of *A. campestris*, *H. articulatum*, and *R. raetam* were subjected to HPLC analysis using a LC-20AD HPLC system (Shimadzu, Japan). A system comprised of a binary solvent delivery system (LC-20AD), a Rheodyne-type injector with a 20 µL sample loop, and a DAD detector (SPD-M 20 A) was applied for the analysis of prepared extracts. Chromatographic separation was conducted on a Capcell Packed C-18, (MGII, 250 mm × 4.6 mm dimensions and 5 µm particle sizes) with an extended guard column using a reverse phase column mechanism. The mobile phase constituted the Methanol-acetonitrile water (40:15:45, v/v/v) with 1.0% acetic acid and isocratic elution for 30 minutes. Software Shimadzu LC Solution was employed to collect and process the data. The Diode array detector was limited to a wavelength range of 240 to 800 nm. 20 µL of the sample volume and reference solution were administered, at a flow rate of 1 mL/min. The peaks were found by monitoring retention times and examining UV spectra. These findings were then verified by running the samples with small amount of the standards and comparing them to reference standards.

2.5. Antimicrobial activity assay

The antimicrobial activity of the different extracts was initially assessed by measuring the diameters of bacterial growth inhibition zones using the agar well diffusion method, as described by Berghe and Vlietinck [15]. After inoculating Luria Broth agar medium (LB) with approximately 10^6 CFU/mL of the tested microorganism, 5 mm diameter wells were punched into the agar. A volume of 100 µL of each extract, at concentrations of 100 and 300 mg/mL, was introduced into the wells. Ampicillin (25 µg/well) was used as a positive control. The antimicrobial activity of each extract was determined by measuring the diameter of the microbial growth inhibition zones around the wells after incubation for 24 hours at 37°C.

The MIC was determined using the method of Eloff [16]. In a 96-well plate, 180 µL of liquid LB medium and 20 µL of each bacterial suspension (10^6 CFU/mL) were added to each well. Then, 100 µL of each extract was added to the first well from which serial dilutions were performed across the plate which was incubated for 24 h at 37°C. Bacterial growth was detected by adding 20 µL of MTT solution ((3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) at 0.5 mg/mL to each well. This reagent reacts with succinate dehydrogenase in live bacterial cells, producing a purple color while dead cells produce a yellow color. The MIC is defined as the lowest concentration of the extract (mg/mL) that completely inhibits visible bacterial growth. The bacterial strains used were: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14759, *Listeria monocytogenes* ATCC 43251, *Salmonella enterica* ATCC 43972, and *Pseudomonas aeruginosa* ATCC 27853.

2.6. Antifungal activity

Three fungal strains *Rhizoctonia solani*, *Fusarium graminearum*, and *Fusarium verticillioides* were used to assess the antifungal activity of the studied ethanolic extracts. These fungal strains were kindly provided by the microbial collection of the Sfax Biotechnology Center (Sfax-Tunisia). Strains were cultured on Yeast-Peptone-Dextrose Agar (YPDA) for 3 to 5 days at 25°C, and the spore count was determined using a Thomas counting chamber. The antifungal activity was evaluated using the microdilution method in a sterile 96-well plate. In each well, 80 µL of liquid YPD medium and 10 µL of a fungal spore solution (10^5 spores/mL) were added. A volume of 100 µL of each extract was placed in the first well, followed by serial dilutions. Negative control wells contained 90 µL of YPD and 100 µL of ethanol. The plates were incubated at 25°C for 3 days. The MIC was defined as the minimum concentration of the extract that completely inhibited visible fungal growth after incubation.

2.7. Antioxidant assays

The antioxidant activity of the different ethanolic extracts was assessed using the 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and reducing power assay. The DPPH radical scavenging capacity was determined using the method described by Bersuder et al. 1998 [17]. A volume of 500 µL of each extract at different concentrations (ranging from 0.1 to 1 mg/mL) was mixed with 375 µL of absolute ethanol and 125 µL of a 0.02% DPPH solution dissolved in the same solvent. A negative control was prepared in parallel without the extract. Butylated hydroxyanisole (BHA) was used as a reference antioxidant (positive control). The DPPH radical scavenging activity expressed in percentage was calculated as follows:

DPPH radical scavenging (%) = $[(A_{control} - A_{extract}) / A_{control}] \times 100$

Where: $A_{control}$: Absorbance of the control reaction at 517 nm, prepared by mixing 875 µL of absolute ethanol

with 125 µL of 0.02% DPPH. $A_{extract}$: Absorbance of the extract at 517 nm.

The reducing power of iron (Fe^{3+}) to ferrous iron (Fe^{2+}) for the different extracts was determined using the method described by Das et al. [18]. A volume of 0.5 mL of each extract at increasing concentrations (ranging from 0.1 to 1 mg/mL) was mixed with 1 mL of 1% potassium ferricyanide and 1 mL of 0.2 M phosphate buffer (pH 6.6). After incubation at 50°C for 30 minutes and subsequent cooling, 1 mL of 10% trichloroacetic acid was added, followed by 15 minutes of decantation. A volume of 1.5 mL of the resulting supernatant was mixed with 1.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. After incubation at room temperature for 10 minutes, the absorbance was recorded at 700 nm. The positive control was represented by a solution of BHA at various concentrations.

2.8. Assessment of *In Vitro* anti-inflammatory activity

2.8.1. HRBC membrane stabilization

Human red blood cell (HRBC) membrane stabilization was used to evaluate the *in vitro* anti-inflammatory activity of *A. campestris*, *H. articulatum* and *R. raetam* extracts, following the method of Gandhidasan et al. [19]. A total of 5 mL of human blood was collected from ten healthy volunteers aged 35-45, who had not used NSAID medications for at least 2 weeks. The blood was centrifuged at 2500 rpm for 5 min, and the supernatant was discarded. Cells were resuspended in an equal volume of isosaline, and centrifuged again at 2500 rpm for 5 min, and this process was repeated 4-5 times until the supernatant was clear. A 10% HRBC suspension was prepared with normal saline buffer and stored at 4°C until use. For the assay, a 4.5 mL reaction mixture was prepared with 1 mL isosaline buffer (pH 7.4), 2 mL hyposaline (0.25% NaCl), varying volumes of the 10 mg/mL extract solution in isotonic buffer, and 0.5 mL of 10% HRBC in normal saline. After incubation at 56°C for 30 min, the mixture was cooled for 20 min and centrifuged at 2500 rpm for 5 min. The absorbance of the supernatant was recorded at 560 nm. Two controls were used: control 1 (1 mL of isosaline buffer instead of extract) and control 2 (1 mL of extract solution without HRBC). The percentage of membrane stabilization was calculated using the following formula:

Percentage of Stabilization= $100-(V_{extract}-V_{Control1}/V_{Control2})\times100$

where V, V1, and V2 are the absorbance values of the tested extract, control 1, and control 2, respectively. Diclofenac sodium served as the drug reference standard, while control 1 corresponds to 100% HRBC lysis.

2.8.2. Secreted PLA_2 ($sPLA_2$) inhibition

The phospholipase activities were assessed using the method outlined by De Araújo and Radvanyi [20]. To evaluate the inhibitory effects of various extracts, dromedary ($DrPLA_2$) and human ($hPLA_2$) groups V and IIA phospholipases were used. 10 µL of each ethanolic extract was mixed with 10 µL of $DrPLA_2$-GIIA or $DrPLA_2$-GV and incubated for 20 min at room temperature. This mixture was then added to 1 mL of substrate, which included lecithin (3.5 mM) dissolved in 100 mM NaCl, 3 mM sodium taurodeoxycholate, 10 mM $CaCl_2$, and 0.055 mM red phenol at pH 7.6. Phospholipase activity was monitored spectrophotometrically by measuring absorbance at 558 nm over 5 min. The percentage of inhibition was calculated based on the residual activity relative to a negative control without extract. IC50 values were derived from the resulting curve. Each experiment was conducted in duplicate.

The $sPLA_2$-GV inhibitor screening assay kit (Cayman test kit 10004883, Cayman Chemical Company, Ann Arbor, USA) was utilized to evaluate the inhibitory effects of four concentrations (25, 50, 100, and 200 µg/mL) of the extracts according to the manufacturer's protocol. This kit includes reaction mixtures and human recombinant $sPLA_2$-GV. Arachidonic thioester phosphatidylcholine (TEPC) served as the positive control. To conduct the assay, 10 µL of each extract was preincubated in a 96-well plate with 25 mM Tris-HCl buffer (pH 7.5) and 10 µL of enzyme. The reaction was initiated by adding 200 µL of 1.66 mM diheptanoylthio-PC, followed by shaking the plate for 30 seconds and incubating for 15 min at room temperature. The reaction was halted by adding 10 µL of 5,5′-dithiobis-(2-nitrobenzoic acid), and the plate was shaken for 1 min. The absorbance of the resulting color was measured at 405 nm using a plate reader. Each experiment was conducted in duplicate.

2.8.3. COX-1/2 inhibition assay

The COX inhibitory activity of the studied extracts was assessed using a colorimetric COX inhibitor screening assay kit (Cayman Chemical Company, catalog no. 560131), following the manufacturer's protocol. Extracts were tested in duplicate at concentrations ranging from 25 to 200 µg/mL. The assay measures the amount of prostaglandin-F2α produced during the COX reaction, with diclofenac sodium used as a positive control for its ability to inhibit both COX-1 and COX-2 enzymes. Each extract (10 µL) was mixed with an equal volume of Tris-HCl buffer (0.1 M, pH 8.0) and preincubated with the enzyme (100 units of ovine COX-1 and human recombinant COX-2) for 15 min at 37°C. Then, 10 µL of AA (10 mM) was added to the reaction mixture, which was then incubated for an additional 2 min at 37°C. The reaction was terminated by adding saturated stannous chloride and 50 µL of HCl (1 N). A sample of the solution was then analyzed spectrophotometrically to quantify the prostanoid produced.

2.8.4. LOX inhibition assay

The inhibition of 5-LOX was evaluated in duplicate using the colorimetric LOX inhibitor screening assay kit (Cayman Chemical Company, catalog no. 766700), following the manufacturer's guidelines. This assay quantifies the amount of hydroperoxide generated by the 5-LOX enzyme acting on its substrate, AA. In a 96-well plate, 90 µL of 5-LOX enzyme was preincubated with 10 µL of each test extract, at concentrations of 25, 50, 100, and 200 µg/mL. After that, 10 µL of 1 mM AA was added to the plate, which was then shaken for 5 min. The reaction was stopped by adding 100 µL of the chromogen and the plate was shaken for 5 min. The absorbance of the resulting color was measured at 490 nm. Nordihydroguaiaretic acid (NDGA) served as the positive control.

2.8.5. Statistical analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation. IC_{50} values were calculated using dose-response curves fitted with non-linear regression analysis.

3. Results

3.1. Determination of extracts' yields and phenols and flavonoids quantification

The extracts' yields of *A. campestris*; *H. articulatum* and *R. raetam* using ethanol as solvent were determined. As presented in Table 1, extraction yields ranged from 2.94% to 6.84% and were comparable to previous findings for *A. campestris* (3.33%±0.09%) [21] and *H. articulatum* (4.27%±0.98%) from Algeria [22]. In contrast, higher yields were reported for *A. campestris* from Morocco (14%) [23] and west-central Tunisia (13.62%±4.92%) [10]. Likewise, *R. raetam* extraction yields ranged between 10.68% and 15.36% [24]. These variations likely stemmed from differences in the availability of extractable components due to the plants' chemical composition, which varies by region, as well as the efficiency of the extraction methods used [25]. Also, the calibration curves for catechin and gallic acid, which are essential for quantifying flavonoids and polyphenols respectively, are illustrated in Figure 1.

The obtained ethanolic extracts (*A. campestris*, *H. articulatum* and *R. raetam*) were then assessed for their total phenol and flavonoid contents, the most important phytochemical compounds commonly associated with diverse biological properties of plants. The total polyphenol (PP) and flavonoid (Fv) quantification data were presented in Table 1 and clearly showed that the ethanolic extract of *A. campestris* exhibited the highest PP content (85.59±2.4 mg GAE/g), followed by *H. articulatum* (70.66±1.7 mg GAE/g) and *R. raetam* (55.68±2.5 mg GAE/g) extracts. On the other hand, the highest Fv concentration was recorded in *R. raetam* extract (34.77±3.09 mg CE/g) while *H. articulatum* and *A. campestris* extracts displayed comparable Fv concentrations of 29.88±2.9 and 28.99±2.6 mg CE/g, respectively (Table 1).

3.2. HPLC analysis

The HPLC chromatograms of the extracts, along with the identified components, were shown in Figure 2. Quantification was achieved using standard calibration methods, with the amounts of the identified compounds presented in Table 2. The present findings demonstrated that the ethanolic extracts were rich in phenolic and flavonoid compounds. In the *A. campestris* extract, sinapic acid was the most abundant compound (4.45 ± 2.2 mg/g), followed by quinic acid (3.47 ± 0.1 mg/g) and vanillic acid (2.64 ± 0.52 mg/g). The *H. articulatum* extract was notably rich in p-coumaric acid (5.56 ± 0.22 mg/g) and quinic acid (5.42 ± 0.32 mg/g), with moderate amounts of caffeic acid (3.23 ± 0.21 mg/g), ferulic acid (2.46 ± 1.1 mg/g), and 1,5-dicaffeoylquinic acid (2.23 ± 0.54 mg/g). In the *R. raetam* extract, salicylic acid was predominant (5.73 ± 0.24 mg/g), followed by quinic acid (4.57 ± 0.12 mg/g), caffeic acid (3.87 ± 0.25 mg/g), caffeoylquinic acid (3.27 ± 1.1 mg/g), and myricetin (2.58 ± 0.52 mg/g). It is worth noting that all of the quantified phenolic compounds in these extracts have recognized therapeutic applications.

3.3. Antimicrobial activity

The antibacterial activity of all extracts was assessed by measuring the bacterial growth inhibition zones using the agar well diffusion method [26] and determining the minimum inhibitory concentration (MIC) through the serial dilution method in a liquid medium [16]. The agar diffusion method offers a quick and qualitative assessment of antimicrobial activity by measuring the zone of inhibition growth, which indicates the sensitivity of the tested microorganisms. The serial dilution method provides a quantitative evaluation, determining the MIC necessary to inhibit microbial growth. The combination of these methods ensures both an initial screening and precise measurement of the antimicrobial potential of the extracts. Three Gram-negative bacterial strains: *Escherichia coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa* and three Gram-positive strains *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes* were used in order to high-

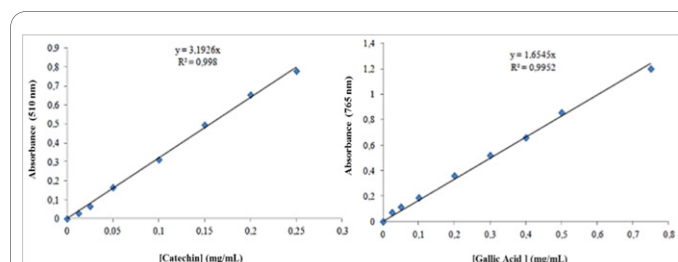


Fig. 1. Calibration curve of catechin (A) and gallic acid(B) for determining flavonoids and polyphenols contents, respectively.

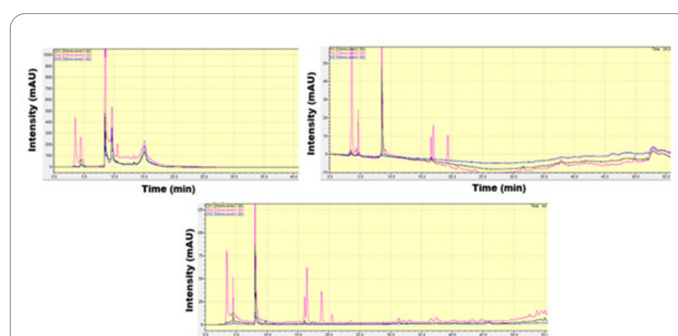


Fig. 2. HPLC chromatogram of ethanolic extract of Artemisia campestris (A.c) (A), Haloxylon articulatum (H.a) (B), and Retama raetam (R.r) (C).

Table 1. Extraction yields, polyphenol and flavonoid contents of *Artemisia campestris* (A.c), *Haloxylon articulatum* (H.a), and *Retama raetam* (R.r) aerial parts using ethanol solvent. The total phenolic content was expressed as milligrams (mg) of Gallic Acid Equivalent (GAE) per gram (g) of dry weight (mg GAE/g DW). The total flavonoid content was expressed as mg of catechin equivalent (CE) per g of DW (mg CE/g DW). Results were presented as the mean value ± standard deviation.

Extract	Extraction yield (%)	Polyphenols content (mg GAE/g DW)	Flavonoids (mg CE/g of extract)
A.c	6.84±1.2	85.59±2.4	28.99±2.6
H.a	4.27±0.98	70.66±1.7	29.88±2.9
R.r	2.94±1.6	55.68±2.5	34.77±3.09

Table 2. HPLC profile of components of *Artemisia campestris* (A.c), *Haloxylon articulatum* (H.a), and *Retama raetam*

Extract	Phenolic/flavonoids compounds	Quantity (mg/g)	Retention time
A.c	Quinic acid	3.47	3.65
	Vanillic acid	2.64	4.53
	cis-Sinapic acid	4.45	8.52
	Cinnamic acid	2.32	9.87
	Apigenin	1.47	10.36
	3,5-dicaffeoylquinic acid	0.21	13.54
	Caffeoyl-quinic acid	1.56	15.52
H. a	Quinic acid	5.42	3.48
	Caffeic acid	3.23	4.21
	p-Coumaric acid	5.56	16.42
	Ferulic acid	2.46	17.02
	1,5-Dicaffeoylquinic acid	2.23	19.21
	Chlorogenic acid	0.15	31.21
	Sinapic acid	0.17	37.98
	m-Coumaric acid	0.12	49.87
R. r	Quinic acid	4.57	3.48
	Caffeic acid	3.87	4.32
	Salicyclic acid	5.73	8.04
	Caffeoyl-quinic acid	3.27	16.23
	Myricetin	2.58	18.87
	Dicaffeoyl derivative	1.01	20.56
	Sinapic acid	0.04	23.48
	Chlorogenic acid	0.03	31.21
	Ferulic acid	0.02	36.63
	Naringin	0.01	43.51
	Isoquercitrin	0.01	44.78

Table 3. Diameter of growth inhibition zones (mm) of bacterial strains treated with 100 and 300 mg/mL of each extract. Minimum inhibitory concentrations (MIC, mg/mL) of Gram-positive strains and three fungal strains: *Rhizoctonia solani*, *Fusarium graminearum* and *Fusarium verticillioides* treated with plant extracts. Results were presented as the mean value with standard deviation.

Extracts/Standard	A.c		H.a		R.r		Ampicillin
Concentration (mg/mL)	100	300	100	300	100	300	25 µg/well
Bacterial strains	**Diameter of growth inhibition zones (mm)**						
Listeria monocytogenes	7±0.6	12±11	10±0.5	17±2	8±1	10±1	24±1
Staphylococcus aureus	9±0.5	15±1.5	4±1.6	12±0.5	8±1	13±2	20±2
Bacillus cereus	5±0.8	9±0.9	6±1	10±1	5±0.5	9±0.5	30±1
Escherichia coli	-	-	-	-	-	-	22±2
Salomonella enterica	-	-	-	-	-	-	35±1
Pseudomonas aerigenosa	-	-	-	-	-	-	21±1.5
			MIC (mg/mL)				
Listeria monocytogenes	9±1.5		9±1		18.75±2		-
Staphylococcus aureus	18.75±1.6		18.75±2		18.75±1.5		-
Bacillus cereus	18.75±2		9±1.8		18.75±1.5		-
Fungal strains	**MIC (mg/mL)**						
Rhizoctonia solani	-		75±3		-		-
Fusarium graminearum	37±2.7		75±2.8		37±3		-
Fusarium verticillioides	75± 3		75±2.5		37±3.5		-

light the spectrum of antimicrobial activity of the extracts and their potential as versatile antimicrobial agents. Table 3 revealed that only the Gram-positive strains displayed sensitivity to the tested extracts, while the Gram-negative strains remained resistant even at the highest concentration of 300 mg/mL. Based on the classification by Ponce et al. [27], strains are considered sensitive when the inhibition zone diameter ranges from 9 to 14 mm, and highly sensitive when the diameter falls between 15 and 19 mm. Results presented in Table 3, therefore, indicated that the three tested strains were sensitive to ethanolic extracts (at a concentration of 300 mg/mL). At a lower concentration of 100 mg/mL, these strains were, however, less sensitive (Inhibition zone diameter less than 8 mm) (Table 3).

Table 3 presented the MIC values of the various extracts, determined within a concentration range of 0.29 to 150 mg/mL. A MIC of 9 mg/mL was observed for both *A. campestris* and *H. articulatum* extracts against *Listeria monocytogenes*, and *Bacillus cereus*. Both extracts inhibited *Staphylococcus aureus* at a MIC of 18.75 mg/mL, while the *R. raetam* extract showed a MIC of 18.75 mg/mL against all three bacterial strains.

The antifungal activity of the different extracts was assessed against three fungal strains (*Rhizoctonia solani*, *Fusarium graminearum*, and *Fusarium verticillioides*) using the agar diffusion and the liquid micro-dilution methods. At 300 mg/mL of extract, no inhibition zones around the wells were observed for all tested fungal strains. Furthermore, to our knowledge, the MIC values of the three extracts were determined, for the first time in the current study, across a concentration range of 0.29 to 150 mg/mL (Table 3). Among the extracts, only *H. articulatum* demonstrated inhibitory activity against *Rhizoctonia solani*, with a MIC of 75±3 mg/mL. All tested ethanolic extracts inhibited the growth of *Fusarium graminearum* and *Fusarium verticillioides*, with MIC values ranging from 37±3 mg/mL to 75±3.5 mg/mL.

3.4. Antioxidant activity

The different extracts from *A. campestris*, *H. articulatum*, and *R. raetam* were tested for their antioxidant activity by determining their ability to scavenge free radicals (DPPH test) as well as their reducing power (FRAP test) and compared and compared to BHA, a reference antioxidant substance.

The DPPH radical is generally one of the most used compounds for the rapid and direct assessment of antioxidant activity due to its stability in radical form and the simplicity of the analysis [17,28]. The DPPH anti-radical activity is typically expressed as the IC_{50} value. As illustrated in Figure 3, the percentage of DPPH radical inhibition increased as the concentrations of the plant extracts as well as the standard (BHA) increased. Interestingly, at all concentrations used, the different extracts were more effective than BHA. At 1 mg/mL, *H. articulatum* extract showed the highest percentage of DPPH inhibition (91.58±0.04%) followed by *R. raetam* extract (90.186±0.07%) and the *A. campestris* extract (70.398±0.03%) whereas the standard, BHA, showed the lowest DPPH inhibition percentage (65.184±0.06%) (Figure 3). As presented in Table 4, *H. articulatum* and *R. raetam* extracts exhibited the highest antioxidant capacity since both recorded the lowest IC_{50} values of about 130 µg/mL. The *A. campestris* extract was less effective showing an IC_{50} of 450 ±10 µg/mL but still lower than that of BHA, (IC_{50} = 500±18 µg/mL) (Table 4).

The FRAP method [18] was used in order to evaluate the extracts reducing power of the Fe^{3+}/ferricyanide complex to its ferrous form (Fe^{2+}). All the extracts, as well as the BHA standard, possessed varying degrees of reducing power in a dose-dependent manner (Data not shown). The antioxidant capacity of the examined extracts was quantified by determining the EC_{50}, which represents the effective concentration that yields an absorbance of 0.5 for reducing power (Table 4). As indicated in Table 4, the iron-reducing efficiency was in the following order BHA > *R. raetam* > *A. campestris* > *H. articulatum*.

3.5. In-vitro anti-inflammatory activity of different extracts

3.5.1. HRBC membrane stabilization

The *in vitro* anti-inflammatory effectiveness of ethanolic extracts of *A. campestris*, *H. articulatum*, and *R. raetam* plants was assessed by measuring the stabilization percentage of HRBC membranes and the inhibition rates of key enzymes involved in inflammation, specifically COX-1, COX-2, 5-LOX, and $sPLA_2$, at various concentrations (25 µg/mL -200 µg/mL).

Data presented in Figure 4 revealed that the *R. raetam* extract, at different concentrations, provided significantly high protection, ranging from 88.25% to 92.5% which was comparable to the effect of diclofenac-sodium used as a positive control. At 200 µg/mL, *A. campestris* and *H. articulatum* extracts exhibited less HRBC protection

Table 4. IC_{50} and EC_{50} values of *Artemisia campestris* (A.c), *Haloxylon articulatum* (H.a), and *Retama raetam* (R.r) extracts and the standard butylated hydroxyanisole (BHA). Results were presented as the mean value with standard deviation.

Extracts	IC_{50} (µg/mL)	EC_{50} (µg/mL)
A.c	450±10	557.95±18.4
H.a	130±9	822.36±19.6
R.r	130±12	498.31±23
BHA	500±18	200±24

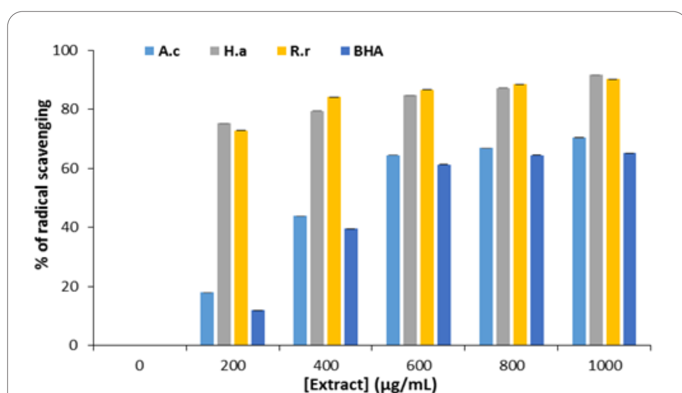


Fig. 3. Scavenging activity of ethanol extracts of Artemisia campestris (A.c), Haloxylon articulatum (H.a), and Retama raetam (R.r) and the standard butylated hydroxyanisole (BHA). Experiments were conducted in triplicate and corresponding results were presented as the mean value with standard deviation.

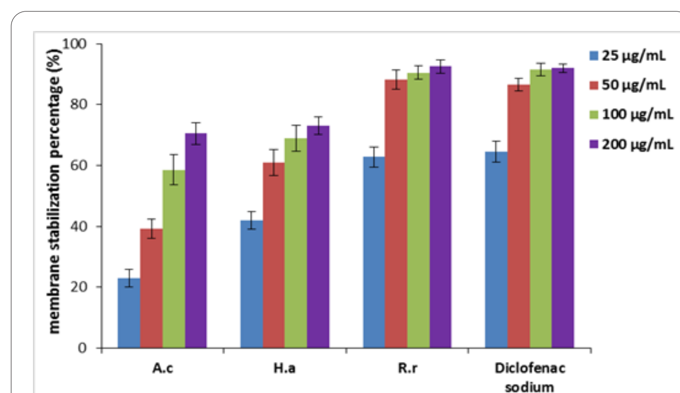


Fig. 4. Degree of membrane stabilization induced by Artemisia campestris (A.c), Haloxylon articulatum (H.a), and Retama raetam (R.r) extracts on human red blood cells, with diclofenac sodium used as the positive control. The results presented are the mean values obtained from three separate replicates.

(70.5±3.53% and 73±2.82%, respectively).

3.5.2. Inflammatory enzyme inhibition

The ethanolic extracts of the three selected plants demonstrated a dose-dependent enzymatic inhibitory potential, with concentrations ranging from 25 to 200 µg/mL, and effectively inhibited the activity of key enzymes associated with the inflammatory process. Particularly, *A. campestris* extract exhibited the most potent efficacy, causing almost complete inhibition of all tested $sPLA_2$s (99%-100%) at a concentration of 200 µg/mL. For comparison, TEPC at 20 µM achieved 98% inhibition (Figure 5.A-C).

Likewise, *H. articulatum* extract effectively inhibited $DrPLA_2$-GV and $hPLA_2$-GV, and to lesser extent, $DrPLA_2$-GIIA reaching up to 80% and 68% inhibition, respectively, at 100 µg/mL (Figure 5. A-C). In contrast, *R. raetam* extract was the least efficacious against all $sPLA_2$ enzymes, with inhibition values ranging between 72% to 79.5% were recorded at the highest tested concentration, 200 µg/mL (Figure 5. A-C). This suggested that while *A. campestris* and *H. articulatum* extracts showed significant potential in targeting enzymes involved in inflammation, the efficacy of *R. raetam* extract was relatively less pronounced. The current findings highlighted the promising role of *A. campestris* and *H. articulatum* extracts in modulating inflammatory responses through their $sPLA_2$ inhibitory activities, particularly targeting GV- and GIIA- phospholipases, which are key mediators in inflammatory pathways [29]. However, the reduced efficacy of *R. raetam* extract needed further investigation into its potential mechanisms and whether its inhibition might be enhanced under different conditions or with synergistic combinations of other inhibitors.

These data indicated that ethanolic extracts could regulate the release of AA from membrane phospholipids, which are produced during tissue injury. Besides, these extracts demonstrated the capacity to inhibit the accumulation of critical inflammatory leukotrienes in the 5-LOX pathway, which plays a significant role in promoting tissue damage [29]. In fact, the three extracts displayed significant inhibition of 5-LOX (Figure 5.D) and COX-1 (Figure 5.E), with less pronounced effects on COX-2 enzyme (Figure 5.F). Remarkably, the *R. raetam* extract fully suppressed the activity of both 5-LOX and COX-1 enzymes at low concentrations, 50 µg/mL (Figure 5.D-E). Similarly, the *A. campestris* extract reduced, in a dose-dependent manner, the activity of COX-1, 5-LOX, and COX-2 up to 76.5±3.35%, 66.5±3.53% and 62.5±3.35%, respectively (Figure 4.E-D). At 200 µg/mL, the *H. articulatum* extract achieved 85.5±3.35% and 71±2.82% inhibition towards 5-LOX and COX-1, respectively (Figure 5.D-E).

A. campestris extract demonstrated exceptional potency, achieving 99%-100% inhibition of all tested $sPLA_2$s, including $DrPLA_2$-GV (100% inhibition) at 200 µg/mL, surpassing TEPC (98% at 20 µM). *H. articulatum* extract showed moderate efficacy, particularly against $DrPLA_2$-GV (80%) and $hPLA_2$-GV (68%), while *R. raetam* extract was less effective (72%-79.5%). These results highlighted the *A. campestris*'s strong inhibitory potential, especially against DrPLA2-GV, as a promising anti-inflammatory agent.

4. Discussion

The therapeutic potential of plants in phytotherapy is primarily linked to their secondary metabolites, especially phenolic compounds. It has been reported that the quality of plant extracts is highly affected by the effectiveness and selectivity of the extraction process. Commonly used techniques include aqueous maceration, infusion, and decoction, each of which influences the yield and purity of the final extract [30,31]. In this study, maceration with agitation was chosen to accelerate extraction, minimize solvent contact time, and preserve bioactivity. The plant material was mixed with solvent, agitated, filtered, and pressed. The liquid was then evaporated and concentrated, with solvent recovered under reduced pressure to maximize yield and prevent heat-induced denaturation [32]. Different solvents were used for polyphenol extraction, with different effectiveness depending on their ability to dissolve specific phenolic compounds. Indeed, the choice of solvent is based on the extraction purpose, component nature, matrix properties, reagent availability, cost, and safety concerns [33]. However, ethanol is the most commonly used solvent for the quantitative extraction of polyphenols from aromatic plants due to its ability to dissolve various phenolic groups [32]. Thus, aerial parts of the three studied plants (*A. campestris*, *H. articulatum*, and *R. raetam*) were extracted using ethanol as a solvent.

The ethanolic extracts (*A. campestris*, *H. articulatum*, and *R. raetam*) were analyzed for total phenol and flavonoid contents, with *A. campestris* showing the highest phenol content (85.59±2.4 mg GAE/g) and *R. raetam* the highest flavonoid concentration (34.77±3.09 mg CE/g). *H. articulatum* and *A. campestris* exhibited comparable flavonoid levels, while phenol content followed the order *A.*

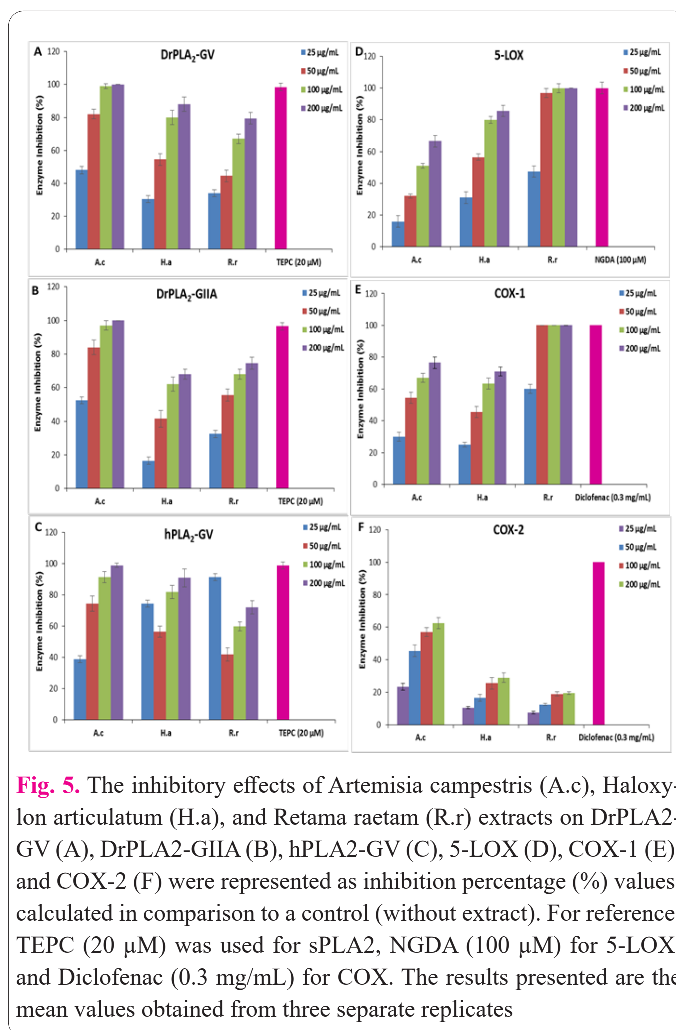


Fig. 5. The inhibitory effects of Artemisia campestris (A.c), Haloxylon articulatum (H.a), and Retama raetam (R.r) extracts on DrPLA2-GV (A), DrPLA2-GIIA (B), hPLA2-GV (C), 5-LOX (D), COX-1 (E), and COX-2 (F) were represented as inhibition percentage (%) values, calculated in comparison to a control (without extract). For reference, TEPC (20 µM) was used for sPLA2, NGDA (100 µM) for 5-LOX, and Diclofenac (0.3 mg/mL) for COX. The results presented are the mean values obtained from three separate replicates

campestris > *H. articulatum* > *R. raetam* (Table 1). Butanolic extract of *A. campestris* previously collected from Algeria showed total PP and Fv contents values of 312.32 mg GAE/mg DW and 11.3 µg QE/mg DW, respectively [21]. A total polyphenol content of 1123 mg CE/g DW in the ethanolic extract of *R. raetam* was also reported [24]. More recently, *R. raetam* extract recorded total PP and Fv contents of 112.12±0.11 GAE/mg DW and 15.93±0.23 mg CE /g DW, respectively [34]. The variation in PP and Fv contents and composition among the same plant species collected from different regions may be attributed to environmental and climatic factors, as well as geographical location, which could promote genetic differentiation between distinct taxonomic groups [34].

HPLC is a widely used method for analyzing phenolic compounds in plant extracts. Given the vast diversity and complexity of plant phenolic, it is not feasible to fully characterize every individual component. However, HPLC allows for the identification of key categories and representative phenolics. In this study, HPLC was employed to further explore the phytochemical profile of ethanolic extracts of *A. campestris*, *H. articulatum*, and *R. raetam* as well as identify and quantify their flavonoids and phenolic compounds with the aim of exploring the diversity of bioactive components known for their biological properties. In comparison with other studies, quinic acid was also identified as a major compound (24 µg/mg DW) in hydro-ethanolic extracts of aerial organs of *A. campestris* from southern Portugal using LC-PDA-MS [35]. The authors additionally reported chlorogenic acid (16 µg/mg DW), caffeic acid (1.6 µg/mg DW), protocatechuic acid (0.27–0.43 µg/mg DW), luteolin (0.19–0.47 µg/mg DW), and coumaric acid (0.17–0.33 µg/mg DW). Meanwhile, HPLC analysis of ethanolic extracts of *H. articulatum* collected from central Tunisia revealed the presence of gallic acid, rosmarinic acid, ferulic acid, 3-hydroxycinnamic acid, and p-coumaric acid at 1.30 mg/g [36]. Similarly, a study on *R. raetam* butanolic extract from the southeastern Sahara region of Algeria highlighted high levels of ascorbic acid (8.43 µg/mg), p-coumaric acid (3.35 µg/mg), and rutin (4.03 µg/mg) [37]. These comparisons illustrate variations in phenolic profiles across geographic regions and extraction methods, contributing to the understanding of the chemical diversity and therapeutic potential of these species.

The antibacterial activity of all extracts was evaluated by measuring bacterial growth inhibition zones via the agar well diffusion method [26] and determining the minimum inhibitory concentration (MIC) using the serial dilution method in liquid medium [16]. The tested extracts were effective only against Gram-positive bacteria, while Gram-negative strains exhibited resistance, even at a maximum concentration of 300 mg/mL (Table 3). Similarly, the ethanol extract of *A. campestris* collected from southeastern Morocco, at 5 mg, inhibited the growth of the Gram-positive strains *Staphylococcus aureus* and *Bacillus anthracis* with inhibition zone diameter of 13 mm and 11 mm, respectively while smaller diameters were observed for the Gram-negative strains *Escherichia coli* and *Pseudomonas aeruginosa* (7 mm and 6 mm, respectively) [38]. *A. campestris* extract collected from west-central Tunisia significantly inhibited *Staphylococcus aureus* (inhibition zone diameter = 12.22 mm) and, to lesser extent, *Pseudomonas aeruginosa* (inhibition zone diameter = 7.66 mm) [10]. Besides, the ethanol extract of *R. raetam* (from northwestern Saudi Arabia), at 0.3 g/mL, showed growth inhibition halos for the strains *Staphylococcus aureus* (5.8 mm), *Pseudomonas aeruginosa* (6.2 mm), and *Escherichia coli* (3.8 mm) [39]. At 10 mg/well, the ethanol extract of *R. raetam* from Palestine exhibited growth inhibition diameters of approximately 6 mm for both Gram-negative and Gram-positive strains [40]. Butanol plant extracts from *H. articulatum* exhibited inhibition zone diameter values ranging between 10 and 20 mm against *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* strains [22]. The MIC values of the different extracts were determined within a concentration range of 0.29 to 150 mg/mL (Table 3) and confirmed that the tested extracts inhibited the growth of Gram-positive bacteria but were ineffective against Gram-negative strains. Such resistance of Gram-negative bacteria could be attributed to their outer membrane's lipopolysaccharides, which block antibiotics and other substances. Gram-positive bacteria are more sensitive because their cell walls are composed of a less restrictive peptidoglycan layer [41].

Several studies reported the antimicrobial activity of these extracts with different MIC values. Indeed, ethanolic extract of *A. campestris* originated from Algeria showed MIC values of 12.5 mg/mL or 25 mg/mL against *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Escherichia coli*, and *Klebsiella pneumoniae* or *Salmonella paratyphi*, respectively [42]. However, no specific MIC values have been reported for *H. articulatum* ethanolic extract so far, except in one study by Jdey et al. [36] who demonstrated that 1 mg/mL of *H. articulatum* (from Tunisia) inhibited *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus*, and *Salmonella enterica* by 96.2%, 55.9%, 80%, and 100%, respectively. These differences in MIC values could be due to the geographical variations that influence plant phytochemical composition, as well as environmental conditions such as soil, climate, and altitude affecting the concentration and diversity of bioactive compounds in plant extracts [34]. Additionally, differences in extraction methods such as solvent used, extraction duration and bacterial strains tested could also explain these variations [25].

The effectiveness of medicinal plant extracts in inhibiting bacterial growth is due to the synergy between their active compounds, which can disrupt bacterial resistance mechanisms and affect their physicochemical properties. Bioactive compounds mainly phenols and flavonoids can affect bacterial cells primarily by targeting the cytoplasmic membrane, altering its structure, integrity, or permeability, which can lead to cell damage or rupture. They may also inhibit membrane pumps and disrupt cell communication, protein interactions, and biofilm formation [43].

The antifungal activity of the extracts was tested against *Rhizoctonia solani*, *Fusarium graminearum*, and *Fusarium verticillioides*, with no inhibition zones observed at 300 mg/mL for any of the strains**.** Similar findings were reported using different concentrations of *H. articulatum* extract from Tunisia against *Aspergillus niger*, *Fusarium graminearum*, and *Fusarium oxysporum* [44]. In contrast, the ethanol extract of *R. raetam* collected from Libya, at concentrations ranging from 5% to 15%, demonstrated an inhibitory effect of 30% to 37% on the mycelial growth of *Fusarium solani*, *Fusarium moniliforme*, *Fusarium semitectum*, and *Macrophomina phaseolina* [45]. The antifun-

gal activity of the various compounds may be attributed to multiple mechanisms, including disruption of fungal cells through inhibition of cell wall formation, interference with cell division, nucleic acid and protein synthesis, and inhibition of transmembrane pumps, as well as mitochondrial dysfunction in the fungus [46].

Antioxidants are recognized as molecules with the potential to neutralize or reduce the damage caused by free radicals [47]. Plants contain high concentrations of antioxidants particularly flavonoids and polyphenols that are considered potential sources of plant-based antioxidants [48]. They have a perfect chemical structure for trapping free radicals [49]. Using DPPH assay, *H. articulatum* and *R. raetam* extracts demonstrated the highest antioxidant capacity, both exhibiting the lowest IC_{50} values around 130 µg/mL. The *A. campestris* extract was less potent, with an IC_{50} of 450 ± 10 µg/mL, though still lower than BHA's IC_{50} of 500 ± 18 µg/mL (Table 4). The higher antioxidant activity of *H. articulatum* and *R. raetam* extracts compared to *A. campestris* may be attributed to their richer phenolic and flavonoid content, as these compounds are key contributors to antioxidant activity. Additionally, differences in their phytochemical composition, influenced by species-specific metabolic pathways and environmental factors, could enhance their free radical-scavenging potential. Previous studies on the macerated ethanolic extracts of *R. raetam* showed significantly lower anti-radical activity compared to the current findings [34,39]. The ethanolic extract of *H. articulatum* plant, a variety collected from Sfax-Tunisia, displayed an IC_{50} of 24 µg/mL against an IC_{50} of 3.2 µg/mL for ascorbic acid [44]. The n-butanolic *A. campestris* extract inhibited DPPH oxidation with an IC_{50} of 2.239 ± 0.32 mg/mL, which is comparable to that of the used standard Ascorbic acid (IC_{50}= 1.824 ± 0.97 mg/mL) [21]. Similarly, the n-butanolic extract of *A. campestris* showed a moderate reducing power at 400 µg/mL [21]. The reducing capacity of the extracts may be partially attributed to their richness in phenolic compounds. Thus, it could be concluded that polyphenols and flavonoids play a crucial role in the chelation of transition metals involved in the Fenton reaction, which leads to the formation of hydroxyl radicals through the reaction of iron with hydrogen peroxide [50].

The FRAP method [18] was utilized to measure the reducing power of the extracts by evaluating their ability to convert the Fe^{3+}/ferricyanide complex into its ferrous form (Fe^{2+}). The iron-reducing efficiency followed the order: BHA > *R. raetam* > *A. campestris* > *H. articulatum* (Table 4). EC50 values of about 900 µg /mL and 380 µg/mL were previously reported for *R. raetam* and *H. articulatum* ethanolic extracts, respectively [34] and compared to BHA (EC_{50} =130 µg/mL), used as positive reference.

The *in vitro* anti-inflammatory activity of *A. campestris*, *H. articulatum*, and *R. raetam* ethanolic extracts was evaluated by HRBC membrane stabilization since the red blood cell membrane is structurally similar to lysosomal membranes, and enzyme inhibition (COX-1, COX-2, 5-LOX, $sPLA_2$) at concentrations of 25–200 µg/mL. The *R. raetam* extract provided strong HRBC protection (88.25–92.5%), comparable to diclofenac-sodium, while *A. campestris* and *H. articulatum* showed lower protection (70.5±3.53% and 73±2.82% at 200 µg/mL). This comparison allows us to establish the relative efficacy of the extracts and their potential as therapeutic agents. The higher protection offered by *R. raetam* extract compared to the other extracts can be attributed to its richer bioactive compound profile, including higher concentrations of phenolics and flavonoids, which are known for their potent anti-inflammatory and membrane-stabilizing effects. Current results underlined the significant *in vitro* anti-inflammatory activity of the three extracts, which was comparable to that of the standard drug, diclofenac sodium, by stabilizing the HRBC membrane given the similarity between lysosomes and HRBC membranes. Indeed, both lysosomal and RBC membranes are composed of phospholipid bilayers lipid bilayer with embedded proteins that are crucial for maintaining cell integrity and regulating transport functions, making them vulnerable to damage under stress or inflammatory conditions. During inflammation, the release of lysosomal enzymes contributes to tissue damage and inflammation. Therefore, compounds that prevent HRBC membrane lysis can be presumed to exert a protective effect on lysosomal membranes in inflammatory cells, reducing the release of inflammatory mediators [51]. The anti-inflammatory properties of the extracts demonstrate that they could be promising candidates for the development of natural anti-inflammatory treatments. Their ability to stabilize cell membranes and inhibit the release of pro-inflammatory mediators suggests they may be effective in managing conditions associated with chronic inflammation, such as arthritis, cardiovascular diseases, and neurodegenerative disorders. These extracts could be explored further for incorporation into topical or oral formulations, offering a plant-based alternative or complement to conventional anti-inflammatory drugs.

The ethanolic extracts showed dose-dependent enzyme inhibition, with *A. campestris* extract achieving 99–100% inhibition of $sPLA_2$s at 200 µg/mL, surpassing TEPC's 98% inhibition at 20 µM. COX-1 /2 inhibition could be due to the interaction of the extract compounds with the key residues of the active site Arg120 in hCOX-1 (Arg106 in hCOX-2) and the phenolic amino acid Tyr 355 (Tyr341 in hCOX-2) responsible for the interaction with the AA carboxylate moiety through ionic and hydrogen bond interactions, respectively [52].

5-LOX requires one non-heme iron atom per molecule, which oscillates between Fe^{2+} (inactive enzyme) and Fe^{3+} (active form) during the catalytic cycle. Its inhibition by the extracts might be linked to the nature of inhibitors present in the extract. These inhibitors could be redox inhibitors or antioxidants, which interfere with the redox cycle of 5-LOX, iron-chelator agents, and non-redox competitive inhibitors, which compete with AA to bind the enzyme active site [5]. Phenolic compounds in the extracts could inhibit 5-LOX indirectly through interacting with 5-LOX activating protein (FLAP), a crucial protein that transfers the substrate AA to 5-LOX resulting to leukotrienes biosynthesis [5].

The ethanolic extracts of *A. campestris*, *H. articulatum* and *R. raetam* demonstrated significant bioactive potential through their antioxidant, antimicrobial, and anti-inflammatory activities. *A. campestris* extract displayed the highest total phenolic content and the most potent anti-inflammatory effects, particularly in inhibiting $sPLA_2$ enzymes ($DrPLA_2$-GV, $DrPLA_2$-GIIA, and $hPLA_2$-GV) with up to 100% inhibition at 200 µg/mL, indicating its strong potential for therapeutic applications in inflammatory conditions. The inhibition of key inflammatory enzymes

(5-LOX, COX-1/2, and $sPLA_2$) highlighted the capacity of these extracts to regulate AA release and leukotriene biosynthesis, with notable effects on COX-1 and 5-LOX pathways. The antioxidant activity, particularly from *H. articulatum* and *R. raetam* extracts, further supported their utility as natural sources of bioactive compounds, capable of scavenging free radicals effectively. These findings highlight the significant potential of these extracts for therapeutic and industrial applications as bioactive ingredients in cosmetics for skin protection, food preservation to prevent spoilage, and in agriculture as natural bio-pesticides. Further research into their active compounds using advanced techniques such as column chromatography, HPLC, or liquid-liquid extraction and mechanisms of action could pave the way for their incorporation into diverse commercial products. The purification and identification of active constituents, allows to be further studied to enhance efficacy and specificity in future applications.

Acknowledgments
The authors extend their appreciation to the researchers' Supporting Project number (RSP2025R237), King Saud University, Riyadh, Saudi Arabia, for funding this work.

Author contributions
All authors contributed to the study's conception and design. All authors have read and agreed to the published version of the manuscript.

Funding
This research received no external funding.

Institutional review board statement
Not applicable

Informed consent statement
Not applicable

Data availability statement
Not applicable

Conflicts of interest
The authors declare no conflicts of interest.

References

1. Saha S, Buttari B, Panieri E, Profumo E, Saso L. (2020) An overview of Nrf2 signaling pathway and its role in inflammation. Mol 25: 5474. doi: https://doi.org/10.3390/molecules25225474
2. Ansari M.Y, Ahmad N, Haqqi T.M. Oxidative stress and inflammation in osteoarthritis pathogenesis: Role of polyphenols. (2020) Biomed Pharmacother 129: 110452. doi: https://doi.org/10.1016/j.biopha.2020.110452
3. Rudrapal M, Eltayeb W.A, Rakshit G, El-Arabey A.A, Khan J, Aldosari S.M, Alshehri B, Abdalla M. (2023) Dual synergistic inhibition of COX and LOX by potential chemicals from Indian daily spices investigated through detailed computational studies. Sci Rep 13: 8656. doi: https://doi.org/10.1038/s41598-023-35161-0
4. Alonazi M.A, Jemel I, Moubayed N, Alwhibi M, El-Sayed N.N.E, Ben Bacha A. (2021) Evaluation of the in vitro anti-inflammatory and cytotoxic potential of ethanolic and aqueous extracts of Origanum syriacum and Salvia lanigera leaves. Environ Sci Pollut Res 28: 19890-19900. doi: https://doi.org/10.1007/s11356-020-11961-z
5. Charlier C, Michaux C. (2003) Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs. Eur J Med Chem 38: 645-659. doi: https://doi.org/10.1016/S0223-5234(03)00115-6
6. Md Idris M.H, Mohd Amin S.N, Mohd Amin S.N, Nyokat N, Khong H.Y, Selvaraj M, Zakaria Z.A, Shaameri Z, Hamzah A.S, Teh L.K. (2022) Flavonoids as dual inhibitors of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX): molecular docking and in vitro studies. BJBAS 11: 117. doi: https://doi.org/10.1186/s43088-022-00296-y
7. Manju S. (2020) Identification and development of thiazole leads as COX-2/5-LOX inhibitors through in-vitro and in-vivo biological evaluation for anti-inflammatory activity. Bioinorg Chem 100: 103882. doi: https://doi.org/10.1016/j.bioorg.2020.103882
8. Lachkar N, Lamchouri F, Bouabid K, Boulfia M, Senhaji S, Stitou M, Toufik H. (2021) Mineral composition, phenolic content, and in vitro antidiabetic and antioxidant properties of aqueous and organic extracts of Haloxylon scoparium aerial parts. JEBM 2021: 9011168. doi: https://doi.org/10.1155/2021/9011168
9. Vyshnevska L, Severina H.I, Prokopenko Y, Shmalko A. (2022) Molecular docking investigation of anti-inflammatory herbal compounds as potential LOX-5 and COX-2 inhibitors. Pharm J 69: 0428-0296. doi: https://doi.org/10.3897/pharmacia.69.e89400
10. Limam I, Ghali R, Abdelkarim M, Ouni A, Araoud M, Abdelkarim M, Hedhili A, Ben-Aissa Fennira F. (2024) Tunisian Artemisia campestris L.: a potential therapeutic agent against myeloma-phytochemical and pharmacological insights. Plant Methods 20: 59. doi: https://doi.org/10.1186/s13007-024-01185-4
11. Ghlissi Z, Sayari N, Kallel R, Bougatef A, Sahnoun Z. (2016) Antioxidant, antibacterial, anti-inflammatory and wound healing effects of Artemisia campestris aqueous extract in rat. Biomed Pharmacother 84: 115-122. doi: https://doi.org/10.1016/j.biopha.2016.09.018
12. Chaouche T.M, Haddouchi F, Ksouri R, Atik-Bekkara F. (2014) Evaluation of antioxidant activity of hydromethanolic extracts of some medicinal species from South Algeria. J Chin Med 77: 302-307. doi: https://doi.org/10.1016/j.jcma.2014.01.009
13. Djeddi S, Karioti A, Yannakopoulou E, Papadopoulos K, Chatter R, Skaltsa H. (2013) Analgesic and antioxidant activities of Algerian Retama raetam (Forssk.) Webb & Berthel extracts. Rec Nat Prod 7: 169. doi: https://www.acgpubs.org/doc/2018080817270027-RNP-1206-84.pdf
14. Rebaya A, Belghith S.I, Baghdikian B, Leddet V.M, Mabrouki F, Olivier E, Kalthoum Cherif J, Ayadi M.T. (2015) Total phenolic, total flavonoid, tannin content, and antioxidant capacity of Halimium halimifolium (Cistaceae). J Appl Pharm Sci 5: 052-057. doi: 10.7324/JAPS.2015.50110
15. Vlietinck A. (1991) Screening methods for antibacterial and antiviral agents from higher plants. Methods in plant biochemistry/Hostettmann K [edit.] 6: 47-69. doi: https://hdl.handle.net/10067/887015162165141
16. Eloff J.N. (2019) Avoiding pitfalls in determining antimicrobial activity of plant extracts and publishing the results. BMC complement med ther 19: 1-8. doi: https://doi.org/10.1186/s12906-019-2519-3
17. Bersuder P, Hole M, Smith G. (1998) Antioxidants from a heated histidine-glucose model system. I: Investigation of the antioxidant role of histidine and isolation of antioxidants by high-performance liquid chromatography. J Am Oil Chem Soc 75: 181-187. doi: https://doi.org/10.1007/s11746-998-0030-y
18. Das K, Dhar T.M, Ghosh M. (2015) A Comparative study of the antioxidative propertiesof the different seed spices available in India. JAPER 5: 44-49.

19. Gandhidasan R, Thamaraichelvan A, Baburaj S. (1991) Anti-inflammatory action of Lannea coromandelica by HRBC membrane stabilization. doi: https://www.cabidigitallibrary.org/doi/full/10.5555/19910305366
20. de Araújo A.L, Radvanyi F.(1987) Determination of phospholipase A2 activity by a colorimetric assay using a pH indicator. Toxicon 25: 1181-1188. doi: https://doi.org/10.1016/0041-0101(87)90136-X
21. Lilia D, Samira B, Faouzia D, Randa D, Wahiba K.A. (2022) In vitro assessment of the bioactive potential of Artemisia campestris L. fractions growing in Khenchela (Algeria). SAJEB 12: 445-455. doi: 10.38150/sajeb.
22. Noureddine T, Nedjimi M.S, Medjdoub A, Belalem M.A, Tlili S, Nacersalah N. (2023) A comparison of the antioxidant and antimicrobial effects of phenolic extracts from plants growing in Oued Souf," Haloxylon articulatum Bioss and Arnebia decumbens Vent Coss and Kral". NeuroQuantology 21: 842. doi: 10.48047/nq.2023.21.7.nq23073
23. Mohammed A, Marghich M, Daoudi N.E, Mamri S, Mekhfi H, Ziyyat A, Bnouham M. (2022) Antiulcer activity of Moroccan Artemisia campestris L. subsp. glutinosa against ethanol-induced gastric ulcer in Mice. AJMAP 8: 20-39. doi: https://doi.org/10.48347/IMIST.PRSM/ajmap-v8i2.29971
24. Rejab A, Ksibi H. (2019) Phenolic and flavonoid contents of some plant extracts from Tunisia southern landscape by using different extraction techniques: the case of Retama reatam. Med Aromat Plants (Los Angeles) 8: 337. doi: 10.35248/2167-0412.19.8.337
25. Sultana B, Anwar F, Ashraf M. (2009) Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Mol 14: 2167-2180. doi: https://doi.org/10.3390/molecules14062167
26. Berghe V, Vlietinck A. (1991) Screening methods for antibacterial and antiviral agents from higher plants. Methods in plant biochemistry 6: 47-68. doi: https://hdl.handle.net/10067/8870151162165141
27. Ponce A, Fritz R, Del Valle C, Roura S. (2003) Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. LWT-Food Science and Technology 36: 679-684. doi: https://doi.org/10.1016/S0023-6438(03)00088-4
28. Bozin B, Mimica-Dukic N, Samojlik I, Goran A, Igic R. (2008) Phenolics as antioxidants in garlic (Allium sativum L., Alliaceae). Food chemistry 111: 925-929. doi: https://doi.org/10.1016/j.foodchem.2008.04.071
29. Scott K.F, Mann T.J, Fatima S, Sajinovic M, Razdan A, Kim R.R, Cooper A, Roohullah A, Bryant K.J, Gamage K.K. (2021) Human group IIA phospholipase A2—Three decades on from its discovery. Mol 26: 7267. doi: https://doi.org/10.3390/molecules26237267
30. Bohui P.S.G, Adima A.A, Niamké F.B, N'Guessan J.D. (2018) Etude comparative de trois méthodes d'extraction des flavonoïdes totaux à partir des feuilles de plantes médicinales: Azadirachta indica et Psidium guajava. J Soc Ouest-Afr Chim 46: 50-58. doi: https://www.researchgate.net/publication/333828837
31. Locarev A, Kuzyakova L, Ohio M, Glazova T, Kovtun E, Nam N. (2020) Comparative analysis of flavonoid content in extraction from complex medicinal plant raw materials using various extraction methods. J Mod Sci 2: 158-165. doi: https:// PYATIGORSK/.doc/VAC/arhiv/2020/2-2020/143-149.pdf
32. Oreopoulou A, Tsimogiannis D, Oreopoulou V. (2019) Extraction of polyphenols from aromatic and medicinal plants: an overview of the methods and the effect of extraction parameters. Polyphenols in plants 243-259. doi: https://doi.org/10.1016/B978-0-12-813768-0.00025-6
33. Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M. (2002) Free radical scavenging properties of wheat extracts. J Agric Food Chem 50: 1619-1624. doi: https://pubs.acs.org/doi/abs/10.1021/jf010964p
34. Najjaa H, Abdelkarim B.A, Doria E, Boubakri A, Trabelsi N, Falleh H, Tlili H, Neffati M. (2020) Phenolic composition of some Tunisian medicinal plants associated with anti-proliferative effect on human breast cancer MCF-7 cells. EBTJ 4: 104-112. doi: https://doi.org/10.2478/ebtj-2020-0012
35. Pereira C.G, Barreira L, Bijttebier S, Pieters L, Marques C, Santos T.F, Rodrigues M.J, Varela J, Custódio L. (2018) Health promoting potential of herbal teas and tinctures from Artemisia campestris subsp. maritima: from traditional remedies to prospective products. Sci Rep 8: 4689. doi: https://doi.org/10.1038/s41598-018-23038-6
36. Jdey A, Falleh H, Jannet S.B, Hammi K.M, Dauvergne X, Magné C, Ksouri R. (2017) Anti-aging activities of extracts from Tunisian medicinal halophytes and their aromatic constituents. EXCLI journal 16: 755. doi: 10.17179/excli2017-244. PMID: 28827992
37. Chouikh A, Alia F. (2021) Phytochemical properties, antibacterial and anti-free radical activities of the phenolic extracts of (Forssk) Webb. & Berthel. collected from Algeria Desert. OUAC 32: 33-39. doi: https://sciendo.com/pdf/10.2478/auoc-2021-0005
38. Al Jahid A, Essabaq S, Elamrani A, Blaghen M, Jamal Eddine J. (2016) Chemical composition, antimicrobial and antioxidant activities of the essential oil and the hydro-alcoholic extract of Artemisia campestris L. leaves from southeastern Morocco. JBAPN 6: 393-405. doi: https://doi.org/10.1080/22311866.2016.1268068
39. Al-Onazi W, Al-Mohaimeed A.M, Amina M, El-Tohamy M.F. (2021) Identification of chemical composition and metal determination of Retama raetam (Forssk) stem constituents using ICP-MS, GC-MS-MS, and DART-MS. J Anal Chem 2021: 6667238. doi: https://doi.org/10.1155/2021/6667238
40. Ali-Shtayeh M, Yaghmour R.M.-R, Faidi Y, Salem K, Al-Nuri M. (1998) Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. J Ethnopharmacol 60: 265-271. doi: https://doi.org/10.1016/S0378-8741(97)00153-0
41. Rabia A, Hussein E.-S, Nabeel S, Asma A.-N, Fatma H. (2012) Antioxidant and antimicrobial properties of five medicinal Libyan plants extracts. J Nat Sci 4 (2012) 12. doi: 10.4236/ns.2012.45045
42. Houicher A, Kuley E, Bendeddouche B, Özogul F. (2016) In vitro antibacterial activity of ethanolic extracts of Mentha spicata L. and Artemisia campestris from Algeria. Microbes in the Spotlight: Recent Progress in the Understanding of Beneficial and Harmful Microorganisms 272. doi: http:// books.google.com
43. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. (2021) Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms 9: 2041. doi: https://doi.org/10.3390/microorganisms9102041
44. Bouaziz A, Mhalla D, Zouari I, Jlaiel L, Tounsi S, Jarraya R, Trigui M. (2016) Antibacterial and antioxidant activities of Hammada scoparia extracts and its major purified alkaloids. S Afr J Bot 105: 89-96. doi: https://doi.org/10.1016/j.sajb.2016.03.012
45. Abushaala F, Ben-Ramadan A, Fahej M. (2017) In vitro antifungal activity of some plant extracts against seed-borne pathogens. J Agric Vet Sci 10: 49-57. doi: 10.9790/2380-1004014957
46. Walker G.M, White N.A. (2017) Introduction to fungal physiology. Fungi: biology and applications 1-35. doi: https://doi.org/10.1002/9781119374312.ch1
47. Kulawik P, Özogul F, Glew R, Özogul Y. (2013) Significance of antioxidants for seafood safety and human health. J Agric Food Chem 61: 475-491. doi: https://doi.org/10.1021/jf304266s
48. Pisoschi A.M, Cheregi M.C, Danet A.F. (2009) Total antioxidant capacity of some commercial fruit juices: electrochemical and

spectrophotometrical approaches. Mol 14: 480-493. doi: https://doi.org/10.3390/molecules14010480
49. Dai J, Mumper R.J. (2010) Plant phenolics: extraction analysis and their antioxidant and anticancer properties. Mol 15: 7313-7352. doi: https://doi.org/10.3390/molecules15107313
50. Ghedadba N, Hambaba L, Ayachi A, Aberkane M, Bousselsela H, Oueld-Mokhtar S. (2015) Polyphénols totaux, activités antioxydante et antimicrobienne des extraits des feuilles de Marrubium deserti de Noé. Phytothérapie 13: 118-129. doi: 10.1007/s10298-015-0944-4
51. Yesmin S, Paul A, Naz T, Rahman A.A, Akhter S.F, Wahed M.I.I, Emran T.B, Siddiqui S.A. (2020) Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (Piper chaba). Clin phytosci 6: 1-10. doi: https://doi.org/10.1186/s40816-020-00207-7
52. Gedawy E.M, Kassab A.E, El Kerdawy A.M. (2020) Design, synthesis and biological evaluation of novel pyrazole sulfonamide derivatives as dual COX-2/5-LOX inhibitors. Eur J Med Chem 189: 112066. doi: https://doi.org/10.1016/j.ejmech.2020.112066