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Liquid chromatography–mass spectrometry phytochemical analysis, antioxidant, anti–inflammatory and analgesic efficacy of *Origanum majorana* L. aerial part extracts collected from North–Eastern of Algeria

Análisis fitoquímico, cromatografía / espectrometría de masas, eficacia antioxidante, antiinflamatoria y analgésica de extractos de partes aéreas de *Origanum majorana* L. recolectados en el noreste de Argelia

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ABSTRACT

Origanum majorana L. (Lamiaceae) has been prescribed in folk medicine for therapeutic purposes such as cancer, asthma, dizziness, rheumatism and inflammatory diseases. To evaluate for the first time the decoction (DecE) and hydro–ethanolic (HEE) extracts of the aerial part of *O. majorana*. The phytochemical analysis (LC–MS/MS), antioxidant, anti–inflammatory and analgesic potentials were analysed. LC–MS/MS analysis identified 17 components in the extracts. The main compounds detected in the aerial parts of *O. majorana* include rosmarinic acid (≥ 50%), elagic acid (≥ 20%), indomethacin along with flavonoids, phenolics, benzopyrone, hydroquinones, indolyl acetic acid. Both extracts showed potent antioxidant effects in various antioxidant assay models. Through the eggs protein denaturation assay, both plant extracts showed high *in vitro* anti–inflammatory effect of the order of 86.91 ± 0.00% for DecE and 85.04 ± 0.00 % for HEE (1.4 mg·mL^{-1}). Acute toxicity measurement revealed no mortality or behavioral changes during the testing period, confirming the two extracts not toxic with an LD_{50} > 2 g·kg^{-1}. The *in vivo* anti–inflammatory effects were found to be statistically significant (P<0.05), reducing the edematous response by 78.40 ± 2.52 % and 77.36 ± 3.88 % in DecE and HEE, respectively. Additionally, at a dose of 400.0 mg·kg^{-1}, both extracts exhibited significant analgesics activity (P<0.05) against acetic acid induced abdominal constriction in mice, with inhibition rates of 78.54 ± 3.30% for DecE and 66.99 ± 1.34% for HEE. The results indicate aerial part extracts of *O. majorana* have potent anti–inflammatory effects and may prove to have potential health benefits.

Key words: *Origanum majorana* L; phytochemical analysis; toxicity; anti–inflammatory activity; analgesic activity

RESUMEN

Origanum majorana L. (Lamiaceae) se ha prescrito en la medicina popular con fines terapéuticos para tratar cáncer, el asma, el mareo, el reumatismo y enfermedades inflamatorias. Aquí se evalúa por primera vez los extractos de decocción (DecE) e hidroetanólico (HEE) de la parte aérea de la *O. majorana*. Se analizó el potencial antioxidante, antiinflamatorio y analgésico mediante análisis fitoquímico (LC–MS/MS). El análisis LC–MS/MS identificó 17 componentes en los extractos. Los principales compuestos detectados en la parte aérea de *O. majorana* incluyen ácido rosmarínico (≥ 50 %), ácido elágico (≥ 20 %), indometacina, flavonoides, compuestos fenólicos, benzopirona, hidroquinonas y ácido indolil acético. Ambos extractos mostraron potentes efectos antioxidantes en diversos modelos de ensayo antioxidante. Mediante el ensayo de desnaturalización de proteínas de huevo, ambos extractos vegetales mostraron un alto efecto antiinflamatorio *in vitro*, del orden del 86.91 ± 0.00 % para DecE y del 85.04 ± 0.00 % para HEE (1.4 mg·mL^{-1}). La medición de toxicidad aguda no reveló mortalidad ni cambios en el comportamiento durante el período de prueba, lo que confirmó la no toxicidad de ambos extractos con una DL_{50} > 2 g·kg^{-1}. Los efectos antiinflamatorios *in vivo* resultaron estadísticamente significativos (P<0.05), reduciendo la respuesta edematosa en un 78.40 ± 2.52 % y un 77.36 ± 3.88 % en DecE y HEE, respectivamente. Además, a una dosis de 400,0 mg·kg^{-1}, ambos extractos mostraron una actividad analgésica significativa (P<0.05) contra la constricción abdominal inducida por ácido acético en ratones, con índices de inhibición del 78.54 ± 3.30 % para DecE y del 66.99 ± 1.34 % para HEE. Los resultados indican que los extractos de la parte aérea de *O. majorana* tienen potentes efectos antiinflamatorios y podrían tener beneficios potenciales para la salud.

Palabras clave: *Origanum majorana* L; análisis fitoquímico; toxicidad; actividad antiinflamatoria; actividad analgésica

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INTRODUCTION

Oxidative stress arises from an imbalance between antioxidant defense systems and pro–oxidants factors [1], whether due to a deficit in defense mechanisms such as antioxidant compounds and antioxidant enzymes [2] or excessive production of free radicals [3]. An antioxidant and anti–inflammatory drugs can prevent or inhibit the generation of toxic oxidants, scavenge those that are already produced, thereby blocking the spreading chain reaction produced by these oxidants [4, 5].

Plants have served as a primary source of medicine, with approximately 80% of the global population relying on herbal remedies for various health issues. The traditional knowledge of plant use varies across different regions, underscoring the importance of documenting this information to preserve it for future generations [6].

In recent years, many pharmaceutical companies have renewed their focus on natural product research, leveraging ethnopharmacological studies to uncover their potential as promising sources of new compounds for therapeutic development [7, 8].

Many of these phytochemicals isolated from plants have potential for drug development, and may act additively, individually, or synergistically to improve health. In contrast, synthetic antioxidants have recently faced approval issues in several developed countries, leading to increased interest in natural anti–inflammatory and antioxidants. This trend has spurred efforts to discover new and beneficial antioxidant and anti–inflammatory agents from medicinal plants [7, 8].

Locally, *O. majorana* is known as Merdeqouch, Arzema or M'loul. This plant contains a high concentration of beneficial substances such as flavonoids, phenolic compounds, cinnamic acid derivatives and flavanones. The components of the plant are being employed in many impoverished nations as a remedy for treatment of a variety of chronic conditions. In folk medicine, is used to treat indigestion, asthma, headache, cramps, depression, rheumatism, and is also considered for its diuretic activity [9]. Traditionally, marjoram leaves are used to manage diabetes [10]. Various parts of plants have been harnessed for therapeutic purposes [5, 11].

Food manufacturers and researchers are increasingly focusing on whole plants and plant parts that contain bioactive phytochemicals in order to develop pharmaceuticals and functional foods. However, there is a paucity of information regarding the phytochemical analysis, antioxidant, anti–inflammatory and analgesic potentials in aerial parts of Merdeqouch plants. This knowledge gap prompted the current study. Furthermore, the phytochemical composition, bioactivity and toxicity of aerial part of *O. majorana* remain poorly documented and warrant further study. To date, only a few scientific reports that they are aware have investigated the phytochemical composition, antioxidant, toxicity, anti–inflammatory and analgesic activities and the safe dose of decoction and hydro–ethanolic extracts of the aerial parts of *O. majorana*. Additionally, there is need to determine whether its use in traditional medicine for inflammation is justified.

MATERIALS AND METHODS

Plant collection and identification

Fresh aerial parts of *O. majorana* were obtained from Bouandas–Setif located in North–Eastern Algeria (5°06′07″ east longitude and 36°29′41″ N) in March–May 2022 during the flowering stage. The aerial parts were air–dried in the shade, then crushed and pulverized with an electric grinder (Germany). The medicinal herb utilized in this investigation was *O. majorana*, which belongs to the Lamiaceae family. Prof. Laouer H., a renowned taxonomist at Setif-1 University, Algeria, authenticated and identified the plant under a voucher specimen (060/DBEV/UFA/22).

Animals

Adult female albino mice (*Mus musculus*) weighing (Aniphy balance) 25.0–30.0 g were used. The animals were procured from Algiers' 'Institut Pasteur d'Algérie'. Mice were housed in cages with a 12:12 light/dark cycle at 25.0 ± 1.0°C for 7 days (d) before to the study. They had unlimited access to water and a regular diet and were kept in compliance with Animals By–Laws No. 425 [12].

Extracts preparation

Preparation of aqueous extract

The plant extracts were obtained using established decoction method [13]. To prepare the decoction extract, 100.0 g of dried aerial parts of the plant were boiled in 1 L of distilled water for 20 min. The resulting extract was then filtered before centrifugation at 3000 G (Sigma 3-30K, Germany) for 20 min. This dried decoction extract (DecE) was then tested for pharmacological characteristics.

Preparation of hydroethanolic extract

The hydroethanolic extract (HEE) of the plant was prepared from 100 g of ground aerial part. The powder was soaked in 1 L of ethanol/water (70/30, v/v) and continuously shaken for 48 h. Then the solution was filtered and the solvent was recovered by evaporation in a rotavapor (Buchi rotavap R-205, Switzeland), at a temperature of 45°C. The dried hydro–ethanolic extract was preserved in 4°C.

Determination of total polyphenol, flavonoids and hydrolysable tannins

The total phenolic content of the extract was assessed using the Folin–Ciocalteu's technique [14]. The polyphenol concentration was expressed as microgram (µg) of gallic acid equivalent (GE) per gram (g) of dry extract, with quantification based on a gallic acid standard curve (ranging from 0.00 to 160 µg·mL^{-1}). The total flavonoid content was determined using the aluminum chloride method [7]. The total flavonoids were reported as µg of quercetin equivalent (QE) per mg dried extract (DE). The total flavonoids in the extracts were evaluated using a quercetine standard curve (0.00 to 40 µg·mL^{-1}). To determine hemoglobin precipitation by tannins, they utilized the method indicated below [15]. The supernatant absorbance was measured at 576 nm, and the precipitation efficiency of the investigated solutions was reported as µg tannic acid equivalent (TE)·mg^{-1} dried extract. To generate the calibration curve, equal parts hemolyzed blood and tannic acid (50-600 µg·mL^{-1}) were combined.

Liquid chromatography–mass spectrometry (LC–MS/MS) phytochemical analysis

The analysis of some selected flavonoids and phenolic acids was performed on AB Sciex QTRAP 4500 LC–MS/MS (Japan), utilizing Hypersil GOLD Dia (100 mm × 4 mm, particle size 3 µm, column oven 40°C). The ethanolic and aqueous extracts were freshly prepared (as 1000 µg·mL^{-1} of each sample separately) by dissolving them in ethanol (99% absolute) and deionized water, respectively. The samples were filtrated by nylon syringe filter (0.45 µm), and 5 µL of each was injected into the QTRAP 4500 system using the developed method. The flow rate was 0.3500 mL·min^{-1} and the mobile phase consisted of 0.1% formic acid in deionized water and, delivered in gradient elution mode. Sample solutions were produced and promptly analysed for phenolic acids and flavonoids using LC–MS/MS. A stock solution consisting of the screened flavonoids and phenolic acids (17 Std: 3,5–dihydroxy–benzoic acid, salicylic acid, caffeic acid, coumarin, ferulic acid, vanillin, 4–hydroxy benzoic acid, chlorogenic acid, rutin, elagic acid, arbutin, trans–2–hydroxy cinnamic acid, P–commaric acid, 3,4–dihydroxy benzoic acid, naringenin, indomethacin, rosmarinic acid) were prepared in HPLC–grade ethanol. Quantitative data for the phenolic compounds were obtained by comparing the samples to the injected mixed phenolic standards.

In vitro anti–oxidant activity

DPPH radical scavenging assays

The free radical scavenging capacity of the extracts were evaluated using the DPPH assay (2,2'–diphenyl–1–picrylhydrazyl) [13]. Absorbance was measured at 517 nm and the scavenging capacity was calculated with the following equation:

$$I\% = \left(\frac{A_b - A_t}{A_b}\right) \times 100$$

Where A_b the absorbance of the blank and A_t the absorbance of the tested sample.

Reducing power assay

The reducing power of *O. majorana* extracts was estimated based on their ability to reduce Fe^{+3} to Fe^{+2} ions [16]. After ten minutes of incubation, the colour intensity was assessed at 700 nm. In this assay, a higher value of absorbance of the solution indicates a greater reducing power of the extract.

In vivo assays

Acute toxicity

The acute oral toxicity of DecE and HEE was tested in mice in accordance with OECD guidelines [12]. The mice were divided into 3 groups, each consisting of five animals. After 12 h of fasting, the mice were orally administered with a single dose of the extracts (2.0 g·kg^{-1}) body weight. The control group received just distilled water. The mice were closely monitored for any indication of toxicity during the first hour of post treatment and regularly over the next 24 h. Subsequently, daily observations were conducted for 14 d to detect any delayed toxic effects.

Xylene–induced ear edema assay

The oral anti–edema effect was also investigated employing xylene–induced ear edema assay [15]. The mice were divided into groups of six. One hour after oral administration of varying doses of DecE and HEE (50 and 200 mg·kg^{-1}), indomethacin (50 mg·kg^{-1}) and distilled water (negative control), edematous was locally provoked in mice ears by applying 30 µL of xylene. A digital caliper (caliper to DIN 862, Germany) was used to measure ear swelling both before and 2 h after edema induction.

Analgesic activity

The analgesic efficacy of the extracts was estimated by induction of abdomen contraction in mice using acetic acid [15]. The mice were divided into groups of five. The negative control group received distilled water, whereas the positive control was administered aspirin (100 mg·kg^{-1}). The test groups were administered with extracts at dosages of 200 and 300 mg·kg^{-1}. Abdominal writhing was caused intraperitoneally by injecting 0.1% acetic acid and 60 minutes post–treatment for all groups. The number of writhes was recorded for each group, starting 5 min after acetic acid injection and continuing for 30 min. The percentage inhibition of the writhing response was determined by applying the following equation:

$$I\% = \left(\frac{C_n - C_t}{C_n}\right) \times 100$$

where C_n and C_t are the mean of constriction' count in mice in the negative control and the treated groups with different concentrations of extracts or aspirin.

Statistical analysis

Results from *in vitro* and *in vivo* were analyzed using one–way ANOVA to determine significance. Data were generated in triplicate and are presented as the mean ± standard deviation. (SD). GraphPad Prism-5 was used to analyze the data collected during this experiment. Significant differences were defined as $P<0.05$.

RESULTS AND DISCUSSION

Extract yield

Origanum majorana L. was extracted using decoction and maceration methods. The extraction yields were calculated, with the HEE showing the extraction yielding 18.04 ± 1.24%, while the DecE had a yield of 17.42 ± 1.87%. The yields of extracts reported here for the aerial part extracts were analogous to leaves extract obtained by Qnais *et al.* [17]. The biological evaluation of *O. majorana* extracts is essential for determining their health–promoting qualities, underscoring the importance of optimizing extraction conditions [18]. The extracts analyzed in this study contained concentrated active principles, likely due to prior extraction with non–polar solvents or at high temperatures [19].

Quantitative phytochemical analysis

Total polyphenols, flavonoids and tannins contents

Quantitative tests were performed to determine the concentration of the phytochemicals in the extracts. The levels of polyphenols,

flavonoids and tannins were quantified using spectrometric methods. Specifically, the total phenolic content (TPC), total flavonoids content (TFC) and total condensed tannins content (CTC) of different *Origanum majorana* L. hydro–ethanolic and decoction extracts were evaluated employing the Folin–Ciocalteu's reagent, aluminium chloride and haemoglobin precipitation methods, respectively. The results are presented in TABLE I. Based on the findings, it is evident that the hydroethanolic extract exhibited the highest concentrations of total phenolics, flavonoids and tannins with values of 302.86 ± 3.90 µg·GE^{-1}; 10.69 ± 0.60 µg·GE^{-1} and 103.87 ± 3.06 µg TE·mg DE^{-1}, respectively.

TABLE I
Total polyphenols, flavonoids and tannins contents of *Origanum majorana* L. extracts

Extracts	Total phenolic content (a)	Total flavonoids content (b)	Tannins content(c)
DecE	283.84 ± 3.18	7.23 ± 0.37	74.14 ± 0.93
HEE	302.86 ± 3.90	10.69 ± 0.60	103.87 ± 3.06

(a): gallic acid equivalent (GE µg) per mg dried extract (DE), (b): quercetin equivalent (QE µg) per mg dried extract (DE), (c): tannic acid equivalent (TE µg) per mg dried extract (DE). DecE: Decoction extract, HEE: hydroethanol extract.

Phenols, flavonoids, and tannins are among the major phytochemicals responsible for antioxidant activity and a variety of biological activities. It is worth noting that phenolics were found to be the most abundant phytoconstituents measured in this study, followed by flavonoids and tannins in HEE when compared to the DecE extracts. The total phenolic content (TPC) of the aerial parts extracts obtained in this study was higher than values reported in previous studies on two oregano leaves [17, 18, 20], aerial part extracts [21] and seeds extracts reported by Dhull *et al.* [22]. Similarly, the total tannin content (TC) of both extracts in this study exceeded that of seeds extracts from *O. majorana* reported by Dhull *et al.* [22].

LC–MS/MS analysis

Seventeen phenolic compounds, including phenolics and flavonoids, which are widespread in edible plants, were analyzed in phenolic–rich the hydro–ethanolic and decoction extracts (TABLE II). Based on the LC–MS/MS results from the current study, the hydroethanolic and decoction extracts of *O. majorana* had very rich phenolic content primarily due to their significant concentrations of rosmarinic acid, elagic acid and other flavonoids and phenolic compounds. Rosmarinic acid was identified as the predominant compound in both extracts, with HEE and DecE showing respective relative areas of 52.46 and 50.16%. Ellagic acid was the second most abundant compound, with an area of 21.05 in DecE and 19.09% in HEE. Notably, 4–hydroxy benzoic acid was not detected in the DecE, unlike in the HEE, (TABLE II).

The present findings revealed the presence 17 phytochemicals in the two extracts: 3,5–dihydroxy–benzoic acid, salicylic acid, caffeic acid, coumarin, ferulic acid, vanillin, chlorogenic acid, rutin, ellagic acid, arbutin, *trans*–2–hydroxy cinnamic acid, *p*–commaric acid, 3,4–dihydroxy benzoic acid, naringenin, indomethacin, rosmarinic acid.Notably, 4–hydroxybenzoic acid which was present only in HEE. In contrast to the present results, Çarıkçı *et al.* [23] reported the absence of vanillin and hydroxy benzoic acid in the different extracts from the aerial part of *O majorana*. Conversely, compounds such as caffeic acid, gallic acid, rosmarinic acid, and chlorogenic acid were present in some extracts by other studies. Additionally, prior research has identified several phenolics and flavonoids from the ethyl acetate extract of the aerial parts of *O. majorana*, including hesperetin, rosmarinic acid, 5, 6, 3′–Trihydroxy–7,8,4′–trimethoxyflavone, hydroquinone, and arbutin [24]. Furthermore, Amaghnouje *et al.* [25] revealed the presence of 12 phytochemicals in the leaves of *O. majorana*: rosmarinic acid, arbutin, quercetin–3–*O*–glucoside, ursolic acid, luteolin–7–*O*–glucoside, quercetin–7–*O*–glucuronic acid, kaempferol–3–0–pentose, kaempferol–3–0–glucuronic acid, catechin, caffeic acid, rutin and quercetin. The genus *Origanum* serves as an excellent source for isolating a wide range of bioactive compounds. Consequently, this genus exhibits significant biological activities and holds potential for addressing a variety of ailments.

TABLE II
LC–MS/MS data for phenolic and flavonoids compounds detected in the decoction and hydro–ethanolic aerial part extracts of *Origanum majorana* L. and their percentage

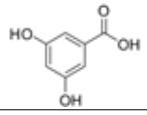
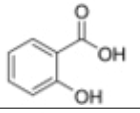
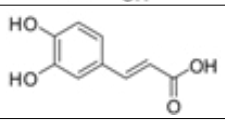
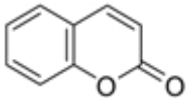
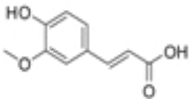
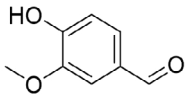
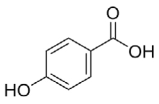
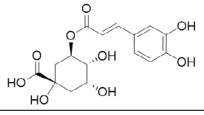
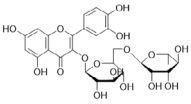
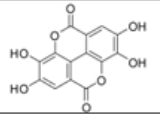
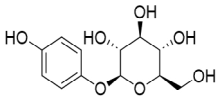
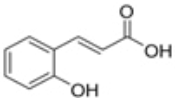
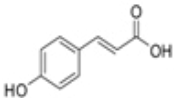
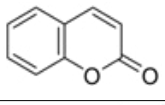
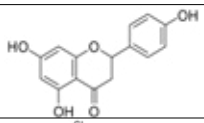
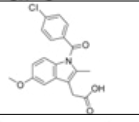
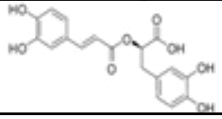
N°	Compounds	Chemical Class	Molecular Formula	Structure	R_t (min)		Area %	
					DecE	HEE	DecE	HEE
1	3,5-dihydroxy–benzoic acid	Phenolic acid	$C_7H_6O_4$		4.756	4.759	4.31	3.56
2	Salicylic acid	Phenolic acid	$C_7H_6O_3$		3.292	3.309	3.31	2.390
3	Caffeic acid	Phenolic acid	$C_9H_8O_4$		10.060	9.986	1.34	1.53

***TABLE II* cont...**
LC–MS/MS data for phenolic and flavonoids compounds detected in the decoction and hydro–ethanolic aerial part extracts of *Origanum majorana* L. and their percentage

4	Coumarin	Benzopyrone	$C_9H_6O_2$		8.861	8.774	3.08	3.24
5	Ferulic acid	Flavonoid	$C_{10}H_{10}O_4$		8.304	8.292	1.52	2.67
6	Vanillin	Phenolic acid	$C_8H_8O_3$		7.319	7.285	2.57	3.17
7	4-hydroxy benzoic acid	Hydroxybenzoic acid derivatives	$C_7H_6O_3$		ND	6.194	ND	1.44
8	Chlorogenic acid	Phenolic acid	$C_{16}H_{18}O_9$		6.161	6.157	7.46×10^{-1}	1.55
9	Rutin	Flavonoid	$C_{27}H_{30}O_{16}$		8.675	8.670	1.64	4.06
10	Elagic acid	Tannins	$C_{14}H_6O_8$		11.997	11.980	21.05	19.09
11	Arbutin	Hydroquinone	$C_{12}H_{16}O_7$		11.576	11.583	4.03	3.90×10^{-1}
12	Trans-2-hydroxy cinnamic acid	Phenylpropanoids	$C_9H_8O_3$		9.759	9.751	2.11×10^{-1}	2.45×10^{-1}
13	*p*–Commaric acid	Phenolic acid	$C_9H_8O_3$		7.959	7.953	01.30	01.29
14	3,4-dihydroxy benzoic acid	Phenolic acid	$C_7H_6O_4$		29.189	29.184	$06.69*10^{-1}$	$06.61*10^{-1}$
15	Naringenin	Flavonoid	$C_{15}H_{12}O_5$		11.576	11.581	03.26	$03.27*10^{-1}$
16	Indomethacin	Indolyl acetic acid	$C_{19}H_{16}ClNO$		12.699	12.653	$02.13*10^{-2}$	$7.28*10^{-5}$
17	Rosmarinic acid	Phenolic acid	$C_{18}H_{16}O_8$		8.681	8.680	50.16	52.46

DecE: Decoction extract, HEE: hydroethanol extract, ND: Not detected

Anti–oxidant effects

DPPH radical scavenging activity

Many plant extracts exhibit significant antioxidant properties due to the presence of phytoconstituents such as flavonoids and phenolic acids. The antioxidant activity of the hydroethanolic (HEE) and decoction extracts (DecE) from the aerial part of *O. majorana* was evaluated against the DPPH assay, with absorbance measured spectrophotometrically at 517 nm. As shown in TABLE III, both HEE and DecE demonstrated high radical scavenging activity with IC_{50} values of 24.55 ± 0.00 and 26.06 ± 0.00 µg·mL^{-1}, respectively compared to the standard. This strong antioxidant activity can be attributed to the high contents of rosmarinic acid (≥50%) and elagic acid (≥20%). Ellagic acid is a potent bioactive compound with numerous industrial and pharmacological uses [26].

TABLE III
Antioxidant activity of decoction and hydroethanolic *Origanum majorana* extracts

	Antioxidant activity / Inhibition concentration (IC_{50})	
Extracts / standards	**DPPH radical scavenging**	**Reducing power**
DecE	26.06 ± 0.00***	39.43 ± 0.00***
HEE	24.55 ± 0.00***	29.50 ± 0.00***
BHT	87.26 ± 0.00	ND
Vitamin C	ND	21. 91 ± 0.48

DecE: Decoction extract, HEE: hydroethanol extract

In agreement with our findings on DPPH and ABTS assays, Erenler *et al.* [24] reported that hydroquinone, hesperetin, rosmarinic acid and arbutin from *O. majorana* exhibited a potent antioxidant in of ABTS$^{\bullet+}$, DPPH$^{\bullet}$, and reducing capacities assays. Similarly, Vasudeva *et al.* [27] reported findings consistent with the present DPPH results, showing that ethanol extracts of root and stem of *O. majorana* also showed high scavenging activity, with an IC_{50} values of 21.05 µg·mL^{-1} and 84.98 µg·mL^{-1}, respectively. Possibly, the high content of rosmarinic acid is the phenolic acid that contributes to the greater antioxidant activity in the hydromethanolic extract of *O. majorana*, as evaluated using FRAP and DPPH [28]. In contrast, Vallverdú–Queralt *et al.* [29] identified protocatechuic acid, syringic acid, and caffeic acid as the primary components in a hydro–ethanolic leaves extract of *O. majorana*, but did not detect rosmarinic acid.

Reducing power assay

TABLE III depicts the antioxidant activity curves for extracts with reducing properties. In this experiment, all extracts indicated the capacity to donate electrons, reducing Fe^{3+} to Fe^{2+}. Among the tested extracts, HEE showed the highest reducing potential with an IC_{50} = 29.50 ± 0.00 µg·mL^{-1} followed by DecE, with an IC_{50} of value of 39.43 ± 0.00 µg·mL^{-1}, respectively. The reducing power of the extracts serves as a strong indicator of their antioxidant activity. Reductions complete the free radical chain reaction by providing hydrogen atoms to the radical molecules. Vasudeva *et al.* [27] found that the ethanol extracts of the root and stem of *O. majorana* exhibited lesser reduction power, in agreement with the current study. By making the conversion or equivalences in the reported units of expression, comparisons can be made, as the majority of the studies has been documented results in terms of absorbance values. Despite this, all extracts demonstrated potential reducing power, likely due to the presence of oxidizable components.

***In vivo* studies**

Acute toxicity

The present results revealed that oral administration of DecE and HEE from *O. majorana* did not cause any mortality in the treated mice over the 14 d experimental period. Furthermore, no behavioural changes or visible signs of acute toxicity were observed. Consequently, the LD_{50} of both extracts was determined to exceed 2 g·kg^{-1} of body weight for mice. Similarly, Qnais *et al.* [17] reported that a single oral administration of methanol extract had an LD_{50}, higher than 2 g·kg^{-1}. In addition, it has been reported that methanolic extract of *O. majorana* leaves is safe and shows no toxicity at the tested doses [25]. However, the administration of high doses of *O. majorana* extracts should be approached with caution.

Xylene–induced ear edema activity

The results of this investigation demonstrated that DecE and HEE, at dosages of 50 and 200 mg·kg^{-1} exhibit strong anti–edema activity in the xylene–induced ear edema assay, as illustrated in FIG. 1. Both extracts exerted significant (P<0.05) and dose–dependent inhibitory effects against the edema reaction produced by xylene at the tested concentrations (50 and 200 mg·kg^{-1}). Additionally, the current findings indicated that DecE and HEE from the aerial part of *O. majorana,* administered at a dose of 200 mg·kg^{-1}, reduces the edema response by 78.40 ± 2.52 and 77.36 ± 3.88%, respectively. These results were comparable to the standard non–steroidal anti–inflammatory drug indomethacin, which exhibited an inhibition rate of 79.01 ± 6.36% at a dose of 50 mg·kg^{-1}.

The current findings show that DecE and HEE obtained from the aerial part of *O. majorana* administered at a dose of 200 mg·kg^{-1},

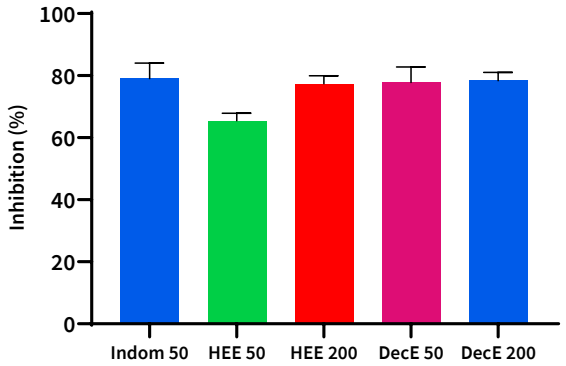


FIGURE 1. Antiinflammatory activity of DecE and HEE by xylene–induced ear edema in mice. Indom 50: Indomethacin: 50 mg·kg^{-1}, HEE 50 / HEE 200: Hydroethanol extract, 50 mg·kg^{-1} / 200 mg·kg^{-1} respectively. DecE 50 / DecE 200: Decoction extract, 50 mg·kg^{-1} / 200 mg·kg^{-1} respectively. The results were expressed as means ± SEM, n= 6

significantly reduced the edema response by more than 75% compared to the nonsteroidal antiinflammatory drug indomethacin (79.01%). In contrast, Ravishankar *et al.* [30] reported lower maximal inhibition (<40%) for *O. majorana* fractions using the carrageenan–induced inflammation model. The anti–inflammatory effect of indomethacin can be attributed to its ability to reduce the production of pro–inflammatory prostaglandins. A variety of flavonoids with diverse chemical structures have been associated with different antiinflammatory mechanistic actions [31]. Additionally, natural products have shown potential in reducing inflammation in various systems, including the skin, joint, cardiovascular, lungs, neuro, and gastrointestinal tract. Furthermore, research exploring the structure–activity relationship of flavonoids and their antiinflammatory properties have been published [32]. Xylene induces vasodilation, increased edema and blood vessel permeability, contributing to inflammation. The chemical and biological mechanisms underlying xylene–induced inflammation include sensory neurons that are sensitive to capsaicin, a phenomena referred to as neurogenic inflammation. This suggests that the extracts may reduce substance P release or decrease its activity [33], potentially through the inhibition of phospholipase A_2, which plays a key role in the pathophysiology of xylene–induced inflammation [34].

Analgesic activity

The present findings indicate that DecE and HEE possess analgesic properties against acetic acid induced abdominal tightness. The results presented in FIG. 2 show that these extracts have a strong antinociceptive efficacy against acetic acid–induced writhing in mice in a dose–dependent manner (P<0.05). At a dose of 400.0 mg·kg^{-1}, HEE and DecE, significantly reduced abdominal constriction by 78.54 ± 3.30% and 66.99 ± 1.34%, respectively. For comparison, the conventional medication, aspirin (100 mg·kg^{-1}), demonstrated a clear inhibitory effect, with a rate of 79.32%.

Injection of acetic acid induces peritoneal irritation, leading to characteristic writhing behaviour. Research has shown that acetic acid indirectly promotes the release of endogenous pain mediators such as prostaglandins, kinins, and histamine, which excite nociceptive neurons that are sensitive to nonsteroidal antiinflammatory drugs [8, 15]. Previous studies have demonstrated that methanolic extract from leaves had a lower antinociceptive effect (≤ 40 %) compared to reference drug [19]. In contrast, our findings revealed a significant antinociceptive effect (≥ 60 %) at a dose of 200 mg·kg^{-1} for both DecE and HEE. These extracts effectively attenuated writhing responses, suggesting that they possess notable antinociceptive properties. Based on the findings of this study, it can be inferred that DecE and HEE obtained from the aerial parts of *O. majorana* have significant antinociceptive properties. Consequently, it is proposed that the observed antinociceptive effects of DecE and HEE could be attributed to their bioactive constituents. In addition, they are likely attributed to the presence of rosmarinic acid, elagic acid, indomethacin, salicylic acid, vanillin, arbutin and other flavonoid and phenolic acids detected in *O. majorana.* Phenolic molecules with both antiinflammatory and antioxidant properties are highly desirable when developing pharmaceutical formulations from any natural resource. Considering these findings, it is evident that *O. majorana* present an antinociceptive properties, which could pave the way for the recovery of natural analgesics.

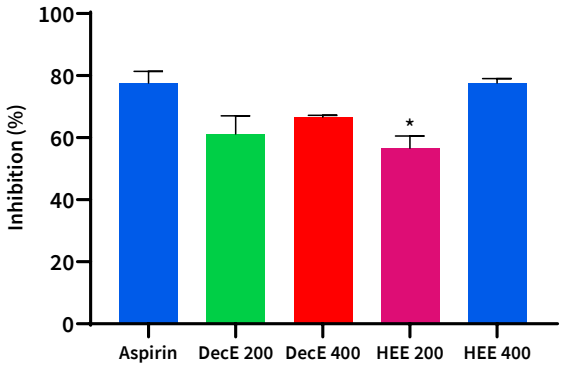


FIGURE 2. Analgesic effect of DecE and HEE against writhing caused by acetic acid in mice. DecE 200 / DecE 400: Decoction extract, 200 mg·kg^{-1} / 400 mg·kg^{-1} respectively. HEE 50 / HEE 200: Hydroethanol extract, 200 mg·kg^{-1} / 400 mg·kg^{-1} respectively. The results were expressed as means ± SEM, *: P<0.1, n= 5

CONCLUSION

Finally, it is worthwhile to screen plants commonly used in the local flora for various biological activities, as some may contain novel bioactive substances. The present investigation revealed that natural extracts the aerial parts *O. majorana* hold promise as antioxidant and anti–inflammatory compounds for treatment human diseases. These results confirm that different phytochemical components exhibit distinct qualitative and quantitative characteristics. The observed biological effects of *O. majorana* aerial part extracts may be attributed to the high presence of phytochemicals such us rosmairinic acid and ellagic acid, and other phenolics and flavonoids compounds. Consequently, the phytopharmaceutical potential of *O. majorana* aerial parts can be further explored to promote human health. However, further research is necessary to ensure its safe and effective application.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Ethical approvals

Experimentation Animale' (http://aasea.asso.dz/articles/) approved experimental assays under statute No. 88-08/1988, which deals with veterinary medical activities and animal health protection (N° JORA: 004/1988).

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