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Anacyclus pyrethrum extracts: examination of bioactive compounds and therapeutic implications through *in vivo, in vitro,* and *in silico* assays

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Abstract

Anacyclus pyrethrum, a native Algerian medicinal plant, demonstrates notable therapeutic efficacy. The bioactive profile of the *Anacyclus pyrethrum* extract was examined using HPLC-UV-VIS, which showed a high concentration of phenolic compounds (33.46 ± 0.57 mg EAG/g EP) and flavonoids (11.08 ± 0.24 mg E Qu/g EP). The *Anacyclus pyrethrum* extract exhibited significant antioxidant activity in iron chelation (IC_{50} = 0.019 ± 0.0006 mg/ml) DPPH (IC_{50} = 0.142± 0.001 mg/ml), $ABTS^+$ (IC_{50} = 0.079 ± 0.0005 mg/ml) and OH (IC_{50} = 0.845 ± 0.052mg/ml) radicals. Anti-inflammatory investigations were conducted using both *in vitro* and *in silico* methods. The *in vitro* testing involved evaluating the denaturation of egg albumin and BSA, while the *in silico* tests focused on measuring the effect of caffeic acid on the COX-2 protein. The anti-inflammatory capacity of the substance was similar to that of Aspirin, with a value of 76.1 ± 1.04% compared to Aspirin's 81.11 ± 1.6%. Our extract exhibited a fatal dose (LD_{50}) of 45.847 ± 1.661 mg/ml and showed significant antibacterial activity with minimum inhibitory concentrations (MICs) ranging from 2.5 to 27.5 mg/ml. The findings emphasize the wide range of medicinal uses of *Anacyclus* pyrethrum, underscoring its significance in both traditional and modern pharmacology.

Keywords: *Anacyclus pyrethrum*, anti-inflammatory, antimicrobial, antioxidant, HPLC-UV-VIS analysis, insecticidal activity

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Introduction

Algeria's geographical location results in a wide range of habitats home to many plant species (Chalane *et al*., 2019; Hemmami *et al*., 2023). It conceals a considerable biodiversity of plant species, a source of many molecules with varied biological properties, likely to be used in different fields (pharmaceutical, perfumery, cosmetics, and agri-food). Among the best-known families, for their biological potential, is the Asteraceae family from the Greek "Aster," which means star, concerning the shape of the flower (Bruneton, 1999). It includes nearly 25,000 species divided into 1300 genera. There are currently 12 subfamilies, which include 43 tribes (Ducombs, 2012). In Algeria, there are 109 genera and 408 species (Quezel & Santa, 1963).

Numerous studies reported that the Asteraceae family is rich in phenolic compounds with powerful antioxidant, antimicrobial, and anti-inflammatory activities, for example, Bursal *et al*. (2021) found that gallic acid from the methanol extracts of *Inula discoidea* (Asteraceae) showed important antioxidant activities. On the other hand, synthetic antioxidants extend the shelf life of foods and prevent degradation. In contrast, many studies have demonstrated that synthetic antioxidants and by-products formed by them can lead to various diseases (Boğa *et al*., 2015). Flavonoids and phenolic acids, a class of polyphenol compounds, are widely distributed in plant kingdoms, whereas over 6000 identified family members in flavonoids. They protect plants from microbial and insect attacks (Bouhafsoun *et al*., 2018).

Anacyclus pyrethrum is an aromatic Mediterranean medicinal plant recognized as African pyrethrum (Bellakhdar, 1997; Hmamouchi, 1999) and widely used in herbal medicine (Hans, 2007). It is native to North Africa; it was cultivated on an experimental scale by imported seeds, whose vernacular names are tigentas, gentas, tigentast, tigenthast, igentas, and gentus (Auhman, 1995). This species includes two varieties, *A. pyrethrum* var. *pyrethrum* (L) and *A. pyrethrum* var *depressus* (Ball) Maire (Jawhari *et al*., 2020; Jawhari *et al*., 2021). The African pyrethrum is a perennial plant that reaches a 30 to 50 cm height and resembles chamomile. Every stalk has a prominent bloom at its end, with a yellow disk, white rays, and a purple hue underneath. The leaves exhibit a sleek texture, arranged in a non-opposite manner, with a feather-like structure, and possess a light green coloration. The roots have elongated, robust, fibrous characteristics, with a brown outside and a white interior. The flavor is strong, and the aroma is subtle (Auhman, 1995). The fruits are glabrous achenes. Sowing in April-May in open ground, African Pyrethrum prefers lean soils, easily reseeds, and flowers from June to September (Hammouch, 1999). *Anacyclus pyrethrum* roots have beneficial therapeutic characteristics (Kishor & Lalitha, 2012). Medicinal properties of the plant include antibacterial, anti-inflammatory, and tonic effects on the nervous system (Tyagi *et al*., 2010; Manouze *et al*., 2017).

The herb *Anacyclus pyrethrum* is widely used in traditional medicine and herbal medicine, and it has been shown to have a beneficial impact on the immune system's ability to regulate itself (Annalakshmi, 2012). Their infusion is used in asthma, colds, rheumatism, and neuralgia (Sharma *et al*., 2010). The roots are also used as an insecticide, antimicrobienne, and anti-inflammatory properties (Selles *et al*., 2012; Kushwaha & Vijay, 2012). According to Duchon *et al*. (2009), Since it demonstrated a powerful KD impact and a powerful exciton-repulsion against resistant mosquitoes, *A. pyrethrum* has the potential to be utilized to control malaria vectors, particularly for the impregnation of mosquito nets and textiles.

This study aims to thoroughly explore the biological capabilities of the indigenous medicinal plant Anacyclus pyrethrum. Our specific objective is to assess the substance's antioxidant capabilities. Anti-inflammatory properties were evaluated using *in vitro* and *silico* experiments. Furthermore, we will evaluate its effectiveness in controlling the green peach aphid, *Aphis gossypii*. These investigations aim to comprehend the therapeutic applications of *Anacyclus pyrethrum*.

Materials and Methods

Plant material

The aerial parts (flowers stems, and leaves) of the plant *A. pyrethrum* L. (Figure 1) were harvested at the flowering stage (June 2021). It was collected early in the morning, in a random way from the region of Hassani Abd Elkarim, Province of Oued Souf. Specimens of this plant have been deposited in the Herbarium of the Faculty of Biology at the University of El Oued Algeria under specimen number L. BIO39MN91. The harvested plant samples were washed from dust, laid on paper, and allowed to air dry at room temperature in a shaded, well-ventilated area.

Animal material

Breeding of *Aphis gossypii* has been established at the Laboratory of Biodiversity and Applications of Biotechnology in the Agriculture Field, University of Chahid Hamma Lakhdar, El-Oued. To provide enough aeration and prevent individual death, the aphid individuals were collected from barley plots and placed on tiller fragments of the same cereal in Petri dishes covered with

Figure 1. *Anacyclus pyrethrum* **L.**

tulle. Conditions for rearing include a temperature of 25°C, a humidity level of 70 percent, and a photoperiod of 16 hours of light and 8 hours of darkness.

Experimental

Preparation of the extract

In 100 mL of hydro-methanolic solution (MeOH/H2O 80:20, v: v), fifty (50) grams of the plant powder were macerated in the dark. The operation was repeated three times, the first for 48 hours and the last two for 24 hours each, with the renewal of the solvent to extract the maximum of the bioactive product. The solvent-vegetable mixture was stirred using a magnetic stirrer. After filtration through filter paper, the filtrate was evaporated by passage through a Rotavapor at 54°C of the Buchi R-200 type (Bouharb *et al*., 2014; Chouikh *et al*., 2018).

HPLC analysis

Our study employed a Shimadzu LC 20 AL High-Performance Liquid Chromatography (HPLC) system fitted with a Hamilton 25 µL universal injector. The analytical column employed was a Shim-pack VP-ODS C18 (4.6 mm×250 mm, 5 µm) manufactured by Shimadzu. The experiment utilized a Shimadzu SPD 20A UV-VIS detector. The mobile phase consisted of a blend of acetonitrile and a 0.1% acetic acid solution. Before use, the mobile phase was passed through a 0.45 µm membrane filter, subjected to sonication, and then pumped from the solvent reservoir to the column at a flow rate of 1 mL/min, as Chouikh *et al*. (2018) described. Subsequently, a 20 µL plant extract solution was injected into the mobile phase flow. We manipulated the elevated force that propels the mobile phase using a pump. The individual chemicals will be identified by passing them through a column for 50 minutes. The mobile phase in the output will be monitored at a wavelength of 268 nm, and the results will be recorded as chromatographic curves by a computer. This study compared the quantification of specific peaks by calibrating standards such as ascorbic acid, gallic acid, chlorogenic acid, caffeic acid, vanillin, p-coumaric acid, and rutin.

Bio-insecticidal effect of A. pyrethrum extract

Four concentrations (200 mg/ml, 150 mg/ml, 100 mg/ml, and 50 mg/ml) of methanolic extract of *A. pyrethrum* L., diluted in 4 ml of acetone, were prepared for the various tests. The control consisted of acetone only. The tests were carried out on wingless forms of *A. gossypii*. Fifteen individuals were placed on barley tiller fragments. Before receiving the insects, the leaves were carefully cleaned with distilled water, soaked in the diluted extracts, and left to dry for 20 minutes to ensure solvent evaporation. An insecticide reference used was Confidor ® OD (Bayer). Five replicates were used for each dose. A binocular loupe was used to examine insects and count dead individuals. To ascertain the mortality rates of aphids exposed to varying concentrations of Pyrethrum extracts, the mortality was assessed and adjusted using Abbott's formula (Abbott, 1925) following a 24-hour treatment period:

$$Mc\% = [(M0\% - MT\%) / (100 - MT\%)] \times 100$$

Mc is the corrected mortality.
M0 is the mortality observed after treatment.
MT is the mortality observed in the control.

Determination of the lethal dose (LD_{50}) and lethal time (LT_{50})

The probit method calculated the lethal dose and time for 50% of the insect population. The mortality percentages were transformed into probits, and the regression of the dose's logarithm according to the mortalities' probits made it possible to determine the LD_{50} for each extract dose.

Antioxidant Activity

DPPH radical scavenging test

The method outlined by Cuendet *et al*. (1997) demonstrates the anti-radical action of the three doses of A. pyrethrum extract on the free radical 2,2-diphenyl-1-pycrylhydrazyl (DPPH). The experiment was conducted in the following way: 1250 µL of DPPH solution (0.004% in methanol) was combined with 50 µL of each test

substance, with concentrations ranging from 0 to 2 mg/mL for the butylated hydroxytoluene (BHT) standard and 0.2 mg/mL for quercetin. The mixes were left unattended at room temperature for half an hour without light. The samples were then tested at 517 nm for absorbance, which was then translated to a percentage. To calculate the radical scavenging activity, we used the formula:

$$\text{Anti-radical activity (\%)} = \{[A_{control} - A_{Sample}] / A_{control}\} \times 100$$

$ABTS^+$ radical scavenging test

Following the methodology outlined by Re *et al.* (1999), the radical scavenging activity of $ABTS^+$ (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic)) was assessed. After reacting seven mM of ABTS in water with 2.45 mM of potassium persulfate (K2S2O8) and letting it stand for 16 hours in the dark, a stock solution of $ABTS^+$ was produced. To get an absorbance of 0.7 ± 0.02 at 734 nm, the ethanol was used to dilute the resultant solution. In brief, 50 μl of each plant extract was mixed with 1000 μl of the ATBTS solution. The absorbance was measured at a wavelength of 734 nm after 10 minutes in darkness. The antioxidant standards utilized were BHT and quercetin. The following formula was used to compute the inhibition rate:

$$I\% = [(A0 - A1)/A0] \times 100$$

A0: Initial absorbance of $ABTS^+$
A1: absorbance of the $ABTS^+$ extract mixture

Iron chelation test

To determine how effective the extracts were as chelators, we used Decker and Welch's (1990) approach, which involves treating the samples with Fe^{2+} ions and then seeing whether they impede the formation of the Fe^{2+}-ferrozine complex. A quick rundown: 40 μL of methanol, 40 μL of extracts, 40 μL of Fe^{+2} (0.2 mM), and 80 μL of ferine were all combined. A reading was taken at 593 nm after incubating the solution for 10 minutes. The standard was EDTA, while the control was a solution of ferrine and $FeCl_2$. The impact of alkylation on iron ions was assessed using the following formula:

$$\text{Iron chelation activity (\%)} = [(AC - AE)/AC] \times 100$$

A_C: absorbance of the white control. AE: absorbance of extract solutions.

OH radical scavenging test

The method of Ates *et al.* (2008) was used to test the extracts' hydroxyl radical scavenging activity. The equilibrium of ferric sulfate (0.0417g) in 100 ml of pure water and hydrogen peroxide (30μl) in 50 ml was established. Afterward, a mixture of 850μl of extract, 150μl of H_2O_2, and 100μl of the mixture was made. After 60 minutes of incubation at 37°C, the absorbance was recorded at 562nm. As a control, vitamin C was utilized.

Antimicrobial Activity

This experiment was conducted using a variety of bacteria and yeasts supplied by the Pasteur Institute of Algeria. The bacteria and yeasts tested included two Gram-positive (Staphylococcus aureus ATCC 2592 and Bacillus subtilis ATCC 6633) and two Gram-negative (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) species. Using physiological saline (0.8% NaCl) broth culture overnight, bacterial inoculums were made to achieve an optical density in the 0.08^{-1} range at 625 nm. Before inoculation, Müller-Hinton agar (MH agar) was added to Petri dishes, solidified, and dried on the surface. The test bacteria-inoculated agars were covered with sterile discs (6 mm) that were then infused with 100 ul of diluted and undiluted extract (25, 50, and 100 v/v DMSO: Dimethylsulfoxide 10%). The negative control was DMSO, while the positive control was a solution containing 15 μg of gentamicin and 25 ug of amoxicillin. The results were obtained by conducting each test three times and then averaging the results. Aerobic incubation was performed on the Petri dishes for 18–24 hours at 37°C. Two studies (Fauchre & Avril, 2002; Dahiya & Purkayastha, 2012) examined and recorded the sizes of the inhibitory zones after incubation. Extract sensitivity was categorized according to the diameter of the inhibition zones as follows: zones less than 8 mm were classified as not sensitive (–), zones 9–14 mm as sensitive (+), zones 15–19 mm as very sensitive (++), and zones larger than 20 mm as extremely sensitive (+++) (Duraffourd *et al.*, 1990; Ponce *et al.*, 2003).

MIC and MBC Determination

Using a broth dilution micro-method in 96-well microliter plates, the antibacterial activity of *A. pyrethrum* extract was evaluated. A 100 mg/mL stock solution was prepared by dissolving the extract in distilled water. The final concentrations ranged from 50 to 2.5 mg/mL, achieved by repeated Mueller-Hinton broth (MHB) dilutions. The wells were further infected with microbial cultures until the final concentration reached 5×10^1 CFU. Each experiment had two independent runs, with MHB alone and MHB combined with distilled water as the control wells. A medicine called gentamicin was used as a positive control. Following a 24-hour incubation period, the minimum inhibitory concentration (MIC) was determined as the extract concentration that completely

halted visible bacteria growth. To find the MBC, 20 µL was transferred from each transparent MIC well onto agar plates. The minimum viable concentration (MBC) of plant extracts that killed 99.9 percent of the bacterial inoculum was established. We conducted three separate experiments to get each result.

Anti-inflammatory Activity

In vitro denaturation of egg albumin

To study the anti-inflammatory activity of *A. pyrethrum* extract, the method of protein denaturation was adopted, according to Bouaziz *et al.* (2020). Fresh eggs were washed and broken carefully, separating the egg whites from the yolks. The volume of the obtained egg whites is measured and then placed in a solution of Tris-HCL (20Mm, pH = 6,8) previously prepared to obtain a diluted solution (1/100, v/v). The solution was shaken gently for 10 minutes and filtered. 2 ml was placed in test tubes containing different concentrations (1.75, 2.5, 5, 10 mg/mL) of the extract or aspirin. After the incubation for 30 minutes at a temperature of 74°C, the absorbance was measured at 650 nm. Inhibition of protein degradation was calculated as follows:

I% = (Abs control – Abs sample)/Abs contro × 100.

In vitro denaturation of BSA

The extract's capacity to prevent albumin denaturation was assessed by adapting the methodology outlined by Chakou *et al.* (2021). In Tris-HCl (0.02 M, pH 6.6), four distinct concentrations were generated for every protein and peptide sample: 1.75, 2.5, 5, and 10 mg/mL. A 2% (w/v) aqueous bovine albumin solution was combined with equal quantities of each sample. Before being heated to 72°C for 5 minutes, the reaction mixture was incubated at 37°C for 15 minutes. We used a spectrophotometer set at 650 nm to measure the samples after they had cooled. The positive control was aspirin. We set up all assays in triplicate. We used the following formula to determine the percentage of albumin denaturation inhibition:

% inhibition = 100 * (Acontrol – Asample)/Acontrol

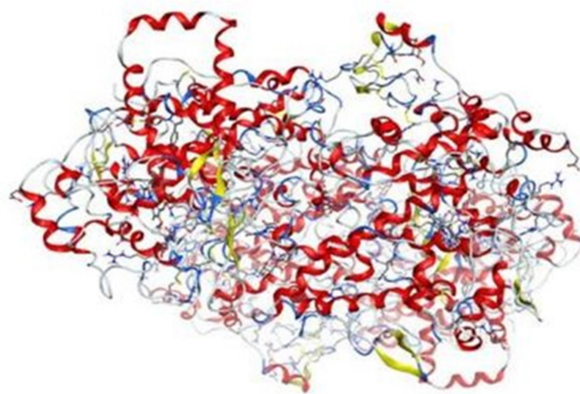

Figure 2. Three-dimensional structure of cyclooxygenase 2 (COX2).

In silico anti-inflammatory activity

The *in-silico* test was performed to evaluate the anti-inflammatory impact of the caffeic acid, one of the products detected in the methanolic extract of the plant Anacyclus pyrethrum, by HPLC-UV analysis, focusing on the COX-2 protein. Prostaglandins have a crucial role in promoting inflammation inside the body. The enzyme prostaglandin synthase, also referred to as cyclooxygenase, catalyzes the conversion of arachidonic acid into other substances, such as prostaglandins PGG2 (Emon *et al.*, 2023; Stachowicz, 2023). The structure of the protein with PDB ID: 1CX2, with a resolution of 3.00 Å, was acquired in PDB format by X-ray diffraction, allowing for the determination of its three-dimensional arrangement. The Autodock Tools software is used to perform molecular docking of conventional medicinal plant extracts with cyclooxygenase-2 (COX) (Figure 2). This process allows us to determine the parameter of this interaction, which is the free binding energy ΔG (Table 1).

Statistical analysis

The data collected for this study were analyzed using R (version 4.2.3) and SPSS (version 20). To determine the plant's efficacy against the green peach aphid over time, researchers compared the means of the examined insecticidal effects of the methanolic extracts. We have run the Kruskal-Wallis test on out-of-the-ordinary data and the ANOVA on normal data. We employed Principal

Table 1. Presentation of the target 1COX2.

Protein ID PDB	Determination Method	Resolution	R-Value	Number of Residues	Co-crystallized Ligand
1CX2	X-ray Diffraction	3.00 Å	0.216	587	1-PHENYLSULFONAMIDE-3-TRIFLUOROMETHYL-5-PARABROMOPHENYLPYRAZOLE

Components Analysis (PCA) to show the efficacy of the examined extract compared to the positive and negative controls and the association between variables and components.

Results

The current study allowed us to record 2.30%, 3.3%, and 3.8% of the yields of total polyphenols in roots, leaves, and flowers, respectively. In addition, the total Phenolic and Flavonoid Content in A. pyrethrum was determined to be 33.46 ± 0.57 mg EAG/ g EP and 11.08 ± 0.24 mg E Qu/ g EP, respectively (Table 2).

The contrast in the number and concentration of chemicals was illustrated by the HPLC chromatogram of *Anacyclus pyrethrum* L. extract shown in Figure 3, which compared the retention time with standards. Table 1 shows the phenolic component concentrations in the investigated extract. Seven compounds—gallic acid, rutin, quercetin, chlorogenic acid, p-coumaric acid, Caffeic acid, and vanillic acid—have been identified in the methanol extract.

Table 2. The concentration of important phenolic compounds in *A. pyrethrum* extract as measured by HPLC (µg/mg extract) (n=3)

Component	$RT_{Reference}$ (min)	$RT_{Extract}$ (min)	Methanol extract (µg/ml)
Gallic acid	5.29	5.254	9.1
Chlorogenic acid	13.392	13.757	0.55
Vanillic acid	15.531	15.448	0.06
Caffeic acid	16.277	16.264	0.09
P-coumaric acid	23.817	23.839	0.25
Rutin	28.37	28.337	9.69
Quercetin	45.047	44.970	2.66

RT: Retention times; min: minute

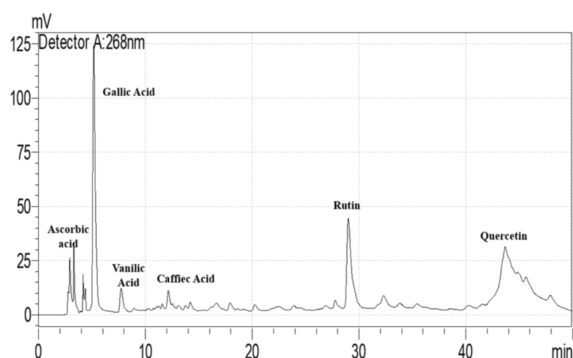

Figure 3. HPLC chromatogram of flavonoids methanol extract of *Anacyclus pyrethrum* L.

Bio-insecticidal activity

The present study showed the presence of an insecticidal effect of the extract of *A. pyrethrum* L. from Oued Souf on the mortality rate of the *A. gossypii* Glover, and the results showed that the mortality rate started as soon as the first day. However, we note that the first dose (D1) showed a mortality of 50% after 24h and that it reached 82% on the third day, while D2 showed a mortality of 20% until it reached 70% on the third day. While (D3 and D4) showed the same mortality rate of 10%, as it became on the third day 55%. The increase continued at all concentrations until the percentage reached 100% on the sixth day. We found that, the mortality of *A. pyrethrum* L. was significantly higher in treated aphids compared to the control group. Statistical data (ANOVA test) revealed a very highly significant difference (P≥0.0000) between doses Figure 4.

Determination of LD_{50} and DL_{90}

The regression line plotted represents the logarithm of the doses tested and the probity-corrected mortality percentages for determining the LD_{50}. Two lethal doses were determined from the regression equations, whereas the LD50 was equal to 45.847 ± 1.661 mg/ml, and DL_{90} was equal to 503.584 ± 2.702 mg/ml. This LD_{50} explained the high toxicity of the plant extract of *A. pyrethrum* L. from Oued Souf on *A. gossypii* Glover Figure 5.

Determination of TL_{50} and TL_{90}

The survival times of the adults exposed to the different concentrations of the extract vary from less than three days to around four days, depending on the concentration. In contrast, in the control group, the adults live an average of eight days. The TL_{50} and TL_{90} are negatively correlated with the concentrations of extract tested in Figure 6.

Antioxidant activity of A. pyrethrum

Four complementary in vitro antioxidant tests were used to investigate the antioxidant activity of the *Anacylcus pyrethrum* root: DPPH, ABTS+, OH radical scavenging, and the Iron Chelation Test. Table 3 shows the concentrations that resulted in a 50% inhibition (IC_{50}).

Take note that lower IC_{50} values indicate more effective protection. In particular, the Iron Chelation and $ABTS^+$ tests demonstrated that the *Anacylcus pyrethrum* extract had comparable and intriguing antioxidant activity, with IC_{50} values of 0.019 ± 0.0006 mg/ml and 0.079 ± 0.0005 mg/ml, respectively. While butylated hydroxytoluene (BHT)

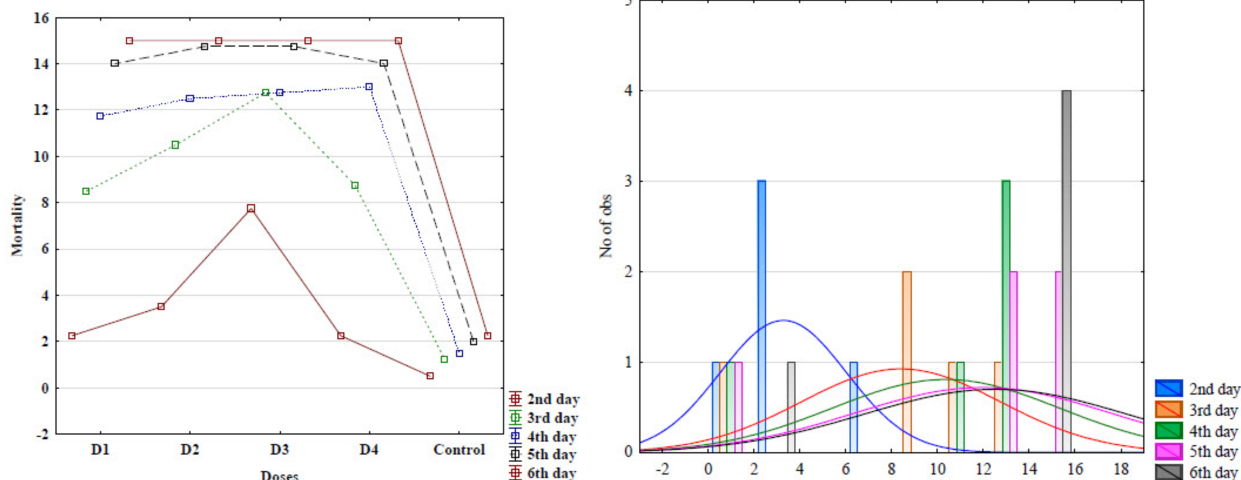

Figure 4. Effect of *A. pyrethrum* on *A. gossypii* depending on time.

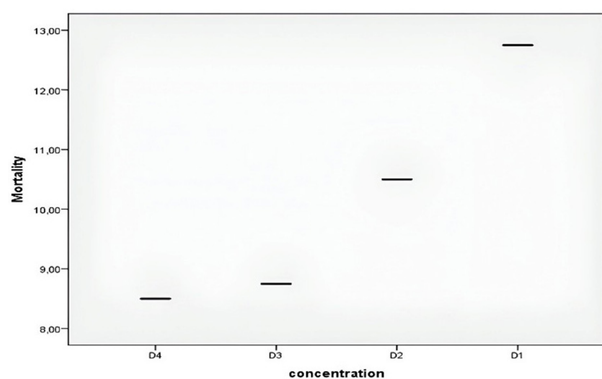

Figure 5. DL_{50} of *A. pyrethrum* extract after six days.

and quercetin were considered reference antioxidants, the extract's antioxidant potencies were much lower (Table 2).

The results showed that the methanolic extract has a significant anti-radical activity ($P < 0.001$) compared to the Vit C standard with an IC_{50} of 0.845 ± 0.052 mg/ml. However, the comparison of the anti-radical. The methanolic extract's ability to scavenge $ABTS^+$ radicals was demonstrated by gradually fading the solution's signature blue-green hue. The results obtained indicate that the extract expresses a strong $ABTS^+$ radical scavenging effect with an IC_{50} estimated at 0.079 ± 0.0005 (Table 2). However, compared with the IC_{50} of the BHT and Trolox standards, they remain significantly different ($p<0.001$) Figure 7.

According to the figure below, all the data were distributed on both axes (100%). In contrast, the total flavonoid (TFC) and phenolic (TPC) content of *A. pyrethrum* were positively correlated with H_2O_2 test successively (Figure 8). Unlike, $ABTS^+$ and DPPH were negatively correlated with the content of this plant.

Antimicrobial activity

The extract of *A. pyrethrum* L shows high activity on Gram-positive and Gram-negative bacteria. The diameters of the inhibition zones vary depending on the strains tested and the dilutions used. The results are illustrated in Table 4.

The best antibacterial power of extract of *A. pyrethrum* was observed at the 100 mg/ml dilution on the *S. aureus* strain with an average inhibition diameter of 19.9± 0.17mm, compared to Gentamicin and Amoxicillin, positive control, presented inhibition zones of 22 mm and 31 mm, respectively (Table 5), and the yeast *C. albicans* with an average inhibition diameter of 19.9± 0.17 mm. The microdilution technique determined the MIC of A. pyrethrum extract. MICs range from 2.5 to 27.5 mg/mL (Table 5). These MICs indicate that the bacterial strains tested are sensitive to the extract of *A. pyrethrum*. This extract exerts good activity on *S. aureus*, *E. coli*, and *P. aeruginosa* strains with a MIC of 2.5, 10, and 12.5 mg/ml, respectively, and the yeast *C. albicans* with a MIC of 2.5 mg/ml. On the other hand, the *Bacillus subtilis* strain is moderately sensitive, with an inhibition zone of 11.5 ± 0 mm and a MIC of 27.5 mg/ml. The MBC of *C. albicans* was 50 mg/mL. On the other hand, the other bacterial strains studied have a value greater than 50 mg/mL.

Anti-inflammatory activity

In vitro results

The results of the anti-inflammatory activity, by thermal denaturation of egg white proteins, indicate a percentage of inhibition equal to 76.1 ± 1.04% with concentrations of 10 mg/ml of the extract. In comparison, acetylsalicylic

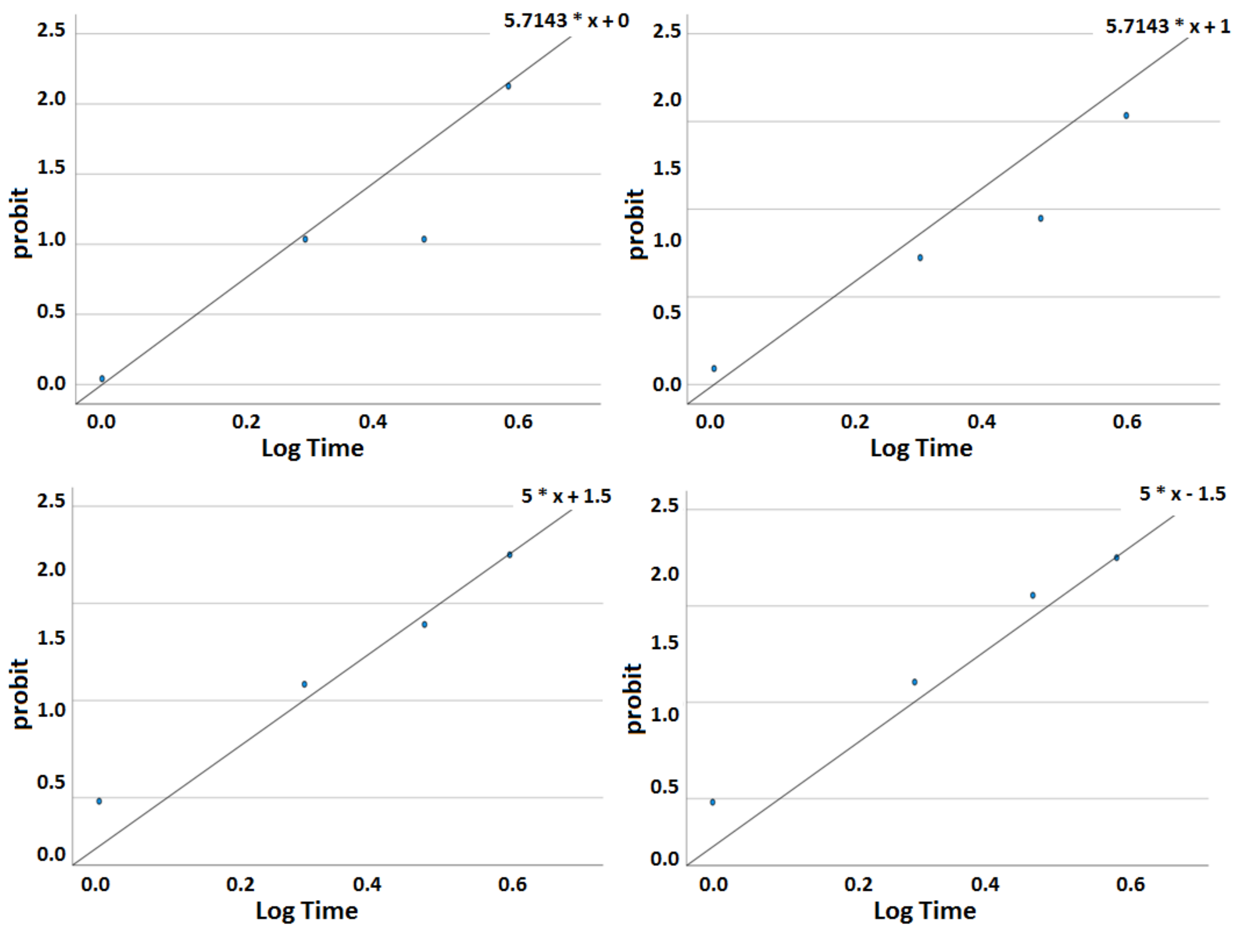

Figure 6. TL_{50} and TL_{90} values (hours) of *A. pyrethrum* extract against adults of *A. gossypii*.

Table 3. A. *pyrethrum*'s antioxidant activity (mg/ml) in comparison to the standards

	DPPH IC_{50}	$ABTS^{+}$ IC_{50}	Iron chelation	Radical OH IC_{50}
Extract	0.142± 0.001***	0.079 ± 0.0005***	0.019 ± 0.0006***	0.845 ± 0.052***
BHT	0.026 ± 0.0006	0.0152 ± 0.003	Nd	Nd
Trolox	Nd	0.0033 ± 0.001	Nd	Nd
EDTA	Nd	Nd	0.0034 ± 0.0005	Nd
Ascorbic Acid	Nd	Nd	Nd	0.017 ± 0.001

***: very highly significant; Nd: Not determined; IC: Inhibitory concentration.

acid presented a percentage of inhibition of 81.11 ± 1.6% at the same concentration (Table 6). Nevertheless, these outcomes are similar to those of a well-used anti-inflammatory drug that, when administered at an equivalent dosage, successfully prevented BSA from denatured by demonstrating a 93.9% ± 0.8% inhibition. The inhibition of BSA is found to be directly proportional to the concentrations of extract. The highest level of inhibition (64.5 ± 2.3%) was achieved at a dose of 10 mg/ml.

In silico results

Using the B3LYP level of theory and the base 6-311++G (d, p), the Gaussian 09 program optimizes the chemical structure of the caffeic acid complex. The characteristics of the interaction between a ferrocene derivative and DNA can be obtained through molecular docking using the AUTODOCK 4.2 docking program. These parameters include the binding constant K and the binding free energy ΔG. The COX2 and caffeic acid ligand-optimized structures were loaded into the AutoDock program. Every docking study was conducted on a Pentium 3.30 GHz microcomputer running Windows 7 with 4.00 GB of RAM. The protein data bank (http://www.rcsb.org./pdb) provided the crystal structure of the COX2 enzyme, which was selected as the receptor to investigate the

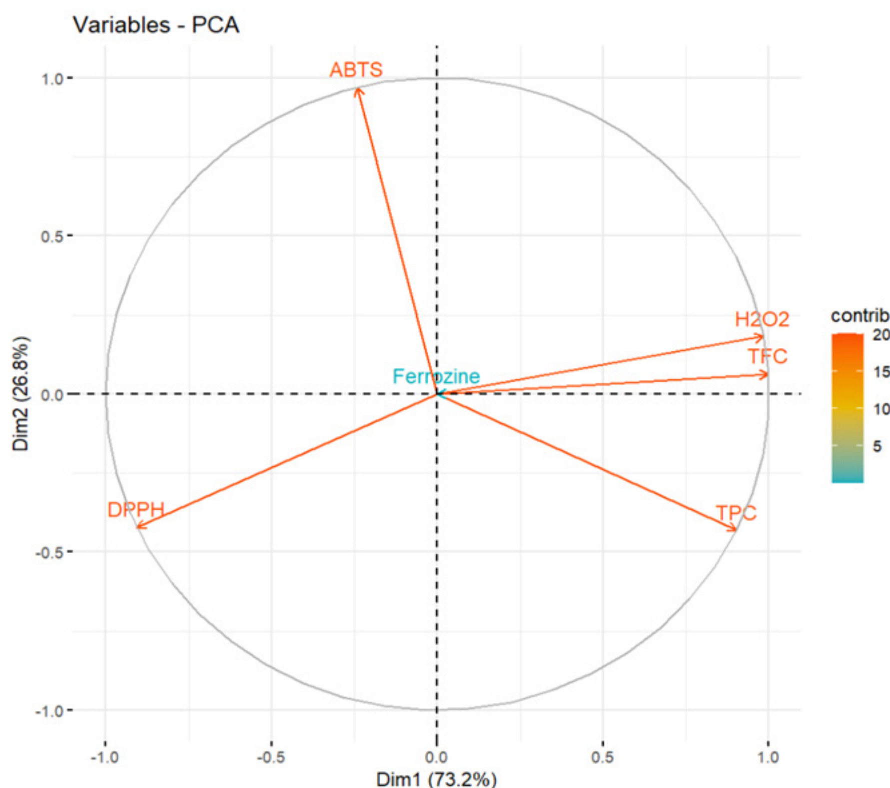

Figure 7. Principal Component Analysis for the antioxidant potential and total flavonoid (TFC) and phenolic (TPC) content of *A. pyrethrum*.

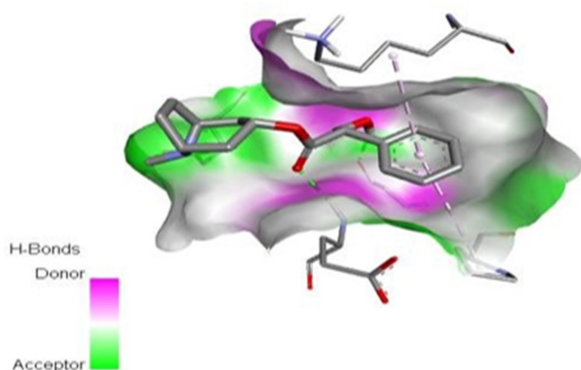

Figure 8. Interaction between caffeic acid and cyclooxygenase-2.

caffeic acid ligand binding. To facilitate molecular docking studies, all hydrogen atoms and charges are added to the structure of caffeic acid. Lamarckian genetic algorithms were utilized for docking calculations, and the grid size was set to 50×50×50 with a step size of 0.372 and a point separated by 1,000 Å centered on the binding site of COX2. Table 7 contained the grid centers. Twenty-five docking trials comprised the docking experiment. The default settings for the other settings were maintained. The conformation with the lowest docking energy was chosen as the optimal one.

Figure 8 depicts a surface perspective, showing how caffeic acid is positioned when docked with cyclooxygenase-2. It demonstrates that caffeine is connected through hydrogen bonding.

The negative values of ΔG indicated the reaction's spontaneity between the caffeic acid and the cyclooxygenase 2 (Table 8).

We used the PLIP database (Protein-Ligand *et al.*) to determine the binding site and the type of interaction formed between caffeic acid and cyclooxygenase 2. The results obtained are shown in Table 7 and Figure 9. Data analysis revealed that all the selected compounds formed electrostatic hydrogen bonds with the target enzyme.

Discussion

Asteraceae is one of the most known and rich families in beneficial compounds. Furthermore, Ak *et al.* (2020) provided important information regarding the impact of extraction methods on the recovery of plant bioactive chemicals. Some species of Asteraceae, such as the *Achillea* genus, are widely used as foods, nutraceuticals, or dietary supplements with health-promoting effects (Yener İsmail *et al.*, 2020) and according to

Table 4. Antibacterial activity of *A. pyrethrum*.

Bacterial Strains	Reference	Extract (mg/mL) 25	50	100	GN	AMX
Staphylococcus aureus	ATCC 25923	11±0	14.2±0.28	19.9±0.17	22	31
Bacillus subtilis	ATCC 6633	6	9.8±0.7	11.5±0	0	16
Escherichia coli	ATCC25922	9.33±1.15	11±0	14.3±0.34	27	26
Pseudomonas aeruginosa	ATCC 27853	6	9.35±1.35	12.75±0.76	25	0
Candida albicans	ATCC 10237	10.6±0.28	13.3±0.25	17.5±0	/	/

Table 5. MIC and MBC of *A. pyrethrum* (mg/mL).

Bacterial Strains	Reference	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus	ATCC25923	2.5	> 50
Bacillus subtilis	ATCC6633	27.5	> 50
Escherichia coli	ATCC25922	10	>50
Pseudomonas aeruginosa	ATCC27853	12.50	>50
Candida albicans	ATCC10237	2.5	50

Table 6. In-vitro inflammatory effects of *A. pyrethrum* extract.

Concen-tration (mg/mL)	EGG Inhibition (%)		BSA Inhibition (%)	
	A. pyrethrum	Aspirin	A. pyrethrum	Aspirin
10	76.1±1.04	81.11±1.6	64.5±2.3	93.9±0.8
5	52.5±2.7	67.9±1.4	62.4±1.2	55.2±0,8
2.5	25.6±1.3	41.6±5.6	33.5±7.1	41.9±2.1
1.75	7.7±1.3	29.1±2.03	28.3±10.5	34.3±0.9

Table 7. Selected docking parameters for cyclooxygenase-2-.

Grid Center			Grid Size (Å)		
X	Y	Z			
42.335	33.591	36.078	75	75	75

Table 8. Binding constants and binding free energy values.

Caffeic acid-COX2	K=1.3577×104 mol^{-1}	ΔG= - 23.594 ($KJ.mol^{-1}$)
Adduct	Binding Coefficient	Gibbs free Energy

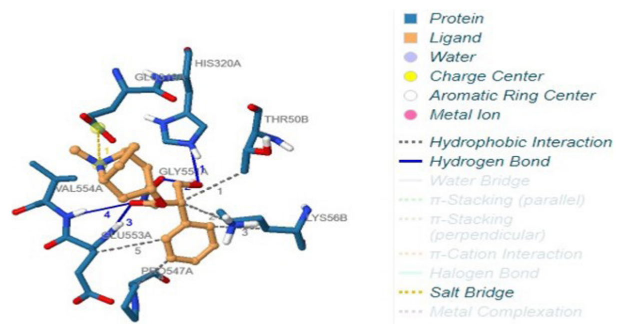

Figure 9. Site of interaction between l-caffeic acid and cyclooxygenase-2.

Usmani *et al.* (2016) and Cherrat *et al.* (2017) mentioned that the phytochemical examination of aerial parts of *A. pyrethrum* revealed the presence of alkaloids, flavonoids, polyphenols, catechin and tannin-reducing compounds. Further compounds found in this plant include gallic tannins, sterols, triterpenes, and coumarins (Akher *et al.*, 2017; Riddick, 2021). Subasri and John (2016) also note the presence of trace elements like Fe, Zn, and Cr. A flavonoid known as quercetin has shown promise as a component in bio-insecticides. A protective mechanism has been linked to its plant metabolism.

The plant can change its nutritional content and taste, making it less digestible or even toxic (Mierziak *et al.*, 2014). Quercetin selectively inhibits the growth and weight gain of nocturnal insect larvae, specifically those of the Hemiptera, Diptera, and Lepidoptera families (Riddick, 2021). El-Nahhal *et al.* (2021) found that quercetin is among the most efficient medications for improving pesticide-induced cardiotoxic illnesses. According to research by Granado-Serrano *et al.* (2012), Gokhan Zengin *et al.* (2018), and Qi *et al.* (2022), quercetin can boost cell antioxidant capacity by activating the intracellular p38 MAPK pathway, increasing intracellular GSH levels, and serving as a source of hydrogen donors for free radical scavenging. On the other hand, chlorogenic acid blocks the insect's cholinergic synapses by inhibiting an enzyme called AChE, which is crucial for the breakdown of acetylcholine. The qualitative study revealed that the roots had abundant alkaloids, whereas the aerial portions, including the leaves and flowers, contained abundant tannins and flavonoids (Cherrat *et al.*, 2017). Anacycline, enetriyne alcohol, hydrocaroline, and volatile oils are present in A. pyrethrum, and chemical analysis has shown that they contain an N-isobutylamide alkaloid known as "pellitorin" and primarily accumulate alkamides, with pellitorin being the main active component (Elazzouzi *et al.*, 2015).

Table 9. Caffeic acid-cyclooxgenase-2-(PLIP) interaction site bungs.

Caffeic acid-cyclooxygenase-2-								
							Hydrophobic Interaction	
Index	Residue	AA	Distance		Atom Ligand		Protein Atom	
1	50B	THR	3.80		21773		5599	
2	56B	LYS	3.90		21773		5660	
3	56B	LYS	3.83		21775		5658	
4	547A	PRO	3.22		21778		5110	
5	553A	GLU	3.48		21779		5156	
				Salt Bridges				
Index	Residue	AA	Distance	Protein Positive?		Ligand Group	Ligand Atoms	
1	319A	GLU		3.18		×	Tertamine	21767

According to multiple studies, gallic acid has antioxidant properties. It can protect against oxidative stress-induced diseases like cancer, heart disease, and metabolic disorders by lowering levels of lipid peroxidation and raising or maintaining normal levels of superoxide dismutase, catalase, and glutathione (Moghtaderi *et al*., 2018; Yang *et al*., 2022). Furthermore, hydrolyzable tannins like gallic acid can interact with metal ions and macromolecules like polysaccharides. In addition to being toxic to insects (Bernays *et al*., 1989) and affecting larval hatching (Nagendrappa, 2005), it can form soluble complexes with proteins that impede digesting enzymes. In addition, as the concentration of gallic acid increased, the larval death rate of *Spodoptera litura* rose, and the adult emergence rate fell (Punia *et al*., 2021). The MeOH extract of Anacylcus pyrethrum exhibited the maximum antioxidant power with an IC50 of 0.056 mg/ml, according to Selles *et al*. (2012), who used the DPPH technique. In a study conducted by Manouze *et al*. (2017), it was found that the *Anacylcus pyrethrum* extracts exhibited antioxidant activity that was comparable to ours. This was especially true in the DPPH test, where the IC_{50} values were 12.38 ± 0.28 μg/ml. The phytochemical components of extracts may be responsible for their antioxidant capacity. Research conducted by Nagendrappa (2005), Halliwell (2008), Stagos *et al*. (2012), Guettaf *et al*. (2016), Elazzouzi *et al*. (2015), and others found that phytochemical screening has confirmed the existence of several compounds such as alkaloids, flavonoids, saponins, tannins, triterpenes, and sterols. This finding aligns with earlier research showing these phytochemicals in *Anacylcus pyrethrum* methanolic extract (Selles *et al*., 2012; Elazzouzi *et al*., 2022). Given the various variables affecting an extract's antioxidant activity, we must try multiple approaches to evaluate our extracts' antioxidant potential (Wong *et al*., 2006).

Baselam *et al*. (2023) found that *A. pyrethrum* aqueous extracts from the Bin El Ouidan region of Morocco had potent antibacterial effects against *S. aureus* and *E. coli* pathogens, with inhibition zones of 17.65 mm ± 1.14 and 17.73 mm ± 0.61, respectively, and MICs of 1.95 and 8.51. Some research has shown that *A. pyrethrum* extracts have an active effect on certain strains of *Escherichia coli* and *Staphylococcus aureus* (Elazzouzi *et al*., 2015; Jawhari *et al*., 2021). *A.* this emphasizes *pyrethrum*'s broad-spectrum antibacterial capability. Furthermore, it effectively inhibits bacterial growth, as evidenced by the bactericidal activity reported at low doses. These findings corroborate earlier research showing that this plant has antibacterial properties against various bacterial strains and the yeast *Candida albicans* (Selles *et al*., 2012; Elazzouzi *et al*., 2022). Selles *et al*. (2012) and Jawhari (2020) found that the antibacterial activities of the extract are due to the presence of secondary metabolites, specifically phenols and flavonoids.

Synthetic anti-inflammatories are known to have a wide range of adverse health effects in humans, such as hepatic damage, ulcers in the gastrointestinal tract, and increased risk of cardiovascular disease and cancer (Than *et al*., 2017). Herbs, with their phytochemicals, may offer a safer and more effective alternative for inflammatory diseases. Both *in vitro* and *in vivo* investigations show a significant anti-inflammatory impact in this study. Manouze and colleagues (2017) examined the anti-inflammatory efficacy of the aqueous and methanolic extract of the *A. pyrethrum* roots in vivo. They found it significantly reduced xylene-induced rat ear edema and Freund-induced total rat claw edema. More than that, another research has demonstrated that *A. pyrethrum*'s aqueous-alcoholic extracts from its various parts—leaves, seeds, roots, and flower heads—have powerful anti-inflammatory effects on rat edema. In the oral treatment groups, the inhibition varied from 61% to 71% after one hour of therapy (Jawhari, 2020).

The *in silico* investigation conducted by molecular docking enhances our work by enabling the production

of different ligand conformations. The choice of the ligand's orientation is determined by the location of the most suitable conformation within the molecular target to create a stable complex. This entails analyzing and simulating diverse potential interactions between the ligand and the target macromolecule. These stages can be iterated multiple times until a desirable outcome is achieved. This theoretical study has validated the spontaneous interaction between the plants and medications being studied and cyclooxygenase-2, as evidenced by the negative values of ΔG (Perez *et al*., 2019). When the active component of the investigated extract attaches to cyclooxygenase-2, it induces conformational changes by creating hydrogen-type electrostatic bonds, thereby inhibiting the enzyme's biological function. Our analysis of the inhibition rate data, compared to typical medications, demonstrates that these extracts effectively block the manufacture of prostaglandins by targeting the enzyme cyclooxygenase (COX-2). This anti-inflammatory activity has been observed both *in vitro* and *in silico*. Hence, it is imperative to exercise caution about the dosage when utilizing these botanical extracts as herbal tea to treat specific inflammations. One prerequisite for the efficacy of any medication is that it must demonstrate, in theory, that it does not pose any carcinogenic, mutagenic, or hepatotoxic hazards. This has been verified for all our examined plants (Garrett *et al*., 1998; Ferrer *et al*., 2019).

Conclusions

The methanolic extract of *Anacyclus pyrethrum*, a plant endemic to Algeria, exhibited substantial antibacterial and anti-inflammatory properties. Additionally, it effectively inhibited iron chelation, DPPH, ABTS, and OH radicals. Moreover, its powerful bio-insecticidal effect on *Aphis gossypii* provides a natural substitute for synthetic pesticides. To fully exploit its capabilities, additional comprehensive research is necessary. The study of formulation techniques to enhance drug absorption into the body, the identification of particular compounds with biological activity, the verification of safety and efficacy through tests on living things, the start of rigorous trials on human subjects, the assessment of the potential synergies between new and existing therapies, and the streamlining of the procedures involved in bringing the treatments to the general public should be the top priorities for future research. In conclusion, we may investigate and separate the bioactive substances in *Anacyclus pyrethrum* to incorporate them into conventional medical practices. This will positively impact global health and help connect traditional knowledge with modern healthcare.

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Conflict of Interest

The authors declare no conflict of interest.

Sample availability

Samples of all the compounds are available from the corresponding author.

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