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Research Article

EVALUATION OF CHEMICAL COMPOSITION, ANTI-INFLAMMATORY, ANTIBACTERIAL ACTIVITY AND SYNERGISTIC EFFECT BETWEEN ANTIBIOTICS AND THE ESSENTIAL OIL OF *ARTEMISIA CAMPESTRIS* L.

Bakhtia Mammeri [1*], Fouad Bahri[1], Mohamed Kouidri[2],
Bouharaoua Boudani[3,4], Fatiha Arioui[5]

[1]*Abdelhamid Ibn Badis University, Faculty of Nature and Life Sciences, Laboratory of Microbiology and Plant Biology, Mostaganem, Algeria.*
[2]*Hassiba Ben Bouali University, Faculty of Nature and Life Sciences, Laboratory of Bio-Resource Naturel, Chlef, Algeria.*
[3]*University of Saida, Faculty of Science, Department of Biology, Laboratory of Water Resources & Environment, Saida, Algeria*
[4]*National Institute of Forestry Research Tenes Station, Chlef, Algeria*
[5]*Abdelhamid Ibn Badis University, Faculty of Nature and Life Sciences, Laboratory of Food Technology and Nutrition Biology, Mostaganem, Algeria.*

**Corresponding Author:*
E-mail: bakhtia.mammeri@univ-mosta.dz

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ABSTRACT. This present study aimed to elucidate the chemical composition of essential oil (EO) which was obtained from the leaves of *Artemisia campestris* (Asteraceae) collected in Djelfa region (Algeria). The *in-vitro* antibacterial activity against six (06) bacterial strains were evaluated, the EO was used alone and associated with antibiotics to assess their synergistic effect. The *in-vivo* acute toxicity and anti-inflammatory activity were evaluated. In this work thirty-three (33) compounds accounting for 100% of total oil were identified by GC and GC/MS analysis of the essential of *A. campestris*. Camphor (41.95%), Chrysanthenone (13.95%), and 1,8-Cineole (13.31%) are found to be the major compounds. From the results of the antibacterial activity using disc diffusion method revealed inhibition zones ranging from 18.33 ±0.57 to 24.33± 0.57mm. Gram-negative was more sensitive to EO compared to Gram-positive bacteria. The combined application of EO of the studied specie with standard antibiotics led to a synergistic effect in some bacteria. At the highest tested dose (5000 mg/kg p.o.) the EO of *A. campestris* did not show signs of acute toxicity. EO of *A. campestris* reduced significantly the paw edema induced by carrageenan in mice at 27.36%, 39.62%, and 56.60% (after 6 hours) at the doses of 100, 200, and 400 (mg/kg) respectively.

Keywords: *Artemisia campestris, essential oil, hydro distillation, chemical composition, synergies, antibacterial activity, acute toxicity, anti-inflammatory activity.*

INTRODUCTION

The emergence of antibiotic resistance is the cause of infectious diseases which pose a real problem for public health [1]. Antibiotics standards are derived from natural

substances produced by some bacteria and fungi to defend other bacteria. Bacteria can synthesize antibiotics at the same time have developed the means to protect themselves, so they are not suicidal [2]. Cushnie et *al.,* [3], have shown that the excessive use of antibiotics can be the major cause of the destruction of the normal intestinal flora and the proliferation of pathogens resistant to antibiotics. Which has directed research towards phytogenic resources to find a solution, so it is a very promoting and effective way to fight against pathogenic bacteria [4]. Plants have been part of human daily life since ancient times since they are used in food and health [5]. It has been reported from Zeggwagh et *al.*, Jakovljević et *al.*, [6,7], that numerous studies carried out on the search for natural products especially in aromatic herbs due to their various pharmacological properties. Different medicinal plants contain various bioactive molecules, such as phenolic acids, flavonoids, tannins, carotenoids, and sterols [8]. According to Shahidi et *al.,* [9], natural products of plant origin, either as pure compounds or as standardized extracts, offer limitless opportunities for a new drug discovered due to the unparalleled availability of chemical diversity. Phytochemicals alone or in combination act as therapeutic agent in various disease complications [10]. In Algeria, traditional medicinal plants have been described as sources of valuable bioactive compounds [11]. Algeria contains a large pool of plants with high bioactive potential that can be used for medicinal applications and for which many of them have not been explored. According to Mabberley [12], Artemisia genus belongs to the tribe Anthemideae of the Asteraceae family and which contains more than 350 species Artemisia which is quite widespread and grows wild over the Northern Hemisphere. 11 species are present in Algerian flora [13]. The specie of *A. campestris* grows on the steppe and desert and is commonly known as dgouft or armoise rouge. For many years the aerial parts of *A. campestris* have been used in traditional medicine especially against digestive troubles, gastric ulcers, and menstrual pains [14]. Indeed, the identification and measurement of all the bioactive constituents of medicinal plants are essential to ensure the reliability of the biological research and the repeatability as well as to ensure the quality control on the pharmacological and/or dangerous advantages [15]; [11]. In Tunisia the essential oil of *A. campestris* has been well isolated and investigated for its chemical composition and biological activities by several authors [16]; [17]. 1.8-Cineol (19.2%) was reported as the major component in northwestern Italian oil [18], and β-pinene (6.9-57.2%) in northern Italian oil [19]. Many chemical compounds which characterize *A. campestris* essential oil such as; alpha and beta-pinenes, *p*-cymene, caryophyllene oxide, spathulenol, limonene, dehydro-1,8-cineole, cadin-4-en-7-ol, gamma-terpinene, (Z)-beta-ocimene, aromadendrene, germacrene D, bi-cyclogermacrene, myrtenol, p-cymen-8-ol, gamma-cadinene, *Ar*-curcumene, del-ta-cadinene, calamenene, alpha-muurolene, gamma-murolene, gamma-cadinene, bisabolene, and endoperoxide, (Z, E)-farnesol, cedrol and verbenone [17,20,21]. The essential oils and solvent extracts from *A. campestris* have been shown to exhibit antioxidant, hepatoprotective, antibacterial, antiviral, insecticide, and allelochemical activities [22,23,24,25]. Ghaleb et al., [26] and Olajuyigbe et al., [27], have been shown that the synergy or the combination of the essential oil with antibiotics; is a therapeutic approach that can lead to a new way of treating infectious diseases. The Treatment of inflammation today requires steroidal anti-inflammatory drugs and non-steroidal drugs. At the same time, although that these substances are effective most often, they can exhibit undesirable effects which can interfere with their long-term use such as hepatic and urinary toxicity, gastrointestinal diseases, and inhibition of platelet aggregation [28]. According to Bengmark [29], inflammation is the body's main response

to aggression and is precisely regulated to limit possible damage to the body's structures, and therefore, improper regulation of this phenomenon can lead to a chronic inflammatory state. Széles et *al.*, [30]; Kang et *al*., [31], have also shown that the inflammatory reaction is an immune defense process of the body in response to an attack originating from physical elements such as; cold, heat, ionizing radiation… or exogenous or endogenous solid elements such as; insect bite, pathogenic microbes, chemical or biological products and compounds resulting from the immune reaction (immune complexes, cytotoxic antibodies, cytokines, etc.). The inflammatory reaction has been reported to be characterized by the cardinal signs of redness, swelling, warmth, pain, and loss of function [32]. In recent years, the appropriate consumption and the use of antibiotics accelerates the selection of multi-resistant bacteria, which currently constitute a real problem in antibiotherapy and in public health. that is why, the research of other solutions and alternatives from natural sources is required and need several studies. The aim of this study is to investigate the chemical composition of *A. campestris* essential oil from North-west of Djelfa (Algeria). EO has been used alone and in combination with standard antibiotics to assess their *in-vitro* antibacterial activities against multidrug-resistant bacteria, and evaluating their *in-vivo* acute oral toxicity and anti-inflammatory properties using the carrageenan-induced paw edema test.

MATERIALS AND METHODS

Plant material

The leaves of *A. campestris* were collected from Hassi Bahbah; located in the Northen-west of Djelfa (Algeria) (longitude: 3°03'59.87" E, latitude: 34°54'10.04" N and altitude: 879 m) (Figure1). The sample was identified by Pr Belhassaini Hachemi, a professor at the University of Sidi Bel-Abbas, Algeria. The specie of *A. campestris* has been collected manually on April 2019. A voucher specimen (A.h a ONA 2018) was deposited at the laboratory of Microbiology and Plant Biology, Abdelhamid Ibn Badis University, Faculty of Nature and Life Sciences, Mostaganem. Plant material was then washed and dried at room temperature in the dark for 15-day [33].

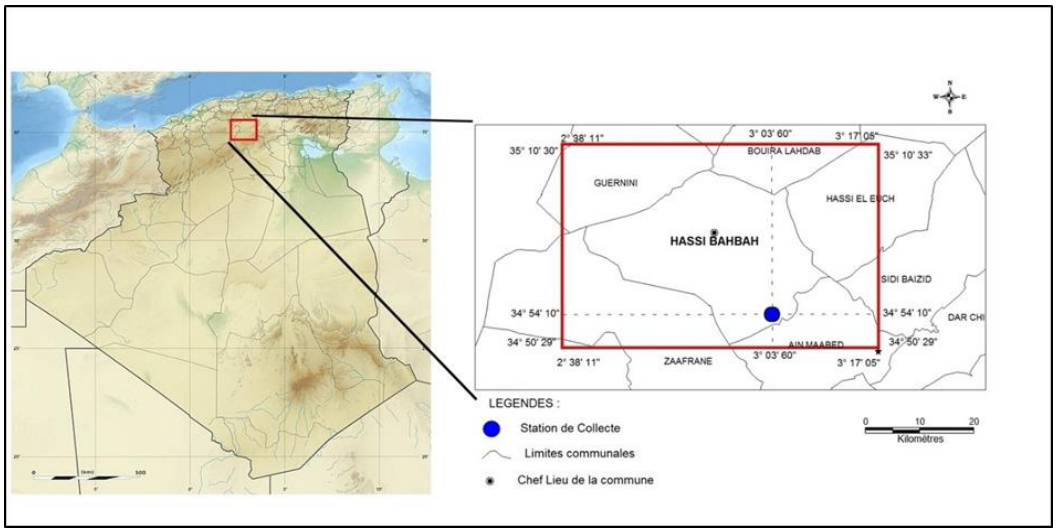

***Fig.1**. Geographical location of the collection station of the studied plant.*

Hydro distillation

The extraction of essential oil from the dry leaves of *A. campestris* was carried out using a Clavenger-type apparatus hydro-distiller. This technique consists of introducing about 100 (g) of the sample which was subjected to hydro-distillation for 4 (h) with 600 mL distilled water. Indeed, the oil obtained was separated from the distillate dried over anhydrous sodium sulfate, and stored in a glass vial in a refrigerator at -18ºC until the moment of analysis [34].

Essential oil analysis (Gas chromatography-Mass spectrometry "GC-MS")

The analyzes were carried out on a gas chromatograph of the Hewlett Packard Agilent 6890 type plus equipped with an HP-5MS capillary column (Stationary phase: 5% Phenyl 95% dimethylpolysiloxane (Dimensions: long 30 m * D int 0.25 mm *) film thickness 0.25µm). The oven temperature was programmed at 60°C for 8 mins, 2°C/mins up to 250°C., Isothermal for 10 mins. Analysis time: 113 mins. The temperatures of the injector and the detector were set at 250°C and 280°C respectively. The carrier gas is: helium, purity: N6.0; with a (High-speed flow): 0.5 mL/min. The EO sample volume injected by Split mode.1;20 is 0.2 µL. The GC-MS analyzes were carried out by a mass spectrometer: Hewlett Packard Agilent 5973 of the quadrupole type. The analysis mode is TIC scan (from 30 to 550). Solvent delay: 3.5 min. The temperatures of the ion source and the interface were set at 230°C and 280°C respectively. The mass spectra were obtained by an Electronic Impact "IE" (type of ionization) of 70 eV (intensity of the filament).

***Table 1**. information on GC-MS performed analysis.*

Column type	HP-5MS
Injection volume	0.2 µL
Injection temperature	250°C
Interface temperature	280°C
Mode of injection	Split.1; 20
Vector gas	Helium

The identification of the chemical compound of our EO was carried out using Thermo-Xcalibur 1.2 software by correlating their respective mass spectra with those of the libraries; Wiley 8th Edition, NIST 08, Adams library [35], Mass Finder terpenoids library [36] and [37]. According to Van Den Dool et *al.*, [38], we can determine the retention indices.

In-vitro studies (Anti-bacterial activity, synergistic effect)

Microorganisms

Antibacterial activity was evaluated using Gram-negative bacteria; *Escherichia coli (E. coli)* ATCC 25922, *Pseudomonas aeruginosa (P. aeruginosa)* ATCC 27853, *Proteus mirabilis (P. mirabilis)* ATCC 35659, *Klebsiella pneumoniae (K. pneumoniae)* ATCC 700603. And Gram-positive bacteria; *Staphylococcus aureus (S. aureus)* ATCC 6538, *Bacillus cereus (B. cereus)* ATCC 10876. All bacterial strains were provided from the (Institute of Pastor Algeria).

Antibiotics

In this study, the antibiotics-standards Carbénicilline 100 (µg) (CAR), Céfazoline 30 (µg) (KZ), Chloramphénicol 30 (µg) (C), Doxycycline 30 (µg) (DXT), Erythromycine 15 (µg) (E), Gentamicine 30 (µg) (CN), Néomycine 30 (µg) (N), Oxacilline 5 (µg) (OX), Pipéracilline 30 (µg) (PRL), Polymyxine B 100 (IU) (PB), Triméthoprime-Sulfamethoxazole 25 (µg) (SXT) were used.

Antibacterial effect of essential oil (Vincent-technique).

The Mueller–Hinton agar medium deposited in sterile petri dishes (90 mm diameter) is seeded in a sheet with the bacterial inoculum. After drying for 30 mins at 37°C, sterile Wattman paper blank discs of 6 mm in diameter were impregnated with 15 µL of essential oil tested and carefully deposited on the surface of the agar previously seeded with the suspension of each pathogenic bacterium. Finally, the dishes are subsequently incubated at 37°C for 24 h. each test was repeated three times. Indeed, after incubation, the absence of microbial growth results in a translucent halo around the disc, the diameter of the inhibition zone is expressed in millimeters [39]. Lesueur et *al.,* [40], indicate that antimicrobial activity of the extracts was determined by the disk diffusion method which is based on the spread of antimicrobial compounds in a solid medium.

Synergistic effect of the combination of the essential oil and antibiotics

The synergistic effect of the combination of the studied essential oil and antibiotics are evaluated using ready discs (antibiotic disc), So 15 µL of essential oil was saturated in the antibiotic disc which is deposited on the surface of the agar, to determine the diameter of the inhibition zone which was measured in millimeters [41].

In-vivo studies (acute toxicity, anti-inflammatory activity)

The *in-vivo* studies were performed on *Swiss albino* mice. In order to evaluate the acute toxicity of essential oil by oral administration and its anti-inflammatory activity by the plantar edema test induced by injection of a phlogogenic agent.

Animal material

Both sexes of *Swiss albino* mice weighing 25 to 30 (g) and originating from the institute Pastor Algiers (Algeria), were used for *in-vivo* testing. These animals were placed in polypropylene cages (fed regularly with free access to water). For a period of two weeks before the experiment, mice were acclimatized to the laboratory environment under controlled temperature conditions 25 ± 2°C with a cycle (light/dark) of 12 H.

Preparation of the solutions to be tested

According to Faria et *al.,* [42], *A. campetris* essential oil was suspended in a mixture of distilled water and 1% Tween 80 so as to obtain a 100 mg/mL solution to be administered orally to mice. Carrageenan (1%) and diclofenac additional agents were dissolved in normal saline (0.9%, w/v) prior to use.

Acute Oral toxicity

According to the OECD (Organization for Economic Cooperation and Development), Guideline N°423 the acute oral toxicity test was performed [43]. First of all, the mice

(females only) were fasted 16 hours before the experiment with free access to water. The studied EO was administered orally to groups of mice (n = 3) at doses of 50, 300, and 2000 mg/kg respectively. Regarding the control group, only received the vehicle (10 mL/ kg of 1% Tween 80). After treatment: The animals were observed continuously for 2 hours before feeding and watering them in order to detect changes in their autonomic or behavioral responses compared to the control group (death, restlessness, breathing, and asthenia). Monitoring for any mortality was continued for 48 hours and then for 7 days. This test was repeated at higher doses (up to 5000 mg/kg) with a new batch of animals (n = 3) when no death has occurred in any group.

Anti-inflammatory effect of EO (Carrageenan-induced plantar edema test)

The study of the anti-inflammatory effect of the studied EO of *A. campestris* was performed according to the protocol described by Winter et *al.*, [44]. Mice of both sexes were fasted for 16 hours prior to the experiment with free access to water. These animals were divided into 5 groups of 6 animals each. The batch I (control) received 10 mL/kg of 1% Tween 80. Batch II (standard) received 10 mg/kg of diclofenac (a reference anti-inflammatory drug). Batch III, IV, and V (experimental) respectively received doses of 100, 200 and 400 mg/kg of EO to be tested. After 30 mins of treatment by oral administration, the edema was induced by injection of 50 µL of carrageenan (1% w/v) into the sub-plantar region of the right hind paw. The thickness of the paw (mm) was measured using a digital caliper before injection (V_0) and every 60 mins for 6 Hours after induction of inflammation [45]. The percentage of inhibition of the edema was calculated according to the following formula;

$$\text{Percentage of inhibition} = [(V_t - V_0)_{\text{control}} - (V_t - V_0)_{\text{tested}}] \times 100 / (V_t - V_0)_{\text{control}}$$

We consider; V_0: volume of the paw before carrageenan injection, V_t: volume of the paw at a fixed time interval after injection of carrageenan

Statistical analysis of the anti-bacterial and anti-inflammatory activity of essential oil of *Artemisia campestris* was performed by software (Stat Box 6.4). Using ANOVA followed by the Newman and Keuls test.

RESULTS AND DISCUSSION

Chemical composition of essential oil

The EO extraction yield of *A. campestris* which has been determined is 4.9% (yield obtained from a test portion of 100 g consisting of dry leaves). The yield of our plant is much larger compared to the one obtained by Akrout et *al.*, [16] for *A. campestris* originating in Beni-Khedache (a mountainous region in the south of Tunisia). Akrout et *al*., [16], indicate that the yield of EO of Tunisian *A. campestris* is 1.2%. A study by Touil et *al.*, [46], shows that the lowest yield of EO of *A. campestris* is recorded for samples taken in spring (0.37%) while the highest level is observed from samples taken in summer with an average value of (0.41%). Consequently, the EO yield of a given species is influenced by intrinsic (such as growth stages) and extrinsic (such as pedoclimatic, conditions, and extraction methods) parameters [46]. The chemical composition of EO of *A. campestris* determined by GC and GC-MS analysis, the

retention time, and the percentage of the identified compounds of this EO was presented in table (2).

Table 2. *Chemical composition of Artemisia campestris essential oil determined by GC and GC-MS expressed in percentage (%).*

N°	Compounds	RI	Area (%)
1	Furfural	800	0.14
2	Camphene	948	0.15
3	α-Phellandrene	1000	0.74
4	1,8-Cineole	1034	13.31
5	cis-p-Menth-2-en-1-ol	1104	1.18
6	β-thujone	1111	6.97
7	trans-Pinocarveol	1116	0.58
8	α-thujone	1122	3.91
9	Chrysanthenone	1122	13.95
10	*cis*-Chrysanthenol	1146	0.21
11	Camphor	1154	41.95
12	*trans*-Pinocarveol	1167	1.96
13	Myrtenol	1177	0.75
14	γ-terpinène	1184	2.62
15	*trans*-Carveol	1195	0.14
16	*cis*-Carveol	1200	0.49
17	Piperitone	1214	0.42
18	Neral	1224	0.38
19	Geraniol	1230	0.60
20	*trans*-Myrtanol	1245	0.24
21	Geranial	1250	0.37
22	Bornyl acetate	1260	0.72
23	Lavandulyl acetate	1276	0.17
24	Thymol	1286	0.74
25	Carvacrol	1310	0.77
26	*trans*-Carvyl acetate	1318	1.30
27	α-Cubebene	1324	2.07
28	Methyl eugenol	1402	0.87
29	*Ar*-Curcumene	1479	0.52
30	Cedrol	1581	0.43
31	Humulene epoxide II	1597	0.21
32	α- Muurolol, epi	1630	0.58
33	β-Eudesmol	1640	0.56

RI: Retention index

Thirty-three compounds were identified representing 100% of the total detected constituent EO. This sample consists mainly of a mixture of monoterpenes, sesquiterpenes, and their oxygenated derivatives. The major compounds of EO of *A. campestris* were camphor (41.95%) and chrysanthenone (13.95%) followed by 1, 8-Cineole (13.31%), β-thujone (6.97%), α-thujone (3.91%). Other components are also found in low amounts such as; γ-terpinène (2.62%) and α-Cubebene (2.07%), trans-

Pinocarveol (1.96%), trans-Carvyl acetate (1.30%), cis-p-Menth-2-en-1-ol (1.18%). The strong predominance of camphor indicates that it represents the EO chemotype of *A. campestris*. Camphor as a major compound has also been reported by Belhattab et *al.*, [47], to have a value of (9%) in the region of Bousaada (Algeria), it is a value much lower compared to camphor of our oil (41.95%). For more comparison, β-pinene represents the major constituent with a high level (45.8%) in a sample collected in Beni-Khedache (a mountainous region in the south of Tunisia), Followed by α-pinene (12.5%) and limonene (7.7%) [16]. (Z, E)-Farnesol (10.3%) has been identified as the major component of EO in the region of Djelfa (Algeria) followed by Cedrol (5.4%), *trans*-Calamenene, Verbenone, Myrcene at contents ranging from (3.0%,3.3%, and 3.8% respectively) [21]. However, a study conducted on *A. campestris* reported that the main chemical profile was characterized by the predominance of germacrene-D, spathulenol, humulene epoxide II, and caryophyllene oxide [48]. On the other hand, according to our results, humulene epoxide has a very low value. Analyzes carried out on *A. campestri* collected in the Batna region also revealed the predominance of EO from the stems by 2-naphthaleneac etaldehyde,1,4-dihydro-α, α-dimethyl-1,4-dioxo- (22.1%) [49].Consequently, the EO of *A. campestris* in Mediterranean countries had different chemical models and differences in the relative amount of chemical compounds in EO. The main constituents recorded in the aerial parts were; γ-terpinene, capillene, 1-phenyl-2,4-pentadiyne, and spathulenol in France. In Italy they were; *Ar*-curcumene, caryophyllene oxide, *p*-cymene, β-pinene, germacrene D bicyclogermacrene, and myrcene. But in Portugal they were; β-pinene, cadin-4-en-7-ol and aromadendrene [50]. It has been reported that the variations in chemical composition encountered in the EOs of the studied species from a qualitative and quantitative point of view can be attributed to one or more factors such as; drying time, storage conditions, extraction method, part of the plant used, stage of development, genotype and presence of chemotype. Consequently, all these factors can influence the pathway of plant biosynthesis and have consequences on the relative proportion of the main characteristic of the compounds [51, 52, 53].

In-vitro studies

The EO of the dry leaves of *A. campestris* has shown that it has an effect against the four tested strains, the greatest zone of inhibition which was obtained against *E. coli* ATCC 25922 (24.33 mm) followed by (21.33 mm) against *K. pneumonia* ATCC 700603, *B. cereus* ATCC 10876 with a value of (21 mm). Then by (18.33 mm) against *P. mirabilis* ATCC 35659. On the other hand, it is inactive against *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 6538 (Table 3). From the antibiogram results presented in (Table 3), we find that the tested bacterial strains showed varying degrees of sensitivity to most of the antibiotics used. Indeed, the antibiotics which recorded the greatest zones of inhibition are as follows; Trimethoprim-Sulfamethoxazol (SXT) against *S. aureus* ATCC 6538 (34.33 mm), *E. coli* ATCC 25922 (31 mm); Neomycin (N) against *B. cereus* ATCC 10876 (26.33 mm); Gentamicin (CN) against *E. coli* ATCC 25922, *B. cereus* ATCC 10876 and *K. pneomonia* ATCC 700603 (24.33 mm, 21 mm and 18.66 mm respectively). Among the antibiotics which are resistant against more than two bacteria are the following; Erythromycin (E), Oxacillin (OX). Carbenicillin (CAR) is against all the tested bacteria. As well as Chloramphenicol (C), Cefazolin (KZ), and Piperacillin (PRL) are resistant against five tested bacteria (table3). The evaluation of the synergistic effect of antibiotics and EO of *A. campestris* by the disk diffusion

method on agar medium allowed us to obtain the results listed in (Table 3). According to the obtained results, the combination of EO of *A. campestris* with Gentamicin (CN) and Chloramphénicol (C) antibiotics showed a synergetic effect against five bacteria. The application of Erythromycin (E) with EO of *A. campestris* led to a synergistic effect on three tested bacteria (*E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *P. mirabilis* ATCC 35659). Followed by Doxycyclin (DXT) against five bacteria and Triméthoprime-Sulfamethoxazol (SXT) against four bacteria (Table.3). A synergistic effect was observed in all bacterial strains when we use Polymyxin B (PB) with the EO of *A. campestris*. The combination of the sample of *A. campestris* with Pipéracillin (PRL); appears to be inactive against all the tested bacterial strains. The association of EO of *A. campestris* with Céfazoline (KZ) antibiotics showed a synergetic effect against three tested bacteria. Finally, the combination of this oil with Carbenicillin (CAR) appears to be inactive against four bacterial strains (Table 3). According to Bertella [54], EO from *A. campestris* was of lower antibacterial activity (*S. aureus* 10.7 mm, *B. cereus* 15.7mm, *P. aeruginosa* 11.3 mm, *E. coli* 11.3 mm, *K. pneumoniae* 9.3 mm, *P. mirabilis* <8mm). On the other hand, our results are superior to this one particularly against *B. cereus*, *E. coli, K. pneumoniae, P. mirabilis*. A study was conducted by Khebri [55], on EO of *A. campestris* shows that the diameters of the zone of inhibition were less than (6 mm) on *E. coli, S. aureus, P. aeruginosa, K. pneumoniae*. The EO of this specie gave diameters of the zones of inhibition against *E. coli, K. pneumoniae, S. aureus* are respectively (18 mm, 10 mm, and 10 mm). On the other hand, these results revealed that *P. aeruginosa* ATCC 27853 was resistant to this oil [16], which confirm our result. Essential oils rich in phenolic components possess high levels of anti-microbial activity [56]. Fillippi et *al.*, [57], shows that the antibacterial power of essential oils can be contributed to their major components such as terpene alcohols which are soluble in aqueous media and therefore very powerful on soluble microbial [57]. It has been reported that the combination of gentamicin with each one of EO, including (*Aniba rosaeodora, Melaleuca alternifolia, Origanum vulgare*, and *Pelargonium graveolens)* shows a combination effect. A synergistic effect against all the tested strains was observed in gentamicin with *A. rosaeodora* and *P. graveolens* [58]. Milenkovića et *al.*, [59], demonstrated that EOs of *C. sylvatica, C. vardarensis*, and *C. nepeta* in combination with antibiotics (gentamicin or ciprofloxacin) exert a synergistic effect. Combination effects suggest that the EOs of the studied species could be used as potential adjuvants of antibiotics. The combination of EO of *T. willdenowii* Boiss with each one ticarcillin, imipenem, gentamicin, and tobramycin increased the antimicrobial activity of the tested antibiotics. As well as, the combination of EO of *O. compactum* with tested antibiotics has a synergistic effect against *P. putida*. A synergistic effect has also been detected against some bacteria when the EO of *C. coronarium* is combined with four tested antibiotics. The combination of Eos which are derived from the five medicinal plants and standard antibiotics have the potential for the development of new antimicrobials, treatment, and reduction of drug resistance which will allow finding the treatment of several diseases caused by microorganisms [60]. Indeed, this synergy could lead to new options for the treatment of infectious diseases and emerging drug resistance. Further studies based on the synergistic interaction molecule are essential to understand the fundamental synergistic mechanism for the development of pharmacological agents to treat bacterial infections using medicinal plants [60]. Finally, according to our bibliographical researches, there were not any previous studies related to the synergistic effect of antibiotics and the essential oil of *A. campestris* against bacterial strains.

Table 3. *Anti-bacterial activity of: essential oil of A. campestris, antibiotics-standard, and the synergistic effect between the essential oil and the tested antibiotics. (Zones of inhibition mm).*

Strains		***E. coli* ATCC 25922**	***P. mirabilis* ATCC 35659**	***B. cereus* ATCC 10876**	***P. aeruginosa* ATCC 27853**	***S. aureus* ATCC 6538**	***K. pneumonia* ATCC 700 603**
EO of *A.c*		24.33± 0.57	18.33 ±0.57	21 ± 1	0	0	21.33 ± 1.15
ATB	CN	24.33±0.57	16±1.00	21±1.00	16.33±0.57	24.33±1.15	18.66±0.57
	C	0	0	19.33±0.57	0	0	0
	E	0	0	0	0	0	0
	SXT	31±1.00	11.66±0.57	21±1.00	0	34.33±0.57	10±1.00
	CAR	0	0	0	0	0	0
	PB	16.66±0.57	16±1.00	12.33±0.57	17±1.00	15±1.00	17±0.00
	KZ	0	0	8.66±0.57	0	0	0
	N	20±1.00	20.33±0.57	26.33±0.57	15.33±0.57	22.66±0.57	14.33±1.15
	PRL	0	0	0	25.66±0.57	0	0
	OX	0	0	0	0	0	0
	DXT	16.66±0.57	16.66±0.57	12±1.00	11.66±0.57	17.33±0.57	11.66±0.57
Synergistic effect (EO of *A. c* + ATB)	CN	14.66±0.57	16±1	0	20.33±0.57	14.33±0.57	13±0.00
	C	24.33±0.57	9.33±0.57	15.66±0.57	16.33±0.57	0	21±1.00
	E	34.66±0.57	16.33±0.57	0	0	0	12.66±0.57
	SXT	18±1	0	16±1	0	10.33±0.57	14±0.00
	CAR	23.33±0.57	12.33±0.57	0	0	0	0
	PB	18±0	17±1	15.66±1. 15	21±1.00	15.33±0.57	14.33±0.57
	KZ	20.66±1.15	0	11±1	15±0.00	0	0
	N	9.33±0.57	0	11.33±0.57	9.33±0.57	12.66±0.57	0
	PRL	0	0	0	0	0	0
	OX	20±1	11.33±0.57	0	0	13.66±0.57	9.66±0.57
	DXT	20±0	12.66±0.57	13.66±0.57	10.33±0.57	0	15.33±0.57

EO of A. c: Essential Oil of Artemisia campestris; ATB: Antibiotic; CN: Gentamicin; C: Chloramphenicol; E: Erythromycin; SXT: Trimethoprim-Sulfamethoxazole; CAR: Carbenicillin; PB: Polymyxin B; KZ: Cefazoline; N: Neomycin; PRL: Piperacillin; OX: Oxacillin; DXT: Doxycycline. E. coli: Escherichia coli ATCC 25922; P. mirabilis: Proteus mirabilis ATCC 35659; B. cereus: Bacillus cereus ATCC 10876; P. aeruginosa: Pseudomonas aeruginosa ATCC 27853; S. aureus: Staphylococcus aureus ATCC 6538; K. pneumoniae: Klebsiella pneumoniae ATCC 700603.

In-vivo studies

Acute Oral toxicity

The assessment of the acute essential oil toxicity of the studied specie (*A. campestris)* was carried out by oral administration in *Swiss albino* mice provided by the Institute of Pastor Algeria. From the results obtained after 24 hours, we observe that the oral administration of this EO of different doses did not change the behavior of the tested mice, as well as there were no death or changes observed in the autonomic responses. Except; we observe very strong breath and asthenia during the first hours after the treatment with the dose of 5000 (mg/kg). According to a study conducted by Dib et *al.*, [61], the EO of *A. campestris* at 2 g/kg treated in animals showed several signs of intoxication such as; tremors, hyperactivity followed by asthenia, convulsions, and irregular breathing. After the first 24 hours, all these signs disappeared. After two weeks of injecting *A. campestris* EO, no significant changes were observed in organs and body

weights. This is the same result of our study. Acute oral toxicity of *A. campestris* EO has been reported that the result of LD_{50} value greater than 2 g/kg body weight. Sefi et *al.*, [62], found that several additional studies on the toxicity of *A. campestris* indicating that intraperitoneal administration of the aqueous extract to mice showed earlier toxicity with LD_{50}=2.5g/kg body weight. Indeed, the performance of an acute toxicity test of the essential oil of *A. campestris* on artemia larvae (Artemia sp.) gave the median lethal dose ranging from 15 to 20 g/mL [50].

Anti-inflammatory activity

The evaluation of the anti-inflammatory activity of *A. campestris* EO was performed by the test for plantar edema induced by injection of carrageenan in mice.

Table 4. *Anti-inflammatory effect of Artemisia campestris essential oil on paw edema induced by carrageenan in mice Swiss albino.*

	Paw diameter in millimeter (mm)				
Hours	**Control**	**Standard**	***Artemisia campestris* essential oil**		
/	(10 ml/Kg)	(10 mg/Kg)	C1(100 mg/Kg)	C2(200 mg/Kg)	C3 (400 mg/Kg)
0H	2.15±0.05	2.03±0.05	1.98±0.09	1.86±0.05	1.95±0.05
1H	2.93±0.1	2.75±0.05	2.75±0.05	2.61±0.04	2.68±0.07
2H	3.26±0.08	2.95±0.05	3.01±0.09	2.88±0.04	2.91±0.07
3H	3.43±0.08	2.68±0.09	3.11±0.04	3.01±0.07	3.05±0.05
4H	3.53±0.08	2.56±0.05	3.15±0.05	3±0.08	3.01±0.07
5H	3.31±0.07	2.33±0.08	2.91±0.07	2.75±0.05	2.70±0.06
6H	3.21±0.07	2.2±0.08	2.75±0.08	2.50±0.08	2.41±0.07

Control: vehicle (Tween 80, 1%); Standard: Diclofenac. Mean ± SEM (n = 6); C: Concentration(mg/Kg).

Table 4 and Figure 2 show the results of the evaluation of the anti-inflammatory effect of paw edema induced by carrageenan in mice (*Swiss albino*). Depending on the hours, the edema volume increases progressively and after four hours of the injection of carrageenan peaking in the control group. Reanmongkol et *al.*, [63], showed that edema induced by injection of carrageenan is a widely used model to assess the anti-inflammatory activity of substances. In fact, the injection of carrageenan causes the release of several chemical mediators which are responsible for the inflammatory process.

In addition, usually, this phenomenon is characterized by a biphasic response: the initial phase (1-2 hours); characterized by the release or evolution of chemical mediators such as serotonin, bradykinin, and histamine. The late phase (3-6 hours); the latter is supported by the release of prostaglandins, leukotrienes, lysozymes, proteases, and nitric oxide (NO) [64,65,66].

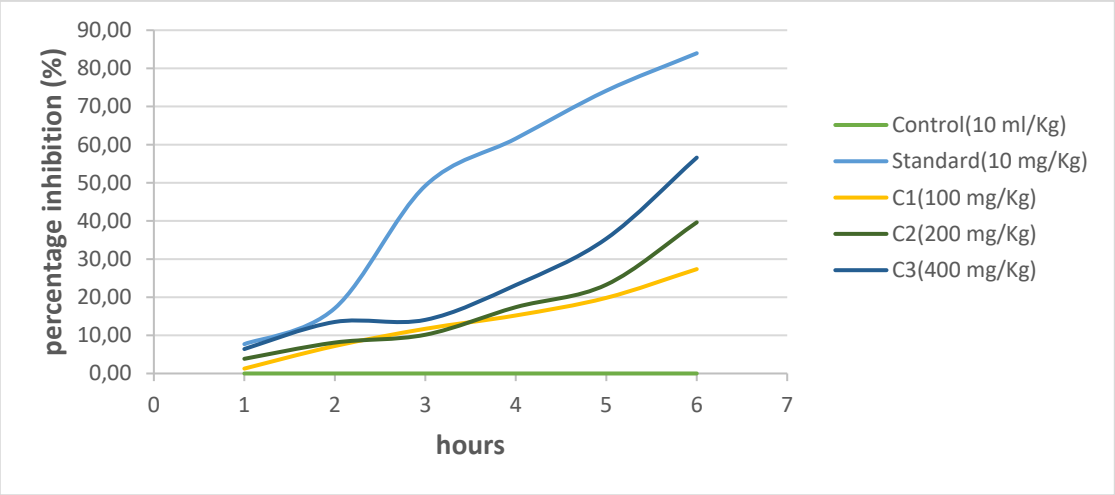

Fig. 2. *The percentage inhibition (%) of paw edema by diclofenac and the essential oil of Artemisia campestris for 6 hours after injection of carrageenan. (Control: 10 ml/Kg; Standard: 10 mg/Kg; C: Concentration mg/Kg (C1:100 mg/Kg; C2: 200 mg/Kg; C3: 400 mg/Kg)).*

A. campestris EO used at different doses for the treatment of mice showed a very significant decrease in the volume of the edema from four hours compared to the control. In comparison with diclofenac, the same phenomenon has occurred but from three hours compared with the control. The anti-inflammatory activity of *A. campestris* EO is attributed to its major compound Camphor and 1,8-Cineole. Indeed, there are minor constituents such as; β-thujone and α-Cubebene can contribute to the anti-inflammatory activity. *A. campestris* EO showed percentages inhibition of 27.36%, 39.62%, and 56.60% at doses of 100, 200, and 400 (mg/kg) body weight respectively after 6 hours on one hand. And on the other hand, these percentages were lower than those observed in the standard Diclofenac 83.96% (the reference anti-inflammatory). ROS (reactive oxygen species) are involved in the pathophysiology of diseases with an inflammatory component (cancer, diabetes, atherosclerosis, arthritis, and infectious diseases). As well as, they induced the release of cytokines (TNF-α, IL-1β, and IL-6) and the activation of pro-inflammatory enzymes (cyclo-oxygenases, lipoxygenase, and nitric oxide synthase) involved in the inflammatory process [67]. Tanas et *al.*, [68], have shown that lipoxygenase catalyzes the biosynthesis of leukotrienes which are pro-inflammatory and allergic mediators. At the same time, the reactive oxygen species oxidize the unsaturated lipids of the cell membrane leading to the formation of lipid peroxides which are cytotoxic and involved in the inflammatory process. Consequently, the activation of lipoxygenase and the formation of lipid peroxides is caused by the injection of carrageenan. Kim et *al.*, Mujumdar et *al.*, Panthong et *al.*, [69, 70, 71], have reported that many herbal remedies exhibit topical anti-inflammatory properties. Several studies show that flavonoids have anti-inflammatory properties and that they can modulate the functioning of the immune system by inhibiting the activity of enzymes that could be responsible for inflammation [72]. They can inhibit histamine [69].

It has been shown that essential oils rich in α-pinene, β-pinene, phellandrene, α-sabinene have anti-inflammatory activity [73,74,75]. Ghlissi et *al.*, [76], found that the anti-inflammatory effect could be due to the presence of phenolic compounds and flavonoids in the extracts. The aqueous extract of the leaves of *A. campestris* administered intraperitoneally shows an anti-inflammatory and analgesic

effect in paw edema induced by carrageenan in rats [76]. It has been reported that very little work has been carried out on studying the anti-inflammatory properties of the plant (*A. campestris*) [77]. According to the results obtained by Boudjouref et *al.*, [77], the study of the anti-inflammatory activity *in-vivo* showed that the extracts of the leaves of *Artemisia campestris* exert a powerful anti-inflammatory effect.

For more comparison, a study carried out shows that the ethanolic extract of the leaves of *Artemisia campestris* L. at a dose of 150 and 300 mg/kg significantly reduced edema from the second hour after administration of the extract compared to the control group. The activity was high at a dose of 300 mg/kg, compared to the activity produced by standard acetylsalicylic acid [78]. On the other hand, in our case, the EO activity was low at a dose of 400 mg/kg, compared to the activity produced by the reference anti-inflammatory diclofenac (83.96%). At the highest dose (300 mg/kg), the percentage inhibition showed that the ethanolic extract of *A. campestris* L. was more active (67.15%) [78] than the percentage inhibition of our EO at the highest dose 400 (mg/kg), and the recorded value was 56.60%. In addition, at the lowest dose (150 mg/kg), the percentage inhibition showed that the ethanolic extract was also superior (34.61%) [78] than the percentage inhibition of our EO at the dose 100 (mg/kg) has a value of (27.36%). Ethanolic extracts from the leaves of *Artemisia campestris* possessed a varying degree of anti-inflammatory activity. While this effect could be explained by the inhibition of the synthesis of pro-inflammatory substances [78]. Numerous additional studies showing that the anti-inflammatory activity of the extract can be explained in part by the presence of polyphenolic compounds such as; tannins and flavonoids in the leaves [79].

CONCLUSION

The essential oil of *A. campestris* analyzed by GC-MS is dominated by Camphor with 41.95%. The variability in the chemical composition of EO can be explained by different extrinsic factors (conditions of growth and development of the plant) or intrinsic (genetics of the plant). This sample has significant antibacterial activity, particularly against Gram-negative strains. The combination of EO of the studied specie with antibiotics may be effective against bacterial infections (origin of multidrug-resistant bacteria MBR). Evaluation of the *in-vivo* activity in *Swiss albino* mice showed that the EO of *A. campestris* when administered orally does not present any risk of acute toxicity. As well, it has demonstrated a considerable anti-inflammatory effect at lower non-toxic doses.

Conflict of Interest. "The authors declared that there is no conflict of interest."

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