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Antioxidant activity, analgesic activity, and phytochemical analysis of *Ammi majus* (L.) extracts

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Abstract: *Ammi majus* (L.) is commonly used to cure many diseases in Moroccan folk medicine, especially vitiligo. This research tries to evaluate the phytochemical constituents of two aqueous extracts (E_1; Maceration; 48 h) and (E_2: Infusion; 1h) and three organic fractions (F_1: Cyclohexane), (F_2: Ethyl acetate (EtOAc)) and (F_3: Ethanolic (EtOH)) of *A. majus* (L.) seeds, as well as to study the antioxidant and analgesic activity of the species. Phytochemical analysis, antioxidant activity (DPPH, FRAP, ABTS, and TAC tests), and analgesic activity (writhing and tail immersion were induced by Acetic acid tests) were analyzed according to the literature. A quantitative phytochemical study indicate that the E_1 had the highest content of total polyphenols (26.95 ± 0.53 mg GAE/g extract) and flavonoids (37.92 ± 0.46 mg QE/g extract), while F_3 showed a promising flavonol content (24.26±0.08 mg QE/g extract). Tannins were found to be high in F_1 (59.27 ± 0.16 mg CE/g extract) and F_2 (57.65 ± 1.18 mg CE/g extract). Antioxidant results reveals that DPPH (IC_{50} = 179.68 ± 0.47 µg/mL) and FRAP (EC_{50} = 367.03 ± 0.12 µg/mL) show to E_1 a high antioxidant activity. Regarding the analgesic activity of the different studied extracts, it was found that E_1 has a high peripheral analgesic effect with 62.32 % and a high central analgesic potential throughout the experimentation at 500 mg/kg. Our studies demonstrated for the first time that *A. majus* seeds extracts have high antioxidant and analgesic activities through different analysis techniques.

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1. INTRODUCTION

Morocco is one of the Mediterranean countries with a long medical tradition and traditional herbal medicine know-how (Bellakhdar, 1997; Bellakhdar *et al.*, 1991; Bourhia *et al.*, 2019). Due to its unique phytogeographical features, it is considered one of the most important reservoirs of biodiversity for many species. More than 4200 species have been identified,

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including 800 endemic and 600 classified as medicinal plants, which remain largely exploited by the Moroccan population, estimated at between 50 % and 90 % (Bellakhdar, 1997; Fennane & Ejdali, 2016; Merrouni & Elachouri, 2020; Rankou *et al*., 2013; Yamani *et al*., 2015).

Ammi majus (L.) (*A. majus*), commonly called "Atrilal" or "Trillane", "Belala", "Rjel l'aghrabe", and "Ich Omla", is a wild medicinal plant which belongs to the family *Apiaceae* (*Umbelliferae*) (Bellakhdar *et al*., 1991; Rhattas *et al*., 2016; Mohamed *et al*., 2013; Azzouzi, 2015). It is a branched annual plant. It achieves a height of 1.5 - 2.0 meters with whitish-tipped slender roots, and hairless stems with fine longitudinal striae. Generally, the leaves are alternate with a long petiole. The inflorescence is shaded with a small whitish, actinomorphic or zygomorphic, bisexual, pentameric, and bracteate flowers (Humans *et al*., 2007).

In Moroccan traditional medicine, *A. majus* (L.) is widely used to treat several diseases, especially vitiligo, due to its potential dermal effect (Rhattas *et al*., 2016; Redouan *et al*., 2020). Besides, herbalists recommend its use in combination with *Anacylus pyrethrum* to enhance the therapeutic effect on vitiligo (Azzouzi, 2015). Evidence from the literature reveals its curative effect on many cardiovascular diseases (Nassiri *et al*., 2016). The Egyptian population uses the fruit as a diuretic, emmenagogue, and blood purifier, as well as for the treatment of leukoderma, urinary tract infections, and reducing kidney stones (Al-snafi, 2013; El Mofty, 1948; Hakim, 1969; Hawryl *et al*., 2000). In addition, the Iranian population uses *A. majus* against psoriasis and vitiligo, while the Chinese population uses it as a diuretic and carminative, also to treat angina pectoris and asthma (Al-Hadhrami & Hossain, 2016; Asadi-Samani *et al*., 2015).

Indeed, pharmacological research have supported the majority of the plant's traditional uses. According to the literature, the plant has a variety of biological actions, including cytotoxicity (Al-Hadhrami *et al*., 2016; Mohammed & El-Sharkawy, 2017), antibacterial (Al-Hadhrami & Hossain, 2016; Al Akeel *et al*., 2014; Fathallah *et al*., 2019)**,** anti-inflammatory (Koriem *et al*., 2012; Selim & Ouf, 2012)**,** antiviral (Selim & Ouf, 2012)**,** antihyperlipidemic, analgesic, antipyretic (Koriem *et al*., 2012), vascular protector (Cao *et al*., 2020), and antioxidant activities (Al-Hadhrami & Hossain, 2016).

The scientific basis of the pharmacological properties of *A. majus* is based on the diversification of its phytochemical constituents. Several bioactive compounds isolated from *A. majus* are cited in the literature. Some compounds have been isolated from the fruit, including bergapten, imperatorin, xanthotoxin (Bartnik & Mazurek, 2016; M. S. Karawya, 1970), isopimpinellin (Bartnik & Mazurek, 2016), ammajin, marmesin (Balbaa *et al*., 1973), umbelliprenin (Abu-Mustafa EA, EL-Bay FK, 1971b) and maurin (Abu-Mustafa EA, EL-Bay FK, 1971a). Numerous others have been isolated from aerial parts, like 6-hydroxy-7-methoxy-4-methyl coumarin, 6-hydroxy-7-methoxy coumarin (Selim & Ouf, 2012), acetylated flavonol triglycosides, especially, kaempferol and isorhamnetin 3-O- [2''- (4'''- acetylrhamnosyl) – 6''-glucosyl] glucosides, and flavonol glycosides, such as isorhamnetin-3-O-rutferinoside-3-O-glucoside, kaempferinoside-3-O-glucoside, and isorhamnetin-3-O-glucoside (Singab, 1998).

The objective of this work is to assess the antioxidant and analgesic activities of seed extracts of *A. majus* from Morocco. Despite its advantageous pharmacological properties, this species is not well exploited. Currently, the only work that evaluates antioxidant activity uses only the DPPH test, which is still insufficient to prove the antioxidant power of this plant. Its analgesic power is inexperienced. Moreover, its phytochemical composition is not elucidated. It can reveal new compounds with strong antioxidant and analgesic power.

2. MATERIAL and METHODS

2.1. Chemicals

Dimethyl sulfoxide (DMSO), Cyclohexane, Ethyl Acetate, Ethanol, Iron (III) Chloride Hexahydrate, Folin-Ciocalteu, Ascorbic Acid, Gallic Acid, Quercetin, Catechin, Aluminum Chloride, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and, Acetic Acid, were purchased from Sigma-Aldrich and Solvachim. All other chemicals used were of analytical grade.

2.2. Animals

Winstar Albino rats (160 – 240 g) and *Swiss albino* mice (20 – 30 g) were obtained from the animal house of the Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco. Animals were reared at 22 ± 2 °C with 14 hours of light and 10 hours of darkness, with unrestricted access to food and water.

All experimental procedures were performed in accordance with the "Principles of Laboratory Animal Care" and were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" of the National Academy of Sciences and approved by the National Institutes of Health (Code of ethics: FMPR0120).

2.3. Plant Materials

The seeds of *A. majus*, had been collected during the fruiting period of May 2019 in Rabat, Morocco (Geographical coordinates: 33° 58' 06" N6° 49' 04" W). Botanical identification of the plant was performed at the Scientific Institute of Rabat. Specimens were stored in the Scientific Institute of Rabat under the herbarium code of RAB111737.

2.4. Preparation of the Extracts

Maceration and infusion

The crushed air-dried seeds of *A. majus* (34 g) were macerated **(E_1)** and infused **(E_2)** in 650 mL of distilled water for 48 h and 1 h, respectively, with intermittent shaking. The extracts were filtered through Whatman filter paper No. 1 (0.45 µm), and then concentrated under vacuum on a rotatory evaporator (BUCHI RE-111 Rotavapor W / 461 Water bath) at 40 °C to constant dryness. The extracts were lyophilized and stored at 4 °C for further analysis.

Soxhlet fractionation

The organic fractions were prepared using a soxhlet apparatus with cyclohexane **(F_1)**, ethyl acetate **(F_2)** and ethanol **(F_3)**, successively for 3 hours for each extraction. At the end of each extraction, the extracts were dried using a rotatory evaporator at 40 °C. Finally, the dried organic fractions were transferred into screw-capped amber vials and stored at 4°C for further analysis.

2.5. Phytochemical Analysis

Total Phenolic Content (TPC)

The determination of total phenolic content was performed using Folin-Ciocalteu method (Poh-Hwa *et al*., 2011). Briefly, we have mixed 200 µL of each extract with 1000 µL of Folin-Ciocalteu reagent at 10 %. Then, we used 800 µL of Sodium Carbonate (Na_2CO_3) at 7.5 % for neutralizing the reaction. The mixture was further incubated in the dark for 30 minutes. The absorbance was determined against a blank at 765 nm with a spectrophotometer (UV-6300PC). A gallic acid standard curve was prepared from a freshly made (From 7 to 125µg/mL) gallic acid stock solution. We have expressed the results as mg of gallic acid equivalents (GAE) /g extract on a dry weight basis (d.w.).

Total Flavonoids Content (TFC-1)

Aluminum chloride colorimetric method was used for the determination of total flavonoids according to the method of (Ordoñez *et al*., 2006). A volume of 0.5 mL of each extract was mixed with 0.5 mL of 2 % Aluminum Chloride ($AlCl_3$) solution. Absorbance was measured 1 h later at a wavelength of 420 nm. Methanol was used as a blank. The calibration curve of Quercetin was obtained in the range of (10 to 60µg/mL). Results were expressed as mg of Quercetin equivalents (QE)/g extract on a dry weight basis (d.w.).

Total Flavonols Content (TFC-2)

The total flavonols content was quantified using the method described by(Yermakov *et al*., 1987). Briefly, 2mL of each extract was mixed with 2 mL of 2 % Aluminum Chloride ($AlCl_3$) solution and 6 mL of 5 % Sodium Acetate solution. Absorbance was measured 2 h 30 min later at a wavelength of 440 nm. Quercetin (15 to 250 µg/mL) was used as standard. The total amount of flavonols was expressed as mg of Quercetin equivalents (QE)/g extract on a dry weight basis (d.w.).

Total Tannins Content (TTC)

The total tannins content (TTC) for each *A. majus* seeds extract was evaluated according to the method described by (Julkunen-Tiitto, 1985). A volume of 50 µL of each extract was added to 1.5 mL of 4 % Methanolic Vanillin solution and 750 µL of concentrated Hydrochloric Acid (37 %). We have incubated the mixture for 20 min in the dark at room temperature. And we have measured the absorbance against a blank (Methanol) at 500 nm. Catechin (100 to 600µg/mL) was used as standard. The tannins content was expressed as mg equivalent of Catechin / g extract (mg CE/g extract).

2.6. Antioxidant Activities

DPPH free radical scavenging activity

To investigate the antioxidant activity of the studied *A. majus* seeds extracts, we used 2,2-diphenyl-1-picrylhydrazil (DPPH) test in similarity with the method established by (Sahin *et al*., 2004). We have mixed 2 mL of Methanolic solution of DPPH at 0.0023 % (60 µM DPPH; Methanol) with 50 µL of each extract at different concentrations. The mixture is vigorously shaken and left to stand for 20 min at room temperature in the dark. The absorbance of the resulting mixture was read at 517 nm. The same procedure was repeated using a control sample (DPPH without extracts). Quercetin (0.38 to 6.09 mg/mL) was used as the standard antioxidant.

The percentage of inhibition was calculated using the formula below. The concentration is plotted as a function of the percentage of inhibition, from the regression equation we calculated IC_{50}.

$$\text{\% Inhibition of DPPH activity} = \frac{\text{Absorbance of Blank} - \text{Absorbance of Test}}{\text{Absorbance of Blank}} \times 100$$

ABTS free radical scavenging activity

The ABTS radical scavenging assay was performed adhering to the method of (Pukalskas *et al*., 2002). The ABTS radical was generated through the oxidation of ABTS with Potassium Persulfate. In brief, the ABTS solution (7 mM) had reacted with Potassium Persulfate (70 mM) solution (mixed in equal volume) for generation of ABTS cations. The mixture was allowed to stand in the dark at room temperature for 16 h. Before being used in the assay, the ABTS radical cation was diluted with Methanol for an initial absorbance of about 0.700 at 734 nm. For the study, different concentrations of each extract (100 µL) were added to 2 mL of ABTS solution.

The absorbance was read at 734 nm and the percentage inhibition was calculated as described earlier for the DPPH test.

Ferric-reducing antioxidant power test (FRAP)

The reducing activity of the studied extracts was measured spectrophotometrically with the method of (Oyaizu, 1986). We have mixed various concentrations of aqueous extracts organic fractions and standard (0.2 mL), with 2.5 mL of 0.2 M Sodium Phosphate buffer (pH = 6.6) and 2.5 mL of 1 % (w/v) Potassium Ferricyanide ($K_3Fe(CN)_6$). After incubation at 50 °C during 20 min, 2.5 mL of 10 % (w/v) trichloroacetic acid was added to the mixture. About 2.5 mL of each concentration was taken and 2.5 mL of distilled water and 0.5 mL of 0.1 % (w/v) Ferric Chloride ($FeCl_3$) were added. The intensity of the blue-green color was measured at 700 nm. We used Catechin (0.65 to 21.39µg/mL) as a positive control.

Total antioxidant capacity test (TAC)

The total antioxidant activity of aqueous extracts and organic fractions was evaluated by the formation of phosphomolybdenum complex according to the method established by (Prieto *et al*., 1999). 2 mL of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added to 0.2 mL of each extract. We have measured the absorbance at 695 nm after boiling at 95 °C for 90 minutes. We have choosed Ascorbic acid (15 to 250µg/mL) as a standard. The total antioxidant capacity was expressed as micrograms of ascorbic acid equivalent (AAE) per grams of extract (mg AAE/g extract).

2.7. Analgesic Activity

Acetic acid induced writhing test

The method used in this test has been described by (Koster, 1959). The mice were weighed and randomly divided into 7 groups consisting of 5 mice in each. Group 1 (control) did not receive any treatment. The other groups received orally E_1, E_2, F_1, F_2 and F_3 at 500 mg/kg and aspirin at 125 mg/kg. After 30 minutes of extracts administration, each mouse was injected with 3 % (v/v) Acetic Acid at the dose of 3.75 mL/kg body weight intraperitoneally. The number of abdominal contortions produced by each mouse was recorded for 10 minutes commencing just 10 minutes after acetic acid injection. The percentage of inhibition of abdominal writhing was calculated according to the following formula:

$$\% \text{ Inhibition} = \frac{1 - \text{Number of contortions of mice in the treated batch}}{\text{Number of contortions of the negative control batch}} \times 100$$

Tail immersion test

The tail immersion test was performed according to the method established by (Sewell & Spencer, 1976). Female rats (160 – 240 g) were used in this test. The distribution of the experimental animals remained similar to that of the abdominal writhing test. Briefly, the animals were treated with aqueous extracts (E_1; E_2) and organic fractions (F_1; F_2; F_3) at 500 mg/kg. Morphine (0.1 mg/kg) was used as a reference standard. The lower 6 cm section of the tail of rats were immersed in a water bath in which the temperature of water was maintained at 55 ± 0.5 °C. The time between tail submergence and tail deflection was recorded at 0, 30, 60 and 120 min after treatment with morphine or extracts using a digital stopwatch. 10 seconds is the time maintained to avoid animal damage.

2.8. Statistical Analysis

Analysis was performed with Graph Pad Prism v8 software. Data shown are the average ± standard deviation of three replicated extractions. Data were subjected to one-way analysis of

variance (ANOVA). Differences between mean values were compared by using Tukey's test at $p \leq 0.05$ probability levels.

3. RESULTS and DISCUSSION

For a rational use of natural resources, the determination of yields has an advantage in deciding the amount of the targeted drug to be taken from nature. The yield results of aqueous extracts and organic fractions of *A. majus* seeds are presented in Table 1.

Table 1. Extraction conditions and yields of obtained extracts from *A. majus* seeds.

Extract Code	Extraction Method	Solvent	Yield (%)
E_1	Maceration (48 h)	Water	17.08 %
E_2	Infusion (1h)	Water	25.47 %
F_1	Soxhlet method	Cyclohexane	8.43 %
F_2	Soxhlet method	Ethyl Acetate	14.44 %
F_3	Soxhlet method	Ethanol	23.35 %

E_1: Aqueous macerated extract; E_2: Aqueous infused extract; F_1: Cyclohexanoic fraction; F_2: Ethyl acetate fraction; F_3: Ethanolic fraction

Regarding the results, the yields decrease with the polarity of the extractive solvents used. Where the higher yield was obtained with the aqueous-infused extract (E_2: 25.47 %), followed by the ethanolic fraction (F_3: 23.35 %), the aqueous-macerated extract (E_1: 17.08 %), the Ethyl acetate fraction (F_2: 14.44 %), and lastly the cyclohexanoic fraction (F_1: 8.43 %). The difference in yields is probably related to the variability of extraction techniques, the time of extraction and the polarity of the used solvents. The comparison of the two aqueous maceration techniques showed that the temperature improves significantly the extractive capacity of water. This could be obviously explained by the fact that the heat causes cell disturbance by increasing the permeability of cell walls, thus enhancing the affinity of water to phytoconstituents. To a certain extent, these results explained the preference of the traditional use of *A. majus* seeds in hot preparations (Bhambri *et al*., 2012; Rhattas *et al*., 2016; Redouan *et al*., 2020).

The objective of the quantitative phytochemical analysis of the studied extracts, using spectrophotometric assays, was to determine their content in total polyphenols, flavonoids, flavonols and tannins. The choice to quantify these phytoconstituents results from its consideration as major contributors to the antioxidant capacity and analgesic potency of medicinal plants. The results obtained are presented in Table 2.

The quantification of total polyphenols revealed that aqueous extracts have significantly high concentrations ($p < 0.05$) in both used extraction methods (E_1: 26.95 ± 0.530 mg GAE/g extract) and (E_2: 23.78 ± 0.420 mg GAE/g extract). In contrast, the organic fractions registered higher tannins content (F_1: 59.27 ± 0.16 mg CE/g extract; F_2: 57.65 ± 1.18 mg CE/g extract; F_3: 16.62 ± 0.28 mg CE/g extract). Significant levels of total flavonoids were observed in aqueous extracts (E_1: 37.92 ± 0.46 mg QE/g extract; E_2: 32.54 ± 0.48 mg QE/g extract), the ethyl acetate fraction (F_2: 33.02 ± 0.29 mg QE/g extract) and the ethanolic fraction (F_3 : 32.36 ± 0.38 mg QE/g extract) with no significant difference ($p < 0.05$). In addition, we found that the ethanolic fraction has a higher concentration of flavonols (F_3: 24.26 ± 0.08 mg QE/g extract) than the aqueous extracts (E_1: 13.91 ± 0.33 mg QE/g extract; E_2: 09.29 ± 0.30 mg QE/g extract), with undetermined value for the cyclohexanoic fraction (F_1) and the ethyl acetate fraction (F_2).

Table 2. Polyphenols, flavonoids, flavonols and condensed tannins contents of extracts from *A. majus* seeds.

Plant Extracts	TPC (mg GAE/g extract)	TFC-1 (mg QE/g extract)	TFC-2 (mg QE/g extract)	TTC (mg CE/g extract)
E_1	26.95 ± 0.53^a	37.92 ± 0.46^e	13.91 ± 0.33^g	7.95 ± 0.02^j
E_2	23.78 ± 0.42^b	32.54 ± 0.48^f	09.29 ± 0.30^h	2.27 ± 0.09^k
F_1	0.71±0.03c	13.48 ± 0.03^g	Nd	59.27 ± 0.16^l
F_2	4.74 ± 0.01^d	33.02 ± 0.29^f	Nd	57.65 ± 1.18^m
F_3	4.79 ± 0.01^d	32.36 ± 0.38^f	24.26 ± 0.08^i	16.62 ± 0.28^n

Data represent the mean ± standard deviation of three independent experiments.
Values in the same column with different superscript letters indicate significant differences (p-value < 0.05)
E_1: aqueous macerated extract; E_2: aqueous infused extract; F_1: Cyclohexanoic fraction; F_2: Ethyl acetate fraction
F_3: Ethanolic fraction; TPC: Total Phenolic Content; TFC-1: Total Flavonoid Content; TFC-2: Total Flavonol Content
TTC: Total Tannins Content; ND: not determined; mg GAE/g extract: mg Galic Acid equivalent per gram of extract; mg QE/g extract: mg Quercetin equivalent per gram of extract; mg CE/g extract: mg Catechin equivalent per gram of extract.

Comparison with literature showed that the obtained results are in accordance to those found for *Ammi visnaga*, which has the same therapeutic properties as *A. majus* and are cultivated under the same agricultural conditions. In fact, (Muddathir *et al*., 2017) reported a total polyphenols content of 34.1 ± 0.97 µg GAE/g extract in the ethanolic fraction of *Ammi visnaga*. This value is lower than that obtained in our study. For total flavonoids, (Aourabi *et al*., 2021) revealed a lower content in the aqueous extract and organic fractions of the aerial parts of *Ammi visnaga* compared to our study. A number of intrinsic and extrinsic factors, including genetic factors, growth conditions, maturation process, sampling techniques, and storage conditions, probably explain this difference. Indeed, the quantification of secondary metabolites in *A. majus* seeds, such as total polyphenols, flavonoids, flavonols and tannins, permits to identify the potential of its pharmacological properties, including the antioxidant capacity.

Given the complexity of the oxidative processes, the use of different tests is necessary to confirm the antioxidant capacity of the analyzed extracts. For this reason, we proceeded with the evaluation of its antiradical capacity with the DPPH, its reducing power of Iron (FRAP) and Molybdate (TAC), and its antioxidant capacity by ABTS.

The results of the evaluation of the antioxidant activity by DPPH assay (Table 3) indicate that the aqueous extracts exhibit significant DPPH-radical neutralization capacity compared to the organic fractions, with IC_{50} of about (E_1: 179. 68 ± 0.47 µg/mL), (E_2: 198.13 ± 0.28 µg/mL), (F_3: 385.80 ± 0.39 µg/mL), (F_2: 565.04 ± 2.60 µg/mL), and (F_1: 3243 ± 3.65 µg/mL), listed in decreasing order. Based on these values, the antiradical effect of the studied extracts on DPPH-radical is lower than that of Quercetin (5.49 ± 0.02 µg/mL), taken as a positive control. Statistical analyses revealed a significant difference ($p < 0.05$) between the IC_{50} obtained with the five studied extracts and Quercetin.

Similarly, the results issued from other research works revealed lower IC_{50} values of Omanian species, reflecting the strong reducing power of these extracts against $DPPH^{\bullet}$ compared to our extracts (R. Al-Hadhrami & Hossain, 2016). In addition, the comparison with the antioxidant power performed by *Ammi visnaga* via DPPH test, indicates that the organic fractions showed a higher antiradical effect compared to the aqueous extracts, contrary to the expressed capacity of our extracts (Aourabi *et al*., 2021; Bencheraiet *et al*., 2011).

Table 3. Antioxidant activity of the aqueous extracts and organic fractions of *A. majus* seeds and standards using DPPH, ABTS, FRAP and TAC assays.

Plant Extracts	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	FRAP EC_{50} (µg/mL)	TAC mg AAE/g of extract
E_1	179.68 ± 0.47 ^{a}	165.07±0.01^{g}	367.03±0.12^{l}	77.25±1.40^{r}
E_2	198.13 ± 0.28 ^{b}	149.93±0.02^{h}	447.31±0.77^{m}	84.68±1.41^{s}
F_1	3243 ±3.65^{c}	3423.8±1.17^{i}	1867.27±1.22^{n}	110.41±2.34^{t}
F_2	565.04 ±2.60^{d}	766.89±9.74^{j}	591.31±4.51^{o}	120.22±1.84^{u}
F_3	385.80 ±0.39^{e}	141.47±0.43^{h}	1128.70±4.58^{p}	120.10±1.13^{u}
Quercetin	5.49 ±0.02^{f}	-	-	-
Ascorbic acid	-	2.52 ±0.02^{k}	-	-
Catechin	-	-	13.90±0.03^{q}	-

Data represent the mean ± standard deviation of three independent experiments.
Values in the same column with different superscript letters indicate significant differences (p-value < 0.05)
E_1: Aqueous macerated extract; E_2: aqueous infused extract; F_1: Cyclohexanoic fraction; F_2: Ethyl acetate fraction
F_3: Ethanolic fraction; IC_{50}: 50% inhibitory concentration of DPPH or ABTS; EC_{50}: Effective concentration that transforms 50 % of Fe^{3+} into Fe^{2+}; mg AAE/g of extract: mg Ascorbic acid equivalent per gram of extract.

The antioxidant capacity against the cationic radical ABTS of the aqueous extracts (E_1; E_2) and the organic fractions (F_1; F_2 ; F_3) was evaluated. Given the IC_{50} values reported in Table 3, the scavenging capacity of ABTS decreases in the following order: Ethanolic fraction (F_3: 141.47 ± 0.43 µg/mL) > Aqueous-infused extract (E_2: 149.93 ± 0.02 µg/mL) > Aqueous-macerated extract (E_1: 165.07 ± 0.01 µg/mL) > Ethyl acetate fraction (F_2: 766.89 ± 9.74 µg/mL) > Cyclohexanoic fraction (F_1: 3423.8 ± 1.17 µg/mL). We conclude that the ethanolic fraction (F_3) and the aqueous-infused extract (E_2) have approximatively the same scavenging capacity of the cationic radical ABTS with no significant difference ($p < 0.05$). Note that the performance of ascorbic acid (2.52 ± 0.02 µg/mL), taken as a positive control, is higher compared to our extracts.

To characterize the reducing power of aqueous extracts (E_1; E_2) and organic fractions (F_1; F_2; F_3) of *A. majus* seeds, two tests were conducted using the Iron (FRAP), Molybdate (TAC) and Reducing Power assessment.

The estimation of the iron reducing power (FRAP) and the total antioxidant capacity (TAC) showed a significant difference ($p < 0.05$) depending on the extraction technique and the solvent used. Based on the results presented in Table 3, the ability of the aqueous extracts (E_1; E_2) to reduce Iron is higher compared to the organic fractions (F_1; F_2; F_3). Thus, this potential decreases in the following order: Aqueous-macerated extract (E_1: 367.03 ± 0.12 µg/mL) > Aqueous-infused extract (E_2: 447.31 ± 0.77 µg/mL) > Ethyl Acetate fraction (F_2: 591.31 ± 4.51 µg/mL) > Ethanolic fraction (F_3: 1128.70 ± 4.58 µg/mL) > Cyclohexanoic fraction (F_1: 1867.27 ± 1.22 µg/mL). These results are consistent with TAC test results, with no significant difference ($p < 0.05$) between the ethanolic fraction F_3 and ethyl acetate fraction F_2.

We found a strong association between the phenolic content measured and the antioxidant ability assessed in the extracts tested, but only with the DPPH radical scavenging test. This finding is explained by the strong scavenging power of radical species and reactive oxygen forms typical to phenolic compounds (Bougandoura & Bendimerad, 2013; Caceres *et al.*, 2020). In addition, this capacity depends not only on the polyphenols and flavonoids content, but also on the variability of their structures and interactions in the extracts (Megdiche-ksouri *et al.*, 2014).

Another pharmacological activity where the potential of phenolic compounds seems to be interesting is the analgesic activity. Since the search for new analgesics is one of the main therapeutic concerns.

Specific study models were conducted to detect and evaluate the analgesic potential of aqueous extracts (E_1; E_2) and organic fractions (F_1; F_2; F_3) of *A. majus* seeds. The Acetic acid induced writhing test is considered a model of visceral pain, as acetic acid-induced pain is similar to peritonitis (Le Bars *et al*. 2001). This test is frequently used to identify substances with a peripheral analgesic effect. Table 4 summarizes the nociceptive response, indicating the number of cramps performed by the animal after intraperitoneal injection of acetic acid and the percentage of cramp inhibition of the studied extracts.

Table 4. Analgesic effect of aqueous extracts and organic fractions of *A. majus* seeds, and Aspirin on acid-induced writhing in mice.

Treatment	Dose (mg/kg)	Number of writhes	% inhibition
E_1	500	14.00 ± 1.30^a	62.32 %
E_2	500	19.50 ± 1.75^b	47.52 %
F_1	500	29.00 ± 2.64^c	28.13 %
F_2	500	24.50 ± 2.38^d	34.06 %
F_3	500	26.00 ± 2.00^e	30.03 %
Aspirin	125	21.66 ± 1.90^b	41.71 %
Negative Control	-	37.16 ± 1.78^g	-

Data represent the mean ± standard deviation of five independent experiments.
Values in the same column with different superscript letters indicate significant differences (p-value < 0.05)
E_1: Aqueous macerated extract; E_2: Aqueous infused extract; F_1: Cyclohexanoic fraction; F_2: Ethyl acetate fraction
F_3: Ethanolic fraction

Regarding the results, the number of acetic acid-induced cramps was significantly ($p < 0.05$) reduced by the aqueous-macerated extract (E_1), administered orally (AO), compared to acetylsalicylic acid (125 mg/kg; AO), taken as a positive control. Besides, at the dose of 500 mg/kg, the antinociceptive activity decreases in the following order: Aqueous-macerated extract (E_1: 62.32%) > Aqueous-infused extract (E_2: 47.52%) > Ethyl Acetate fraction (F_2: 34.06%) > Ethanolic fraction (F_3: 30.03%) > Cyclohexanoic fraction (F_1: 28.13%). Accordingly, we can confirm that the studied extracts of *A. majus* seeds act at the peripheral level. Cytokines, histamine, serotonin and prostaglandins are mediators among several mediators produced by the inflammatory cells under the effect of acetic acid injection, this method is accompanied to the increase of PGE2 and PGF2a in the peritoneal fluid and the production of lioxygenase (Derardt *et al*., 1980). The activity of our extracts may be due to the inhibition of the above mentioned elements. Another study showed that the response induced by acetic acid and depend strongly on both peritoneal macrophages and mast cells (Ribeiro *et al*., 2000).

The tail immersion test was also used to evaluate the analgesic potential of the studied extracts. It is a sensitive model to central analgesics, particularly those with a spinal site of action (Daniel *et al*., 2001). The obtained results, expressed as the mean time of the tail withdrawal reflex at 30, 60 and 120 min, after extracts and morphine treatment at a dose of 500 mg/kg and 0.1 mg/kg, respectively are gathered in Table 5.

Table 5. Analgesic effect of aqueous extracts and organic fractions of *A. majus* seeds, and morphine on nociceptive responses in the tail immersion test.

Treatment	Dose (mg/kg)	Reaction time in seconds			
		0 min	30 min	60 min	120 min
E_1	500	2.43±0.24^a	5.50±0.79^b	8.11±0.65^f	4.39±0.36^l
E_2	500	2.44±0.10^a	5.45±0.29^b	7.39±0.43^g	5.84±0.79^m
F_1	500	3.18±0.14^a	4.82±0.97^d	4,27±0.79^h	3.51±0.44^p
F_2	500	3.21±0.46^a	3.18±0.51^e	3.55±0.77^k	4.24±1.07n
F_3	500	2.88±0.24^a	4.53±0.61^c	4.78±0.24^i	2.5±0.34^p
Morphine	0.1	2.53±0.43^a	6.46±0.13^b	6.75±0.12^j	7.70±0.18^o
Negative control	-	2.35±0.32^a	2.62±0.39^c	2,63±0.47^k	2.03±0.31^p

Data represent the mean ± standard deviation of five independent experiments.
Values in the same column with different superscript letters indicate significant differences (p-value < 0.05)
E_1: Aqueous macerated extract; E_2: Aqueous infused extract; F_1: Cyclohexanic fraction; F_2: Ethyl acetate fraction
F_3: Ethanolic fraction

Based on previous presented results, the oral treatment of rats with the aqueous extracts (E_1; E_2) and organic fractions (F_1; F_2; F_3) significantly ($p < 0.05$) increased the reaction time to the nociceptive thermal stimulus (Fig 2). This antinociceptive effect starts from 30 min and persists throughout the experiment, with a maximal effect at 60 min for the aqueous extracts (E_1: 8.11 ± 0.65 sec) and (E_2: 7.39 ± 0.43 sec) at the dose of 500 mg/kg, comparatively to morphine. The organic fractions showed an antinociceptive effect over the experimental period. The action peaks of cyclohexanoic (F_1: 4.82 ± 0.97 sec), ethanolic (F_3: 4.78 ± 0.24 sec) and ethyl acetate (F_2: 4.24±1.07 sec) fractions were observed at 30, 60, and 120 min, respectively. Antinociceptive activity against temperature-induced pain may be due to stimulation of the opioid receptor or processes facilitated by monoaminergic neurotransmitters such as dopamine, serotonin and norepinephrine that control pain in the dorsal horn (Benarroch, 2008). The intensity of pain can be reduced by activating the downward pain suppression pathway (Dale *et al*., 2005). Several *A. majus* compounds have been identified in the extract of seeds as having central analgesic activity, such as bergapten and, imperatorin (Singh *et al*., 2019). (Koriem *et al*., 2012) found that the ethanolic extract of *A. majus* has a central analgesic activity that confirms what we found.

To a certain extent, this study allowed us to confirm the antioxidant and analgesic properties of the different extracts of *A. majus* seeds, and to scientifically validate the use of this plant in traditional medicine against diseases related to oxidative stress as well as for relieving clinical pain.

The previously presented results indicate that the seeds of this plant contain bioactive compounds with effective antioxidant and analgesic activities *in vitro* that could be used in conventional medicine.

The therapeutic properties of these extracts open a promising way in the fight against oxidative stress and clinical pain. They can constitute a safe and acceptable alternative for human use. For this reason, the evaluation of its toxicity could be considered to assess the risks of their long-term administration. In addition, complementary tests of other pharmacological activities should be conducted to confirm and elucidate the therapeutic performance of this plant.

4. CONCLUSION

The seeds of *A. majus* growing in Morocco could be regarded a source of bioactive chemicals, according to this study. The biological activities of the extracts tested revealed that *A. majus* seeds have a significant anti-oxidant impact and are a prospective source of both peripheral and central analgesics. Given the preceding findings, it appears that A. majus seeds could be used as a source of natural chemicals that could be used in foods, pharmaceutical items, or cosmetics. In perspective to make the best use of the extracts studied, toxicological studies, the chemical composition of each extract and the isolation of certain compounds will be necessary for a future study.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors. **Ethics Committee Number**: National Institutes of Health-FMPR0120.

Authorship Contribution Statement

Otman El-Guourram: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing - original draft. **Soufiane Drioua:** Investigation, Resources. **Mouna Ameggouz**: Investigation, Resources, Visualization, Methodology. **Najoua Salhi:** Investigation, Methodology. **Karima Sayah:** Resources, Visualization, Software. **Ahmed Zahidi:** Investigation, Supervision, Writing-Reviewing. **Anass Doukkali:** Investigation, Writing-Reviewing. **Gokhan Zengin:** Investigation, Writing-Reviewing. **Hanane Benzeid**: Investigation, Supervision, Writing-Reviewing

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