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EVALUATION OF ANTIOXIDANT, ANTIBACTERIAL, ANTALGIC AND ANTI-INFLAMMATORY PROPERTIES OF *CRATAEGUS AZAROLUS*

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The purpose of this study was to examine the potential effects of a Crataegus azarolus extract on antioxidant, antibacterial, analgesic, and anti-inflammatory activities. Additionally, a phytochemical analysis was conducted to identify the chemical constituents of the extract. The antioxidant potential was assessed using DPPH, FRAP, and β-carotene assays, while the antibacterial activity was evaluated against five different microorganisms by measuring the diameter of inhibition zones. The anti-inflammatory and analgesic effects were examined by inducing paw edema with carrageenan and intraperitoneal injection of acetic acid, respectively. The results indicated that the extract exhibited strong antioxidant activity, and the highest antibacterial activity was observed against Bacillus subtilis, with an inhibition zone diameter of 18 mm. Additionally, administering 400 mg/kg of the extract resulted in a strong inhibition of paw edema, showing a percentage reduction of 42.28% compared to the control group. Similarly, the methanolic extract (600 mg/kg) of Crataegus azarolus showed inhibition of pain caused by acetic acid in mice. The findings of the study propose that the extract has the potential to provide bioactive compounds, and that its individual constituents may act together in a synergistic manner. Therefore, Crataegus azarolus extract could be used as an alternative to synthetic anti-inflammatory and analgesic compounds in the pharmaceutical industries in the future.

Keywords*: Crataegus azarolus, antioxidant, antibacterial, antalgic, anti-inflammatory*

INTRODUCTION

Hawthorn, which belongs to the rose family and the hawthorn genus[1&2], comprises over 280 varieties that are widely distributed in Asia and Europe[3&4]. It serves not only as a direct food source but also finds application in processed forms such as jams or jellies, owing to its high pectin content[5]. Additionally, it is utilized in snack food processing due to its pleasant sweet and sour taste and abundant nutrients[6].

Various parts of the hawthorn plant, including its berries, flowers, and leaves, are rich in nutrients and have long been associated with numerous health benefits, medicinal properties, and nutraceutical effects[7]. Hawthorn is extensively utilized as a natural fruit in the wild, serving purposes in both culinary and medical investigations. The fresh or dehydrated hawthorn fruits are employed in the production of preserves, teas, and dietary supplements. Extracts derived from hawthorn berries, leaves, and flowers are employed in the prevention of hypertension and heart failure[8&9]. In addition,

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hawthorn has a long history of use in traditional medicine, dating back centuries, due to its potential therapeutic effects on various aspects such as tonifying the spleen[10] and regulating blood glucose and lipid metabolis[11]. These effects are attributed to the presence of abundant biologically active substances in hawthorn, including flavonoids [12], phenolics, terpenoids, and pectin. As a result of its medicinal properties and nutritional value, hawthorn is also recognized as a fruit with medicinal and food-related benefits in Algeria.

As far as we know, some researchers have demonstrated the beneficial effects of hawthorn in terms of its anti-inflammatory and antioxidant properties. However, these investigations utilized the fruit of distinct hawthorn species, rather than a combination of leaves and flowers from *Crataegus azarolus*. As of yet, no published research has examined the potential preventative effects of the combination of *Crataegus azarolus* leaves and flowers. This investigation should provide more information about the pharmacological and therapeutic effects of *Crataegus azarolus*. Furthermore, exploring the utilization of plant residues, such as stems, leaves, and flowers, that are usually commercially discarded, may lead to significant advancements in the field of medicinal plants due to their great chemical and nutritional composition.

MATERIAL AND METHODS

Chemicals

Carrageenan, linoleic acid, ascorbic acid, butylated hydroxytoluene, DPPH, Tween 40, potassium ferricyanide, DPPH, ferric chloride, β-carotene, methanol, aspirin, acetic acid used in the experiment were procured from Sigma (Sigma-Aldrich, Germany).

Plant material

The collection of *Crataegus azarolus* leaves and flowers was carried out in October from northeast Algeria when they were in the flowering stage. Specifically, they were collected from Constantine, located at UTM coordinates 32S, Latitude 36° 21' 36.558" N, and Longitude 6° 38' 32.7588" E. After being identified by Professor Toumi from the Department of Biological and Environmental Sciences at ENS Kouba, Algeria, the harvested leaves and flowers were thoroughly cleaned and air-dried at room temperature before being finely crushed.

Animal material

Swiss albino mice with a weight range of 25-30 grams were obtained from the Pasteur Institute in Algiers and kept in standard cages.

Extraction of phenolic compounds by maceration

A powder mixture of leaves and flowers (20 g) was subjected to extraction using 600 mL of methanol at room temperature for 48 hrs. The resulting mixture was filtered using filter paper, and the methanol was removed through evaporation under reduced pressure using a rotavapor (BÜCHI) at 40 °C. The dried extract was kept in a dark environment at 4°C until it was used in subsequent experiments.

High Performance Liquid Chromatography (HPLC) analysis

The analysis of phenolic compounds in the methanolic extract of *Crataegus azarolus* was conducted using the High-Performance Liquid Chromatography (HPLC) technique. The sample was analyzed using an Agilent 1260 Infinity system equipped with a UV-VIS detector DAD. The analysis was performed in reverse phase using a C18 column (5 μm, 250 × 4.6 mm).The elution was performed at room temperature of 23 °C in gradient mode using a mixture of binary solvents composed of water acidified with 5% hydrochloric acid (solvent A) and 100% methanol (solvent B). The samples were injected with a volume of 20 μL and the mobile phase was set at a flow rate of 0.6 mL/min. Detection was performed at 254 nm, 280 nm, 290 nm, 320 nm and 370 nm. The identification of phenolic compounds was carried out by comparing their retention times with those of the standards and confirming their characteristic spectra.

Antioxidant Activity

DPPH radical scavenging activity

To evaluate the DPPH free radical activity, the method outlined by Musa et al. (2011)[13] was employed. In short, varying concentrations of the extract (1 mL) were combined with a methanol solution of DPPH (0.04 %, 1 mL). After a period of thirty

minutes, the absorbance of sample was measured at 517 nm. Reference standards of BHT and Ascorbic acid were utilized as benchmarks. The percentage of inhibition activity was calculated using the equation below:

$$\% \text{ Inhibition} = (Ac - As/Ac) \times 100$$

In the given equation, Ac corresponds to the absorbance of the control, while as denotes the absorbance of the sample solution. The antiradical activity of the samples was quantified by determining their IC_{50} value, which reflects the concentration of the sample necessary to eliminate 50 % of DPPH radicals.

Reducing power

The method for determining the reducing power was based on the protocol established by Yen and Chen (1995)[14]. To summarize, varying concentrations of the extract in 1 mL of distilled water were combined with 2.50 mL of a 0.2 M sodium phosphate buffer (pH = 6.6) and 2.50 mL of 1% potassium ferricyanide ($K_3Fe\ (CN)_6$), and then incubated in a water bath at 50°C for 20 minutes. Subsequently, 2.50 mL of 10 % trichloroacetic acid were added to the mixture, followed by centrifugation at 3000 rpm for 10 minutes. The supernatant (2.50 mL) was mixed with 2.50 mL of distilled water and 0.50 mL of 0.1% ferric chloride solution, and the absorbance was measured at 700 nm. BHT and Ascorbic acid were employed as reference standards. The reduction power of the extract was expressed in terms of EC_{50} value, which represented the concentration at which the absorbance reached 0.5.

β-carotene bleaching assay

The method for evaluating the antioxidant activity using the β-carotene bleaching assay was carried out based on the procedure outlined by Miraliakbari and Shahidi (2008)[15]. Initially, a mixture of β-carotene and linoleic acid was prepared by combining 0.5 mg of β-carotene in 1 mL chloroform, 25 µL of linoleic acid, and 200 mg Tween 40. The chloroform was removed under vacuum and 100 mL of oxygenated distilled water were added to the residue. The test tubes were individually added with 350 µL of the sample solution to 2.5 mL of the mixture mentioned above. Afterward, they were placed in a water bath at 50°C and incubated for 2 hrs. The absorbance of each sample was measured at 470 nm at 30-minute intervals for 2 hrs. The percentage of inhibition of the samples was determined using the following formula:

$$I\% = (A\ \beta\text{-carotene after 2-h assay} / A\ \text{initial}\ \beta\text{-carotene}) \times 100$$

Where, A β-carotene after 2-h assay refers to the absorbance measurements of β-carotene remaining in the samples after the 2-hr assay, and A initial β-carotene represents the absorbance value of β-carotene at the commencement of the experiments.

Antibacterial activity

Disc diffusion method

The disc diffusion method was employed in this study to assess the antimicrobial activity. A total of five bacterial strains were used, including two Gram positive strains (*Staphylococcus aureus* CIP7625, *Bacillus subtilis* ATCC6633) and three Gram negative strains (*Klebsiella pneumoniae* CIP82.91, *Escherichia Coli* CIPA22, *Pseudomonas aeruginosa* IPA200). These strains were provided by the microbiology laboratory at E.N.S de Kouba.

The assay was conducted with slight modifications to the disc agar diffusion method [16]. The tested strains were cultured on Müller-Hinton agar at 37°C for 18 hrs for bacteria, and their suspension was prepared in a saline solution (0.9% NaCl) to achieve a turbidity equivalent to 0.5 MacFarland standards (108 CFU/ml). This suspension was used to inoculate Petri plates with a diameter of 90 mm, containing the aforementioned medium. Sterile discs made of Whatman paper N°1 (6 mm in diameter) were impregnated with 5 µL of *Crataegus azarolus* extract. Prior to incubation, the Petri dishes were kept in the dark at 4°C for 1 hr to allow for the diffusion of the essential oil. Incubation was performed at 37°C for 24 hrs for bacteria. The antibacterial activity was evaluated by measuring the diameters of the inhibition zones.

Acute toxicity

Several concentrations of methanolic extract of *Crataegus azarolus* (600, 1000, 2000, 4000 mg/kg) were tested, diluted in physiological saline solution (0.9 % NaCl). Five groups were formed, each containing six mice. The first group received physiological saline solution orally (control -), and the other groups were treated with various concentrations of the methanolic extract (600, 1000, 2000, 4000 mg/kg), which were then observed for 72 hrs for toxicity symptoms and mortality rate[17].

Acute anti-inflammatory activity

The method used to assess the anti-inflammatory activity is by measuring the inhibition of carrageenan-induced mouse paw edema [18]. Animals were fasted for 12 hrs prior to the experiment and weighed. At the time of the experiment, five groups of six mice were randomly formed. The initial thickness of the hind paw of each mouse was measured using a digital caliper before administering the methanolic extract orally. The mice were treated with the following concentrations:

- Control group: a solution of physiological saline
- Reference group: an aspirin solution with a concentration of 100 mg/kg
- Treatment group 1: a solution of the extract with a concentration of 200 mg/kg
- Treatment group 2: a solution of the extract with a concentration of 400 mg/kg
- Treatment group 3: a solution of the extract with a concentration of 600 mg/kg.

After one hr of administering the treatment, a subcutaneous injection of 5 µL carrageenan solution at a 1% concentration was administered at the aponeurosis level of the left hind paw of the mouse. The thickness of the paw was then measured every hr until the fourth hr.

$$\% \text{ reduction of edema} = [(\Delta T - \Delta E)/\Delta T] \times 100$$

Where:-

ΔT: the difference between the average thickness of the hind paws (right - left) for the control group - (physiological saline solution 0.9% NaCl). ΔE: the difference between the average thickness of the hind paws (right - left) for the test group (methanolic extract or control +).

Analgesic activity (writhing test)

The study was performed in accordance with the Collier et al. Method[19]. It consists of reducing pain caused by an irritating substance capable of inducing twisting movements in mice by using an analgesic substance.The analgesic activity was evaluated on torsions induced by intraperitoneal administration of acetic acid solution to mice. The animals were fasted for 12 hrs before the experiment and weighed. In this study, five groups were formed, each containing six mice. The different extract concentrations were orally administered using a gastric tube according to the concentrations mentioned below:

- Control group: a solution of physiological saline
- Reference group: an aspirin solution with a concentration of 100 mg/kg
- Treatment group 1: a solution of the extract with a concentration of 200 mg/kg
- Treatment group 2: a solution of the extract with a concentration of 400 mg/kg
- Treatment group 3: a solution of the extract with a concentration of 600 mg/kg.

One hr after oral administration of methanolic extract of *Crataegus azarolus*, 0.2 mL of a 3 % acetic acid solution was intraperitoneally injected into the mice. The number of writhings for each mouse was counted ten minutes after the injection of acetic acid for ten minutes.

$$\% \text{ Reduction of pain} = [(\Delta T - \Delta E)/\Delta T] \times 100$$

Where:

ΔT represents the average number of writhings in the control group treated with physiological water 0.9 % NaCl, while ΔE represents the average number of writhings in the test group treated with either the methanolic extract or positive control.

Statistical study

The mean values of the data were presented as means ± standard deviation (S.D.). The differences between groups were analyzed using a one-way analysis of variance (ANOVA) test, followed by the Tukey test.

RESULTS AND DISCUSSION

Results

Determination of the yield

The findings indicate that the methanolic extract obtained from *Crataegus azarolus* yielded 26.96 % in comparison to the initial dry plant material.

High Performance Liquid Chromatography (HPLC) analysis

HPLC chromatography was utilized to analyze the phenolic composition of the methanolic extract of *Crataegus azarolus*. In order to identify our separated compounds, we used several standards of phenolic acids and flavonoids of different natures **(Fig. 1)**. By comparing the retention times and UV spectra of the peaks obtained from the extract with those of the standards, we were able to identify several phenolic compounds present in the *Crataegus azarolus* extract, including rutin, epicatechin, gallic acid, chlorogenic acid 5, *naringin*, caffeic acid, kaempferol, and apigenin **(Fig. 2, Table 1)**.

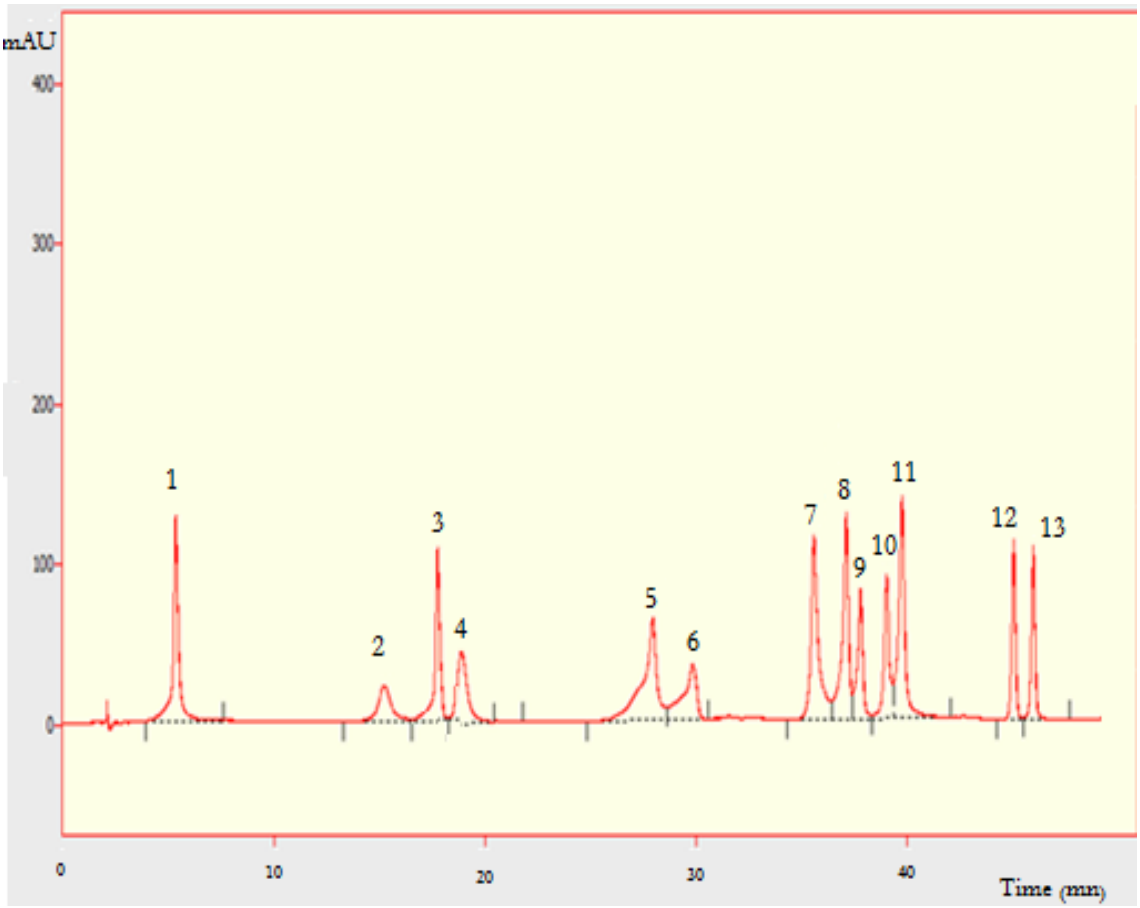

Fig.1: HPLC chromatogram of standard substances: (1): Gallic acid, (2): Caffeic acid, (3): Chlorogenic acid 5, (4): Epicatechin, (5): rutin, (6): Naringin, (7): quercetin, (8): Naringenin, (9): Hesperetin, (9): Quercetin, (10): Kaempferol, (11): Apigenin, (12): Flavon, (13): Methoxyflavone.

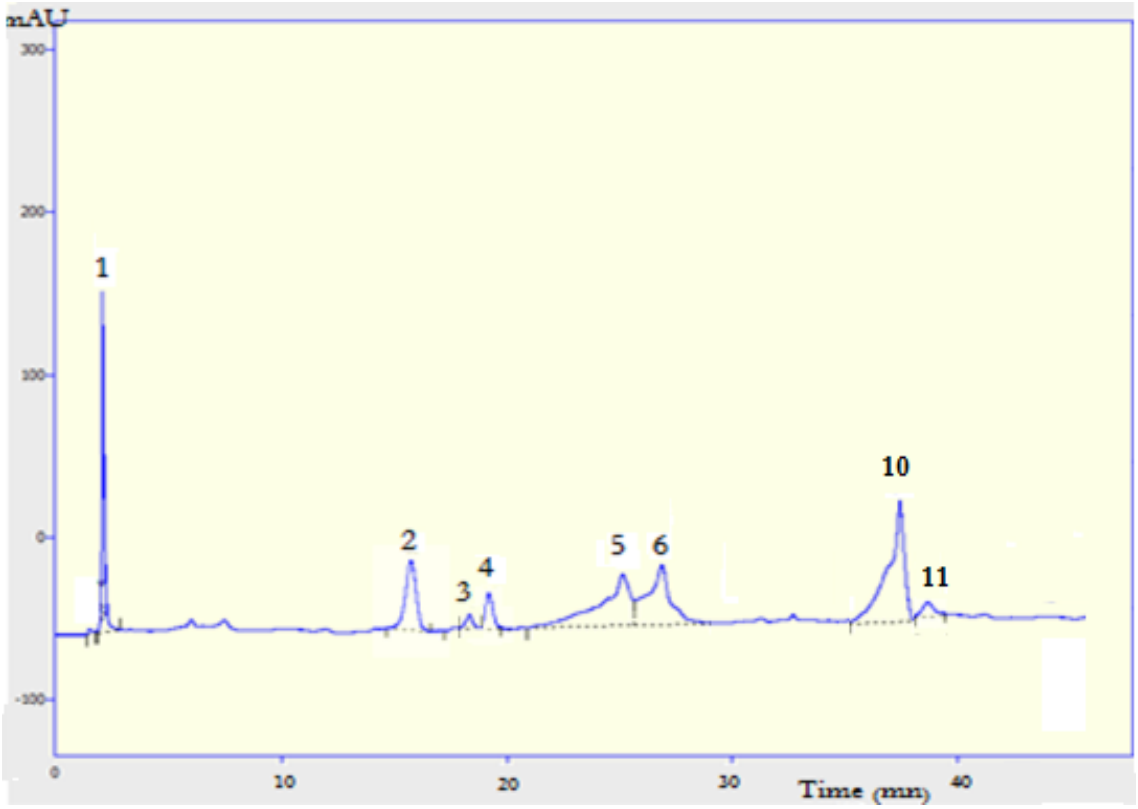

Fig.2: HPLC profile of *Crataegus azarolus* extract. Phenolic compounds identified: (1): Gallic acid, (2): Caffeic acid, (3): Chlorogenic acid 5, (4): Epicatechin, (5): Rutin, (6): Naringin, (10): Kaempferol, (11): Apigenin.

Table 1: The phenolic compounds identified by HPLC-DAD in methanolic extract of *Crataegus azarolus*.

N	Compound	Retention time (min)	λUV max (nm)
1	Gallic acid	5.34	270
2	Caffeic acid	15.25	329
3	chlorogenic acid 5	17.76	280.5
4	Epicatechin	18.84	280
5	Rutin	27.98	352
6	Naringin	28.64	373
10	Kaempferol	39.02	365
11	Apigenin	39.79	342

Antioxidant activity

The DPPH assay is a widely used method for evaluating the antioxidant properties of plant extracts. In this test, a stable free radical, DPPH, is reduced by antioxidants in the extract, leading to a decrease in absorbance at 517 nm. The results of the radical scavenging efficacy of the methanolic extract of *Crataegus azarolus*, compared with BHT and ascorbic acid, are presented in **Table 2**. The methanolic extract showed a strong scavenging effect, with an IC_{50} value of 20.96 ± 0.340 µg/mL, indicating its potent antioxidant activity. The IC_{50} values of BHT and ascorbic acid were 20.74 ± 0.120 and 2.23 ± 0.006 µg/mL, respectively. Based on the findings, it can be inferred that the methanolic extract exhibits the ability to transfer electrons to active free radicals, which ultimately leads to the conversion of these radicals into stable, non-

reactive entities. This process effectively terminates the chain reaction caused by free radicals.

Furthermore, the FRAP assay was employed to investigate the reducing power of the extract. This assay evaluates the capacity of extracts or substances to reduce a ferric salt to a ferrous salt via electron transfer. As indicated in **Table 2**, the methanolic extract displayed a reducing power with an EC_{50} value of 40.47 ± 1.59 mg GAE/g. Ascorbic acid and BHT demonstrated strong reducing power, with EC_{50} values of 6.54 ± 0.038 µg/mL and 11.29 ± 0.114 µg/mL, respectively.

During the β-carotene blanching assay, linoleic acid undergoes oxidation and generates free radical hydroperoxides when the reaction emulsion is incubated at 50°C. These hydroperoxides attack the β-carotene, causing it to bleach. **Table 2** presents the antioxidant activity of the *Crataegus azarolus* extract as determined by the β-carotene bleaching assay. The findings indicate that the methanolic extract effectively inhibited linoleic acid oxidation with an inhibition percentage of 43.00 ± 0.974 %. On the other hand, ascorbic acid showed a low antioxidant activity, with an inhibition percentage of only 14.85 ± 0.183 %.

Antibacterial activity

The antibacterial activity of the extract obtained from *Crataegus azarolus* was evaluated against five different microorganisms. The potency of the extract was determined by measuring the diameters of inhibition zones. Based on the results presented in **Table 3**, the extract demonstrated a wide-ranging antibacterial effect, with inhibition zone diameters ranging from 10.66 mm to 18 mm. The highest antibacterial activity was observed against *Bacillus subtilis*, with an inhibition zone diameter of 18 mm. On the other hand, the lowest inhibition zone was observed against *E. coli*, measuring 10.66 mm.

Table 2: Antioxidant activity of *Crataegus azarolus* extract.

	IC_{50} (µg/mL)	**EC_{50} (µg/mL)**	**Blanching inhibition (%)**
Methanolic extract	20.96 ± 0.34	40.47 ± 1.59	43.00 ± 0.974
Acide ascorbique	2.23 ± 0.006	6.54 ± 0.038	14.85 ± 0.183
BHT	20.74 ± 0.120	11.29 ± 0.114	80.53 ± 0.770

Table 3: Antibacterial activity of extract methanolic of *Crataegus azarolus* tested by disc diffusion method.

Microorganisms	**Extract of *Crataegus azarolus* (inhibition zone mm)**
Staphylococcus aureus CIP7625	15 ± 0.34
Bacillus subtilis ATCC6633	18 ± 0.006
Escherichia Coli CIPA22	10.66 ± 0.120
Klebsiella pneumoniae CIP82.91	12± 0.008
Pseudomonas aeruginosa IPA200	12± 0.010

Acute toxicity

The results obtained indicated that there were no signs of toxicity or mortality observed in mice that were orally administered with different concentrations of the methanolic extract of *Crataegus azarolus*, including 600, 1000, 2000, and 4000 mg/kg.

Anti-inflammatory activity

The findings of the anti-inflammatory activity are presented in **Fig. 3**, which show the variations in the kinetics of reduction of edema in the left hind paws of mice in different tests (control + and methanolic extract) compared to the negative control (physiological saline). The injection of 100 µL of 0.6 % carrageenan into the left hind paw of the mice causes visible inflammation in the different groups. One hr after injection, this edema gradually decreases over time until the end of the experiment (four hrs). The negative control, treated with physiological saline, showed the highest swelling thickness. During this kinetic study, pretreatment of mice with the methanolic extract induced a strong inhibition of inflammation with a percentage reduction of 42,28 % at a concentration of 400 mg/kg.

Analgesic activity (writhing test)

This study involves inducing algogenic action by intraperitoneal administration of 1% acetic acid to mice. This injection causes a pain sensation in the mouse, which is manifested by stretching of the hind limbs and twisting of the dorsoabdominal musculature, known as abdominal contortions. The analgesic effect is evaluated by counting these cramps for 30 minutes after the injection of the algogenic agent. During the test, a strong inhibition of pain was observed with the methanolic extract of *Crataegus azarolus* at 600 mg/kg, with a pain inhibition percentage of 84.59 % **(Fig. 4)**.

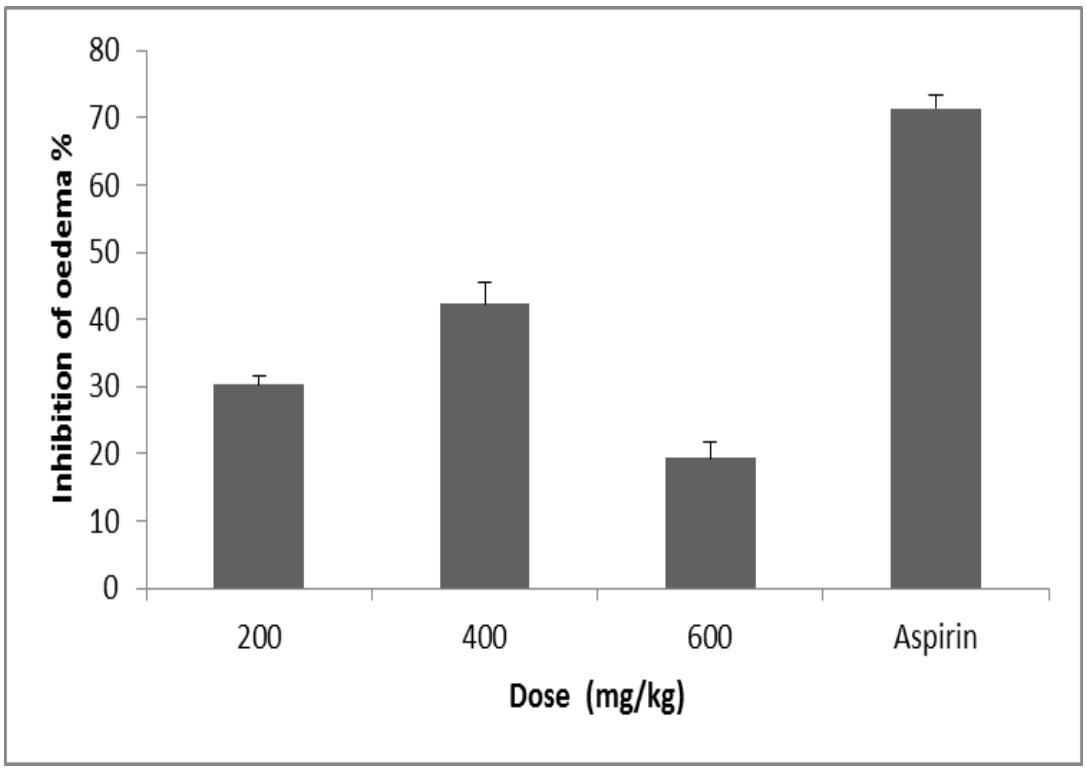

Fig. 3: Anti-inflammatory effect of *Crataegus azarolus* in carrageenan-induced paw edema.

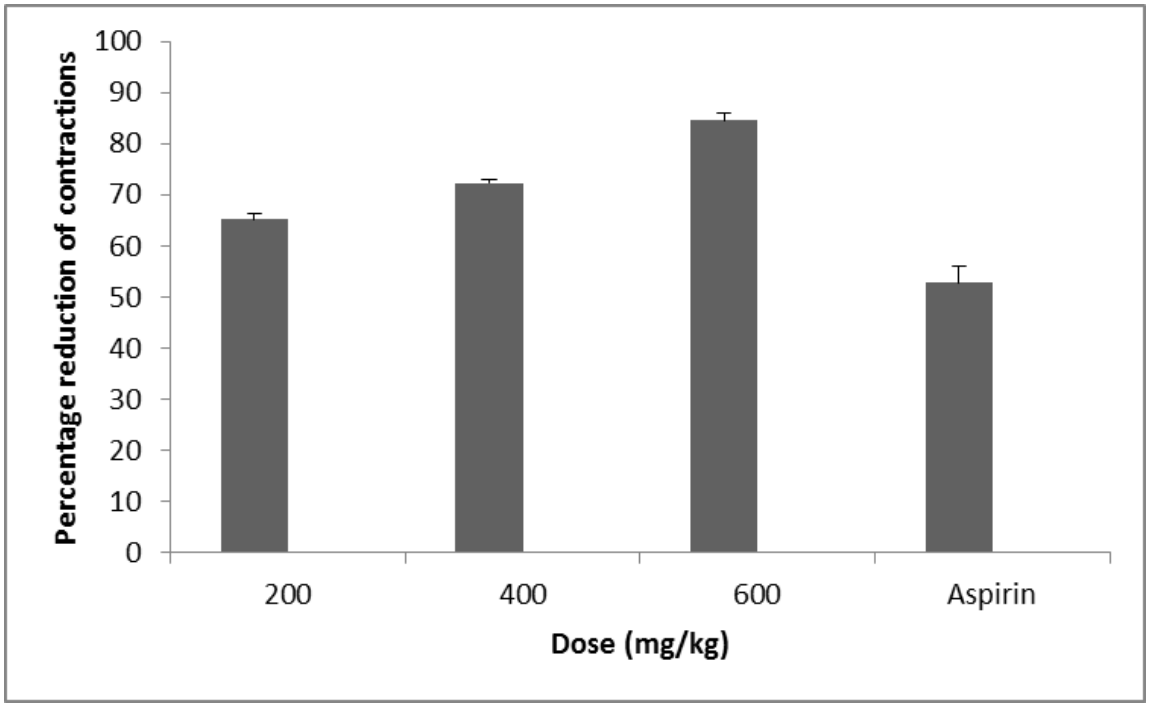

Fig. 4: Effect of *Crataegus azarolus* on acetic acid-induced writhing in mice.

Discussion

The findings of the chromatographic analysis indicated the existence of multiple compounds, including gallic acid, caffeic acid, rutin, epicatechin, chlorogenic acid 5, *naringin*, kaempferol, and apigenin. These findings are consistent with previous research on *Crataegus* genotypes in China, which found that hawthorn leaves primarily contained chlorogenic acid and rutin as flavonoids, while vitexin and quercetin were present in smaller amounts and sometimes absent in certain species. Furthermore, the methanolic extracts of both leaves and fruits of *Crataegus monogyna* were found to contain chlorogenic acid, rutin, isoquercetin, hesperidin, and catechin[19&20].

Rutin, the major compound, was detected in all parts of the plant material, with flower samples of *Crataegus almaatensis* showing slightly higher rutin content (0.66 mg/g DW) compared to *Crataegus oxyacantha* (0.53 mg/g DW). This is in line with the average rutin content (0.097-1.186 mg/g) found in *Crataegus azarolus* var aronia. However, smaller amounts of this flavonoid diglycoside were found in *Crataegus azarolus* (0.161-0.615 mg/g DW) [21]. Additionally, it was discovered that *Crataegus azarolus* flowers contain a higher concentration of the flavonol quercetin, with levels ranging from 0.02 to 0.18 mg/g DW).

Antioxidant activity

To evaluate the antioxidant potential of natural substances, foods, or dietary supplements, chemical-based antioxidant activity assays are employed. These assays provide initial indications of the antioxidant capacities of extract. A study conducted by Abu-Gharbieh and Shehad[22] examined the DPPH radical scavenging potential of *Crataegus azarolous* var. *eu-azarolous* leaves and reported that the ethanol extract exhibited an IC_{50} value of 129.2 µg/mL. By comparing Tunisian wild *Crataegus azarolus* and *Crataegus monogyna* leaves, the DPPH and ferric reducing-antioxidant assay were used to measure their antioxidant activity, which was determined to be in the range of 166.50-168.18 µmol/g fw and 365.32-378.07 µmol Fe^{2+}/g fw, respectively[36]. Bahri-Sahloul et al. (2009)[23] observed that the antioxidant activity of DPPH radical showed results ranging from 317-893 µmol Trolox/100 g DW. The antioxidant activity of hawthorn is primarily attributed to the presence of total and individual phenolic compounds. Several studies have shown that the radical-scavenging activity of hawthorn floral bud extracts is mainly due to the presence of phenolic compounds such as chlorogenic acid, hyperoside, rutin, spiraeoside, quercetin 3-glucoside (isoquercetin), quercetin, epicatechin, and

procyanidin B2[22]. Furthermore, Agregan et al. (2019)[24] and Roselló-Soto et al. (2019)[25] reported a strong correlation between the content of phenolic compounds and the antioxidant activity of plant constituents.

The presence of a large number of phenolic substances in hawthorn allows it to effectively scavenge peroxy free radicals by providing sufficient hydrogen atoms. The antioxidant activity of hawthorn is closely linked to the total phenols and total flavonoids it contains [26]. In vivo, hawthorn may exert its antioxidant effects by increasing the levels of endogenous antioxidant enzymes[27]. In a study using an 80 % ethanol extract of hawthorn, which included compounds like procyanidin B2, epicatechin, chlorogenic acid, hyperoside, and isoquercitrin, it was found that the extract significantly enhanced the activities and mRNA levels of CuZn-SOD and CAT while reducing the levels of the peroxide product MDA. These findings suggest that hawthorn has the potential to protect against oxidative damage[28].

It is important to note that excessive production of free radicals is often associated with conditions such as cancer, aging, and neurodegenerative diseases. However, it is challenging to solely rely on antioxidant treatment to inhibit the progression of tissue damage once oxidative injury has occurred, as other factors play significant roles in the pathology[28]. Therefore, taking preventive measures to avoid oxidative damage through the consumption of natural food products is crucial. Hawthorn, with its abundance of phenols and flavonoids, holds promise as a dietary supplement for the prevention and improvement of diseases related to oxidative stress.

Antibacterial activity

The antibacterial activity of *Crataegus azarolus* was assessed by measuring the diameters of inhibition zones against various microorganisms. In comparison to the findings of Belkhir et al.(2013)[29], it was observed that Tunisian azarole leaves and fruit peel extracts exhibited strong potential in inhibiting the growth of various bacteria, particularly *Staphylococcus aureus* and *Streptococcus faecalis*. Another study also demonstrated significant antimicrobial activity of *C. monogyna*, primarily against Gram-positive bacteria, while no effect was observed on Gram-negative strains. This suggests that Gram-positive strains were more susceptible to the antibacterial effects. The active substances responsible for inhibiting the growth of *S. aureus* strains in *C. monogyna* were proposed to be different classes of phenolic compounds, such as flavonoids, proanthocyanidins, and phenolic acids[30&31]. Nortjie et al (2022)[32] discussed the mechanisms associated with the interaction between these active phytochemical groups and cellular enzymes. The penetration rate of bioactive compounds into microbial cells relies on membrane permeability, and disruption of cell membranes can lead to cell death by compromising cellular integrity.

Anti-inflammatory activity

Acute inflammation induced in mice by carrageenan injection is a standard and practical model widely used for evaluating the anti-inflammatory properties of various agents. According to our results, the methanolic extract of *Crataegus azarolus* exhibits remarkable anti-inflammatory activity. Hawthorn is rich in polyphenols and flavonoids, which are bioactive compounds known for their significant effects in preventing and treating inflammation. According to Liu et al. (2020)[33], flavonoids extracted from hawthorn using 70 % ethanol demonstrated anti-inflammatory properties by alleviating TNF-α-induced intestinal epithelial barrier deficit in Caco-2 cells. This was observed through the improvement of epithelial permeability, as shown in **Fig. 3**. Additionally, oligomeric proanthocyanidins extracted from hawthorn have been found to reduce the expression of proinflammatory factors IL-1β, IL-6, and TNF-α, leading to the alleviation of proinflammatory immune responses in rats[34]. These findings highlight the potential of hawthorn's polyphenols and flavonoids in mitigating inflammation and its associated effects.

In a study conducted by Han et al. (2016)[35], the effects of hawthorn polyphenols were investigated in mice with high-fructose diet-induced liver injury. The findings revealed that the polyphenols derived from hawthorn exhibited protective effects against inflammation and oxidative stress, as evidenced by a reduction in the production of

IL-6 and TNF-α. The possible mechanisms underlying these effects involve the regulation of AMPK/SIRT1/NF-κB signaling by hawthorn polyphenol extract, leading to a decrease in the gene and/or protein expressions of inflammatory-related factors[36]. These results suggest that hawthorn polyphenols have the potential to modulate inflammatory responses and alleviate oxidative stress through their impact on key signaling pathways.

Analgesic activity (writhing test)

A writhing model induced by acetic acid was employed to evaluate the peripheral analgesic effects of a methanolic extract derived from *Crataegus azarolus*. The introduction of acetic acid via intraperitoneal injection leads to inflammation in the peritoneum, which is characterized by writhing and an increase in prostaglandin E2 and prostaglandin F2a levels in the peritoneal fluid [37]. Notably, the oral administration of various doses of the methanolic extract of *Crataegus azarolus* significantly reduced the abdominal writhing responses induced by acetic acid injection. These findings indicate that the extract has the ability to inhibit lipoxygenase and/or cyclooxygenase enzymes in peripheral tissues, thereby interfering with the transduction mechanism in the primary afferent nociceptor. The observed analgesic effect can be attributed to the activation of opioid receptors by phenols, leading to a central anti-nociceptive effect. This activation subsequently leads to a reduction in the release of pain mediators, including substance P[37&38].

Conclusion

This study examined the effectiveness and chemical makeup of the methanolic extract of *Crataegus azarolus*, and evaluated its potential antioxidanh, antibacterial, anti-inflammatory and analgesic properties. Results indicate that this particular Hawthorn species is rich in flavonoids and phenolic compounds, suggesting its potential as a natural source for these beneficial compounds. The results indicate that the plant extract could be a feasible substitute for conventional medicine in the food preservation and pharmaceutical sectors.

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تقييم الخصائص المضادة للأكسدة و البيكتيريا والألم والالتهابات لنبات Crataegus azarolus

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تهدف هذه الدراسة إلى التحقق من تأثيرات مستخلص نبات الزعرور الأحمر (Crataegus azarolus) كمضاد للأكسدة، و مضادة للبيكتيريا المسكن للالام، ومضاد للالتهابات، بالإضافة إلى تحديد مكوناته الكيميائية من خلال تحليل فيتوكيميائي. تم تقييم النشاط المضاد للأكسدة باستخدام اختبارات DPPH، FRAP و β-carotene، في حين تم تقييم النشاط المضاد للبكتيريا ضد خمسة أنواع ميكروبية مختلفة من خلال قياس قطر مناطق التثبيط. كما تم تقييم الأنشطة المسكنة للالم والمضادة للالتهابات باستخدام نموذج حقن الحمض الخليك في البطن و نموذج الورم في القدم الناتج عن الكاراجينان، على التوالي. أظهرت النتائج أن المستخلص يمتلك نشاطًا قويا مضادًا للأكسدة، وأن جميع الجرعات من المستخلص أدت إلى تثبيط تورم القدم. وتم ملاحظة أعلى نشاط مضاد للبكتيريا ضد Bacillus subtilis، حيث بلغ قطر منطقة التثبيط ١٨ مم.وأثبت ان جرعة ٤٠٠ ملغ/كغ من المستخلص الميثانولي لنبات الزعرور الأحمر تثبط تورم القدم بنسبة ٤٢,٢٨٪. بالإضافة إلى ذلك، أظهر المستخلص الميثانولي (٦٠٠ ملغ/كغ) لنبات الزعرور الأحمر تثبيطًا للألم الناتج عن الحمض الخليك في الفئران. تشير الدراسة إلى أن المستخلص يمثل مصدرًا محتملًا للمركبات الحيوية، وأن مكوناته قد تحتوي على تأثيرات تآزرية. وبالتالي، يمكن استخدام مستخلص نبات الزعرور الأحمر كبديل للمركبات المسكنة والمضادة للالتهابات الاصطناعية في صناعة الأغذية والأدوية في المستقبل.