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Research article

Chemical profiling, antioxidant, and antibacterial activities of *Juniperus procera* and *Cinnamomum camphora* essential oils, alongside their insecticidal properties against *Aphis craccivora*

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Abstract: Over the past decade, the therapeutic effects of essential oils have become a research interest. This study describes the chemical composition, antimicrobial, antioxidant, and insecticidal properties of *Juniperus procera* and *Cinnamomum camphora* essential oils. Essential oils were extracted by the steam distillation method and identified by gas chromatography-mass spectrometry (GC-MS), revealing the presence of monoterpenes and sesquiterpenes as the main components of *J. procera*, together with α-pinene, endo-Borneol, and 1,8-cineole. *C. camphora* showed high concentrations of α-pinene, trans-Pinocarveol, and 1,8-cineole. The antimicrobial investigation revealed that both essential oils displayed significant antibacterial efficacy against all tested organisms. *J. procera* demonstrated antibacterial activity ranging from 30.5 ± 0.70 to 24.0 ± 1.41 mm, surpassing that of *C. camphora*, with inhibition zones ranging from 19.5 ± 0.70 to 12.5 ± 0.70 mm. The essential oils were comparable to the reference antibiotic (chloramphenicol). Gram-positive bacteria exhibited higher susceptibility than Gram-negative bacteria. The insecticidal effects of essential oils on *Aphis craccivora* were studied, and results indicate increased death rates in a concentration-dependent manner when exposed to essential oils. The use of *J. procera* and *C.*

camphora oils at concentrations below lethal levels was found to affect important biological factors in *A. craccivora*, including generation time, net reproductive rate, intrinsic rate of increase, and finite rate of growth. This suggests the potential of these oils to be effective agents for controlling pests. The multifaceted bioactivities of *J. procera* and *C. camphora's* essential oils highlight their promising potential as natural alternatives for antimicrobial and pest control applications.

Keywords: chemical compositions; GC-MS; essential oil; bacteria; fungi; *Aphis craccivora*; natural insecticides; antioxidant

1. Introduction

Plants have always provided all the necessary human needs, such as food, shelter, clothing, spices, scents, medicines, insect repellents, and other critical items. Historically, it is believed that plants were the primary source of medicine in the prehistoric era and beyond, based on evidence from fossil records unearthed in Iraq in 1960. These records date back approximately 60,000 years, indicating that humans utilized Hollyhock (*Alcea rosea* L.) as a medicinal remedy [1,2]. Throughout the history of medicine, medicinal plants have played a crucial role, serving as traditional remedies in various cultures and as trade commodities meeting the needs of distant markets. Recently, the WHO reported that traditional medications, primarily sourced from plants, are used by most of the world's population to cure different illnesses. Traditional medicine constitutes the main form of healthcare for almost 60% of the global population, with 80% of those in poor nations relying on medicinal plants for primary healthcare (Parveen et al., 2020) [3,4].

The global health landscape faces important challenges such as the escalating prevalence of chronic and infectious diseases, which are increasingly regarded as serious concerns. Scientific literature provides plentiful evidence of the utilization of traditional medicinal plants for curative, preventive, or palliative purposes in the context of significant chronic ailments, such as cardiovascular disease, diabetes, cancer, arthritis, dementia, and asthma. A substantial number of these reports originate from Asian countries, where traditional medicine continues to play an integral role in healthcare services. Among some noteworthy medicinal plants, garlic (*Allium sativum*), turmeric (*Curcuma longa*), ginseng (*Panax ginseng*), green tea (*Camellia sinensis*), *Ginkgo biloba, Astragalus membranaceus, Apocynum venetum, Codonopsis pilosula, Huperzia serrata, Fallopia multiflora, Lycium barbarum, Stephania tetrandra, Trichosanthes kirilowii*, and *Lycium chinense* are particularly noteworthy [5]. Moreover, infectious diseases, particularly those caused by microbial infections, are a prominent global cause of mortality, and the rise of antibiotic resistance poses a grave threat to public health worldwide, as it is believed to lead to the proliferation of superbugs that are impervious to existing antibiotics. This leads to the belief that we may have to revert to a time before antibiotics, and widespread outbreaks of severe epidemic diseases might occur [6]. On the other hand, insect pests present a substantial threat to global food security, resulting in considerable economic losses and diminished crop yields. Conventional pest control approaches have predominantly depended on synthetic insecticides, yet the excessive use of these chemicals has given rise to insecticide-resistant pests, environmental pollution, and health risks for both humans and non-target organisms. Consequently, there is an urgent demand for innovative and efficient pest management strategies, which include the exploration and development of natural insecticidal agents [7,8].

Therefore, medicinal plants hold promise as a natural source for novel drug development, and ongoing research aims to uncover their biological activities.

The primary origin of several bioactive compounds lies in secondary metabolites, commonly referred to as phytochemical compounds. Phytochemicals are utilized by plants to address temporary or ongoing environmental threats, regulate vital functions of growth and reproduction, and notably exert physiological effects on animal cells. Among these bioactive phytochemicals, alkaloids, glycosides, phenolics, flavonoids, tannins, volatile oils, and resins are included [9]. *Juniperus procera* Hoechst. Ex Endl. is an aromatic plant belonging to the family Cupressaceae, which encompasses 70 distinct plant species [10]. Aromatic plants have historically been employed in traditional agricultural practices, particularly in regions with limited access to synthetic insecticides, such as certain developing economies. However, the scientific understanding of *J. procera* and *C. camphora,* two species with demonstrated insecticidal potential, remains critically underexplored. This scarcity of comprehensive studies highlights a significant research gap, particularly regarding their efficacy, mechanisms of action, and practical applicability as botanical insecticides. Synthetic pesticides have led to environmental pollution, pest resistance, and harm to non-target organisms (including humans and beneficial insects) [11]. Botanical insecticides, such as those derived from *J. procera* and *C. camphora*, offer a biodegradable and less toxic alternative [12]. Botanical extracts may be integrated into pest management programs to diminish dependence on synthetic chemicals [13]. *C. camphora* extracts exhibit synergistic benefits when utilized in conjunction with other biopesticides [14].

Juniperus procera is found in the southern region of Saudi Arabia and exhibits a widespread distribution throughout this area, where it is commonly referred to as "Arar" in Arabic. It is also indigenous to the mountains of eastern Africa, spanning from Eastern Sudan to Zimbabwe, and extending to the southwestern Arabian Peninsula [15]. *Juniperus* species have been employed in traditional medicine for the treatment of conditions such as hypoglycemia, anti-inflammatory disorders, intestinal worms, cancer, liver disease, bronchitis, pneumonia, wounds, ulcers, and tuberculosis [16]. *Cinnamomum camphora* (L.), belonging to the Lauraceae family, is an esteemed aromatic plant renowned for its diverse applications in traditional medicine, such as rheumatism, bronchitis, sprains, asthma, diarrhea, indigestion, muscle pains, and menstrual disorders, as well as chills and colds [17]. Saudi Arabia is an excellent source of various medicinal and aromatic plants. Many Saudi plants differ from the same plants growing in other areas and climates in their chemo-diversity and therapeutic effects because of the distinct geographical location of the country [18].

Camphor oil is extensively utilized in medicine, yet limited research has been conducted to explore its potential as a bio-pesticide in plant protection studies. Prior studies utilized camphor oil as a repellent against stored-product insects, including *Sitophilus granarius* and *Tribolium castaneum* [19]. It also showed larvicidal activity against the sheep bot fly, *Oestrus ovis* [20]. Little is known about *J. procera* and *C. camphora* growing wild in Saudi Arabia. Therefore, the current study aims to evaluate the chemical components of the essential oils by gas chromatography–mass spectrometry (GC-MS) and assess their antibacterial effectiveness against both Gram-positive and Gram-negative bacteria, comparing their performance with that of a conventional antibiotic (chloramphenicol). Additionally, the study aims to investigate the toxicity and fatal effects of different doses of *J. procera* and *C. camphora's* essential oils against *Aphis craccivora* (cowpea aphid) and to ascertain whether these oils can function as environmentally sustainable biopesticides for insect control in agriculture. Finally, the study aims to evaluate the insecticidal efficacy of these essential oils against *Aphis craccivora*, analyzing concentration-dependent mortality and their effects

on critical biological metrics, including generation time, net reproduction rate, intrinsic rate of increase, and finite rate of growth.

2. Materials and methods

2.1. Plant materials

Green leaves of *Juniperus procera* and *Cinnamomum camphora* were manually collected from Al-Rass city in Qassim region, Saudi Arabia, at Winter, in 2023. Voucher specimens of these plant materials were subsequently deposited in the herbarium of the College of Sciences and Arts at Al-Rass, Qassim University, Saudi Arabia. The authentication process was carried out by botanists affiliated with the Department of Science Laboratories within the College of Science and Arts at Al-Rass, Qassim University, Saudi Arabia.

2.2. Study region climate

The town of Rass has a typical desert environment, known for its chilly winters and hot and arid summers, with minimal humidity. The average winter temperature is between −3 and 21 °C. The hottest months are June, July, August, and September. In summer, the temperature can reach severe levels (between 39 and 50 °C).

2.3. Plant extraction

The leaves of the plant samples underwent a thorough cleaning process, initially being flushed with tap water and subsequently with distilled water. Following this, leaves were air-dried for three days in a dark environment at room temperature. Dried leaves were finely powdered using a commercial blender and subjected to percolation in warm distilled water, allowing them to rest overnight at room temperature (25–35 °C). A conventional method, steam distillation, was employed to collect the essential oils (Aziz et al., 2018). The extraction process involved utilizing a simple distillation system and a separating funnel to extract essential oils from the percolate. The resulting essential oils were collected in high-quality glass sample tubes.

2.4. Gas chromatography–mass spectrometry (GC–MS) analysis

The chemical composition of essential oil samples was analyzed using a GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 μm film thickness). The column oven temperature was initially held at 60 °C, increased by 5 °C/min to 250 °C, followed by a 2 min hold, and finally increased to 300 °C at 30 °C/min. The injector temperature was kept at 270 °C. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The solvent delay was 4 min, and 1 μL of diluted samples were injected automatically using Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of 50–650 m/z in full scan mode. The ion source and transfer line were set at 200 °C and 280 °C, respectively. The components were identified by comparison of their mass spectra with those of WILEY 09 and NIST14 mass spectral databases [21,22].

2.5. Antioxidant activity assays

2.5.1. DPPH free radical scavenging test

The method developed by [23,24] was utilized to determine how effective the extracts were in neutralizing the free radical DPPH. The reaction was carried out in a 96-well microplate using 40 µL of each extract at a range of concentrations and 160 µL of a solution of the radical DPPH that was dissolved at 1 mM in methanol. The mixture was incubated at room temperature and in the dark for 30 min. The absorbance was determined with a microplate reader at 517 nm. Trolox was chosen as the standard for this research. The samples' capacity to eliminate DPPH was evaluated based on their inhibition percentages, which were calculated using the following equation:

$$\text{Inhibition (\%)} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/(\text{Abs}_{\text{control}}) \times 100 \quad (1)$$

Graphical calculations were performed using the curve of inhibition percentages at various concentrations to obtain the IC50 values. An IC50 value is defined as the concentration of the sample (in µg/mL) that results in a 50% reduction of the radicals.

2.5.2. Ferric reducing ability (FRAP) assay

The FRAP assay was carried out according to the method of [25] with slight modifications. Briefly, 190 µL of freshly prepared TPTZ reagent (300 mM acetate buffer, pH = 3.6, 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$, in a ratio of 10:1:1 v/v, respectively) was mixed with 10 µL of sample in a 96-well plate (n = 6). The reaction was incubated at room temperature for 30 min in the dark. At the end of the incubation, the resulting blue color was measured using the microplate reader FluoStar Omega at 593 nm. Data is represented as mean ± SD. The ferric reducing ability of the samples is presented as a µM TE/mg sample using the linear regression equation extracted from the calibration curve (linear dose-response curve of Trolox).

For the Trolox standard for FRAP assay, a Trolox stock solution of 5 mM in methanol was made, and 10 serial dilutions of 4000, 3000, 2000, 1000, 800, 600, 400, 200, 100, and 50 µM were made.

2.6. Microorganisms

This investigation included a total of four bacterial strains that have been identified as standards. These bacteria include two Gram-positive strains (*Staphylococcus aureus* ATCC BAA-1026 and *Bacillus cereus* ATCC 10876) and two Gram-negative strains (*Pseudomonas aeruginosa* ATCC 10145 and *Escherichia coli* ATCC 9637). The bacterial isolates were obtained from the stock culture kept at the Department of Laboratory Sciences, College of Sciences and Arts at Al-Rass, Qassim University, Saudi Arabia.

2.7. Antibacterial activity assay

The antibacterial efficacy of the essential oils extracted from *Juniperus procera* and *Cinnamomum camphora* leaves was assessed through the modified agar-well diffusion assay, as described by Abdellatif et al. [26] with minor modifications. In brief, bacterial strains were sub-

cultured and adjusted to 0.5 McFarland turbidity (10^6 CFU/mL) prior to the antibacterial screening. Chloramphenicol (2.5 mg/mL), a traditional antibiotic, served as the positive control. Autoclaved nutrient agar was dispensed into 25 mL glass bottles, which were then placed in pre-sterilized Petri plates and allowed to solidify at room temperature. Wells were created on the plate surface using a sterile 6-mm cork borer. The agar plates were coated with 100 µL of the adjusted bacterial culture (10^6 CFU/mL). Subsequently, 50 µL of the essential oil samples (100% v/v) and the positive control were added to separate wells. Another well on the same plate received 50 µL of chloramphenicol (2.5 mg/mL). Autoclaved distilled water was poured into a well on some plates as a negative control. The loaded plates were then incubated overnight at 35 ℃. After incubation, the inhibitory zone diameter, measured in millimeters, was employed as an indicator of antibacterial activity. The mean of two replicates (in mm ± standard deviation) was calculated. Antibacterial activity was classified as weak if the zone was less than 10 mm, moderate between 10 and 13 mm, and strong if exceeding 13 mm.

2.8. Insect rearing

The *Aphis craccivora* specimens utilized in the study were initially obtained from a field of broad bean (*Vicia faba)* in Assuit Governorate, Upper Egypt. They were then transferred to the Insect Research Laboratory at Assuit, Plant Protection Institute, Agricultural Research Center (ARC). An *A. craccivora* stock culture was cultivated on broad beans under controlled conditions. The temperature was maintained at 20 ± 1 ℃, with a photoperiod of 16 hours of light and 8 hours of darkness, and a relative humidity of 65% ± 5%. For all trials, the insects were placed on newly grown broad bean plants that were cultivated in miniature pots measuring 8 cm in diameter. Each pot had only one plant. The insects were individually housed in glass cylinders that were 10 cm in diameter and 22 cm long. The tops of the cylinders were covered with muslin, which was secured with rubber bands.

2.9. Insecticidal activity

The insecticidal activity of *J. procera* and *C. camphora* on adult *A. craccivora* was assessed using a leaf dipping bioassay [27–29]. Six concentrations of *J. procera* (1000, 2000, 4000, 6000, 8000, 10,000 ppm) and eight concentrations of *C. camphora* (1000, 2000, 4000, 6000, 8000, 10,000, 12,000, 14,000 ppm) were used. The essential oils were produced with the addition of two drops of Triton X-100 as an emulsifier. Twenty mature aphids were meticulously moved onto a bean leaf disc placed on a sterile Petri dish with a moist filter paper. Precautions were taken to ensure the safety of the aphids during their transfer to the leaf discs. Once the aphids had settled on the leaf disc, they were immersed in essential oils of different concentrations for 10 s. Then, the leaf discs with aphids were left at room temperature for 10 min to dry. A double layer of muslin cloth was placed on each Petri dish, which was covered with a manually perforated lid to avoid suffocation while prohibiting escape of the aphids. The negative control treatment was prepared using distilled water and Triton X-100 (1%) without essential oils. Distilled water was used as positive control. All Petri dishes were incubated under controlled conditions of 20 ± 1 ℃ and 65% ± 5% relative humidity with a photoperiod of 16/8 h light/dark. The number of dead aphids was counted after 24 h. Dead aphids were identified by gently striking the pest with a small brush and observing any movements of legs or antennae. Three replicates for each concentration were performed. Percent mortality was calculated. The LC_{50} and LC_{95} values and slopes were calculated by Probit analysis [30].

2.10. Life table study

In order to examine the effects of *J. procera* and *C. camphora*'s essential oils on the life cycle of cowpea aphids, recently hatched wingless females (< 24 h) were isolated on Petri dishes. A group of adult aphids was subjected to a sublethal concentration of *J. procera* (2552.41 ppm) and *C. camphora* (6213.94 ppm) using the leaf dipping technique. Some groups were treated with water alone and considered the positive control, and others with distilled water + Triton X-100 (1%) and considered the negative control. Every treatment started with a cohort of 30 wingless female insects, and the broad bean leaves were replenished as necessary every 2 days. Petri dishes were positioned in an incubator set at 20 ± 1 ℃, with a photoperiod of 16/8 h light/dark and a relative humidity of 65% ± 5%. Subsequently, the aphid adults who received treatment were examined daily throughout their entire lifespan to document the number of offspring produced per adult until the death of all adults. Adult life span, fecundity, and daily reproduction on each treatment were recorded. Data were subjected to statistical analysis using an F-test, and means were compared according to Duncan's multiple range test of significances at a 0.05 level of probability. The collected data were utilized to compute various features of the fecundity table as per Birch, 1948 [31]. The calculations were performed using the QBASIC software, as described by Jervis and Copland, 1996 [32].

1. Net reproductive rate (R_0) = $\Sigma\, l_x.m_x$.
Number of females (apterae) offspring that replace each female (aptera) of the previous generation.
2. Generation time (GT) = $\Sigma\, l_x.m_x$. X / $\Sigma\, l_x.m_x$.
Mean time from birth of apterous to birth of offspring;
3. Population doubling time (DT) = Log e $2/r_m$;
Days for the population to double.
4. Intrinsic rate of natural increase (r_m) = Log e R_0/T;
Difference in birth rate and death rate in a population with a stable age distribution.
5. Finite rate of increase (λ). = anti log rm;
Number of individuals added to the population per aptera per day.
6. (X) Age of individuals in days.
7. (L_x) Number of individuals alive at age (x) as a proportion of one;
8. (m_x) = Number of female offspring produced per female at age intervals X.

2.11. Statistical analysis

Experimental results are provided as the mean value ± standard deviation (SD). The statistical analysis was performed utilizing IBM SPSS Statistics software version 23. One-way analysis of variance was used to make mean comparisons. A significance level of $p < 0.05$ was used to determine statistical significance.

3. Results

3.1. Antibacterial capacity

The antibacterial efficacy of the essential oils extracted from *Juniperus procera* and *Cinnamomum camphora* leaves was assessed through the determination of inhibition zones against

various bacterial strains. Comparative analysis of the results, specifically the zone of inhibition, was conducted between the bacterial strains and the control (chloramphenicol 2.5 mg/mL). The findings, detailed in Table 1 and Figure 1, indicate that both essential oils demonstrated notable antibacterial activity against all tested organisms, with recorded zones of inhibition exceeding 13 mm. The sensitivity levels varied, and statistical significance ($p < 0.05$) was observed, highlighting the substantial efficacy of these essential oils. In detail, *J. procera* exhibited higher antibacterial activity than *C. camphora*, with results comparable to the reference antibiotic. Gram-positive bacteria displayed greater susceptibility than Gram-negative bacteria. The most susceptible bacterium to *J. procera* essential oils was *S. aureus* (30.5 ± 0.70 mm), followed by *B. cereus* (34.5 ± 0.70 mm), *E. coli* (28.5 ± 0.70 mm), and *P. aeruginosa* (24.0 ± 1.41 mm). The most susceptible bacterium to the essential oils of *Cinnamomum camphora* was *B. cereus* (19.5 ± 0.70 mm), followed by *S. aureus* (15.0 ± 0.0 mm), *E. coli* (15.0 ± 0.70 mm), and *P. aeruginosa* (12.5 ± 0.70 mm).

Table 1. Assessment of antibacterial activity of essential oils from leaves of *Juniperus procera* and *Cinnamomum camphora*.

Bacterial strain	Mean zone of inhibition (mm)*		
	J. procera EO (100% v/v)	*C. camphora* EO (100% v/v)	Chloramphenicol (2.5 mg/mL)
Staphylococcus aureus	30.5 ± 0.70	15.0 ± 0.0	34.5 ± 0.70
Bacillus cereus	34.5 ± 0.70	19.5 ± 0.70	33.5 ± 0.70
Escherichia coli	28.5 ± 0.70	15.0 ± 0.70	27.6 ± 2.12
Pseudomonas aeruginosa	24.0 ± 1.41	12.5 ± 0.70	13.5 ± 0.70

*Results are expressed as mean ± standard deviation (SD) from two independent measurements, encompassing the inhibition zone diameter using a 6 mm well. The well was filled with 50 µL of the tested compound.

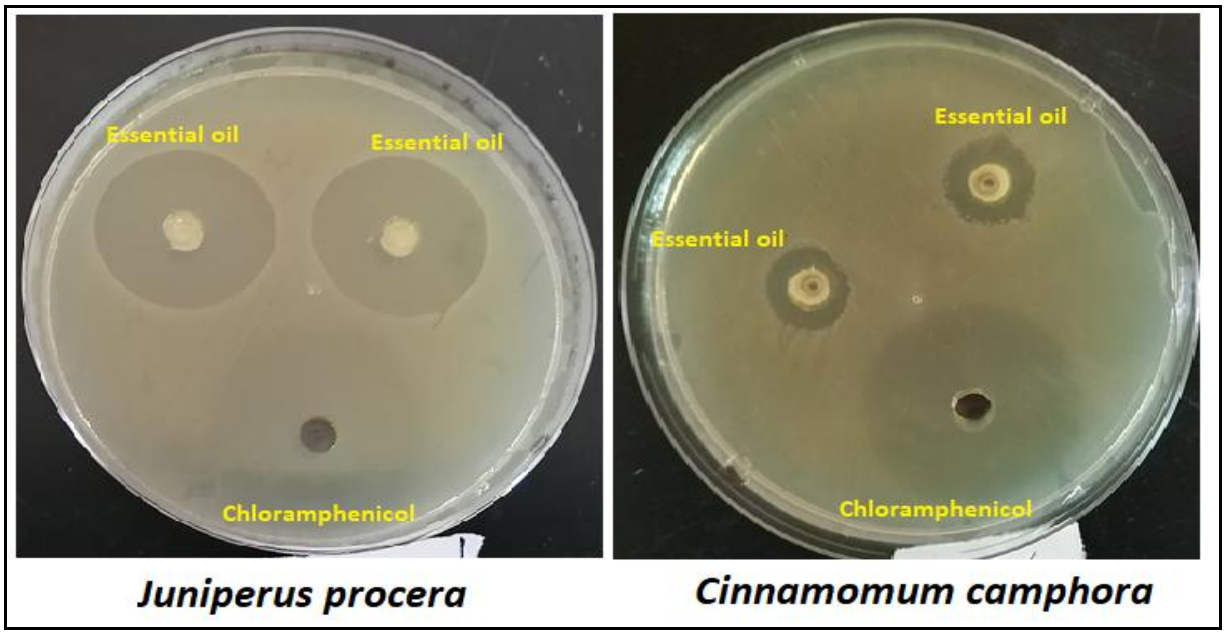

Figure 1. Representative photo comparing both essential oils against *E. coli.*

3.2. Toxicity of essential oils against *A. craccivora*

Table 2 demonstrates the efficacy of the essential oils utilized in this study against *A. craccivora*. Particularly at higher concentration levels, there was a corresponding rise in the mortality rate. The mortality rate at the highest concentration of *J. procera* (10,000 ppm) was 98.78%. With *C. camphora* at the highest concentration (14,000 ppm) there was a 96.67% mortality rate. The mortality rate at the lowest concentrations (1000 ppm) of *J. procera* and *C. camphora* was 16% and 3.39%, respectively. The LC_{50} and LC_{95} values for *J. procera* were 2552.41 ppm and 8320.24 ppm, respectively (Table 2 and Figure 2A and 2B). For camphor oil, the LC_{50} and LC_{95} values were 6213.94 ppm and 14887.53 ppm, respectively. These results prove the higher efficiency of *J. procera* compared to *C. camphora*.

Table 2. Toxicity of *J. procera* and *C. camphora* in adults of *A. craccivora.*

Tested oils	Concentration (ppm)	Mortality (%)	Lethal concentration		Slope ± SE	Regression equation
			LC_{50}	LC_{95}		
J. procera	10,000	98.78	2552.41	8320.24	3.94 ± 0.15	Y = −15.11 + 3.94X
	8000	88.89				
	6000	78.57				
	4000	65.91				
	2000	20.83				
	1000	16				
C. camphora	14,000	96.67	6213.94	14887.53	3.03 ± 0.00797	Y = −10.52 + 3.03X
	12,000	81.25				
	10,000	71.67				
	8000	62.50				
	6000	37.70				
	4000	12.50				
	2000	8.06				
	1000	3.39				

3.3. Effect of tested essential oils on adult longevity and fecundity

Figure 2 shows that there were no significant variations in adult longevity across treatments during the pre-reproductive and post-reproductive stages of *A. craccivora*. There are notable variations in the reproductive time of cowpea aphids when treated with essential oils compared to control treatments. The mean time to give birth by adults in the control (+), control (−), *C. camphora* and *J. procera* groups was 16.80, 15.53, 5.04, and 1.33 days, respectively. This suggests that the essential oils being evaluated have an impact on the reproductive organs of the insect, resulting in a shorter reproductive time compared to the control treatments. Longevity followed the same trend: adults treated with *J. procera* and *C. camphora* lived much less (2.11 ± 1.02 and 5.55 ± 3.60 days, respectively) than those in control (+ and -) treatments (17.47 ± 6.01 and 16.27 ± 5.74 days, respectively). There was no significant difference in the longevity of the cowpea aphid when treated with the tested essential oils. The number of offspring per female was significantly different between

the control (+), control (-), *C. camphora,* and *J. procera* treatments: 54.40 ± 16.79, 52.40 ± 20.08, 9.22 ± 7.01, and 3.28 ± 1.87 nymphs/female, respectively (Figure 2).

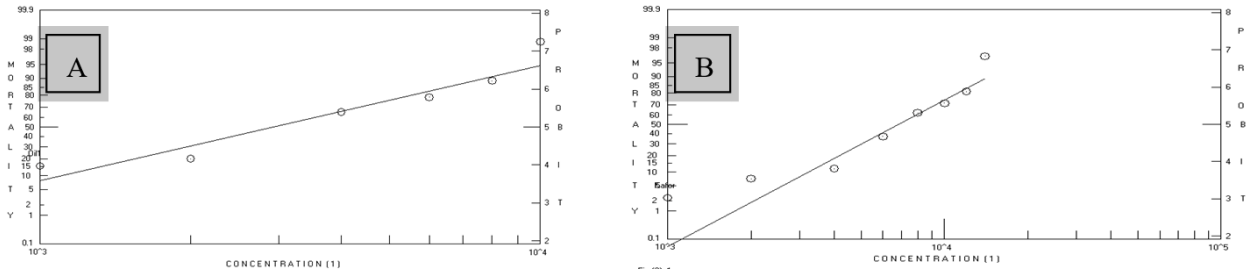

Figure 2. Relationship between the probit-transformed mortality percentages and the log concentrations (%) of *J. procera* (**A**) and *C. camphora* (**B**) against adult *A. craccivora*.

3.4. Sublethal effects of essential oils on the life table parameters of *A. craccivora*

The effects of the two essential oils on the biological parameters of *A. craccivora* were compared to those of the control groups (Table 3). *J. procera* and *C. camphora* treatments led to reduced R_0 (1.75 and 3.52 nymphs/female, respectively) in comparison with 60.40 and 52.35 nymphs/female in the control (+) and control (−) treatments, respectively. Also, *A. craccivora* had the minimum R_0 when treated with *J. procera* essential oils. Doubling time (DT) and mean generation time (GT) were affected by essential oils (Table 2). The r_m value was reduced (0.0608 and 0.1018 day^{-1}) for *J. procera* and *C. camphora*, respectively, when compared with the control (+) (0.2369 day^{-1}) and control (−) groups (0.2336 day^{-1}). Both *J. procera* and *C. camphora* reduced the aphid's finite rates of population increase (1.0627 and 1.1072 day^{-1}, respectively), compared with the control treatments (Table 2). This indicates that a population of ten wingless cowpea aphids might grow to approximately 16, 20, 52, and 51 individuals when treated with *J. procera*, *C. camphora,* control (+), and control (−), respectively, within a one-week period.

The effect of sublethal concentrations of the tested essential oils on age-specific survivorship (lx) and age-specific fecundity (m_x) of *A. craccivora* was compared with control treatments (Figure 3). It could be noted that the survivorship (lx) for *A. craccivora* females was lower when treated with *J. procera* and *C. camphora* than in control treatments. The survival patterns declined to 50% after 1 and 2 days of treatments with *C. camphora and J. procera*, respectively, whereas it took about 20 days in both control treatments. Also, age-specific fecundity per day (m_x) of cowpea aphids was the highest on the control (+ and -) treatments (5.86 and 5.92 females/female/day, respectively) on the seventh day. However, when treated with *J. procera,* the maximum reproduction rate per female per day (m_x) reached 1.47 females/female/day on the first day; after that, the population was still alive for 6 days without any births (Figure 4C). The m_x value reached 3.36 females/female/day on the second day after *C. camphora* treatment, as shown in Figure 4D. Furthermore, the age-specific fecundity curves (m_x) indicated that the reproductive phase of *A. craccivora* persisted for about 22 days under normal conditions, whereas it lasted for just 6 and 13 days under sublethal concentrations of *J. procera* and *C. camphora*, respectively (Figure 4A, B, C, and D). It can be inferred that the reproductive period of females is shortened following the use of essential oils.

3.5. Chemical composition of *Juniperus procera* and *Cinnamomum camphora*

GC-MS was used for essential oil identification of *J. procera* and *C. camphora* (Table 4). The major components identified in *J. procera*'s essential oils were α-pinene (9.08%), myrcene (14.60%), endo-Borneol (16.42%), and 1,8-cineole (14.40%). *C. camphora*'s essential oils showed high concentrations of α-pinene (16.56%), trans-Pinocarveol (11.54%), and 1,8-cineole (34.40%). As such, these essential oils mainly consist of mono- and sesquiterpenes, while oxygenated derivatives are only minor constituents.

3.6. DPPH radical scavenging activity

The capacity of essential oils of *J. procera* and *C. camphora* to serve as antioxidants was evaluated using the DPPH method (Table 5). The free radical DPPH reacts with an odd electron, producing a significant absorbance at 517 nm and a purple color. An FRS antioxidant combines with DPPH to generate DPPH-H, which has a lower absorbance, as the number of electrons absorbed increases. Decolorization has a considerable effect on reducing capacity. The essential oils of *J. procera* and *C. camphora* showed free radical scavenging activity, with an IC50 value of 44.46 ± 0.68 and 64.78 ± 0.98 µg/mL, respectively, compared with that of Trolox 21.14 0.89 µg/mL. The results of previous in vitro studies on the antioxidant activity of *J. procera* and *C. camphora* support our findings [28,29].

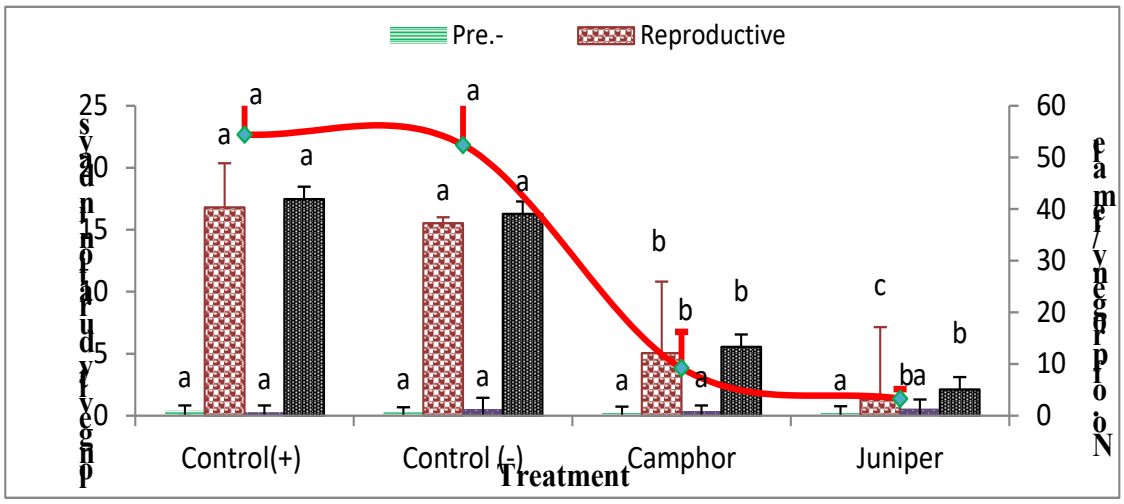

Figure 3. Mean durations (days) and fecundity (progeny/female) of the adult stage of *A. craccivora* at different treatments. Data are presented as mean ± SE; different letters are significantly different at $p < 0.05$.

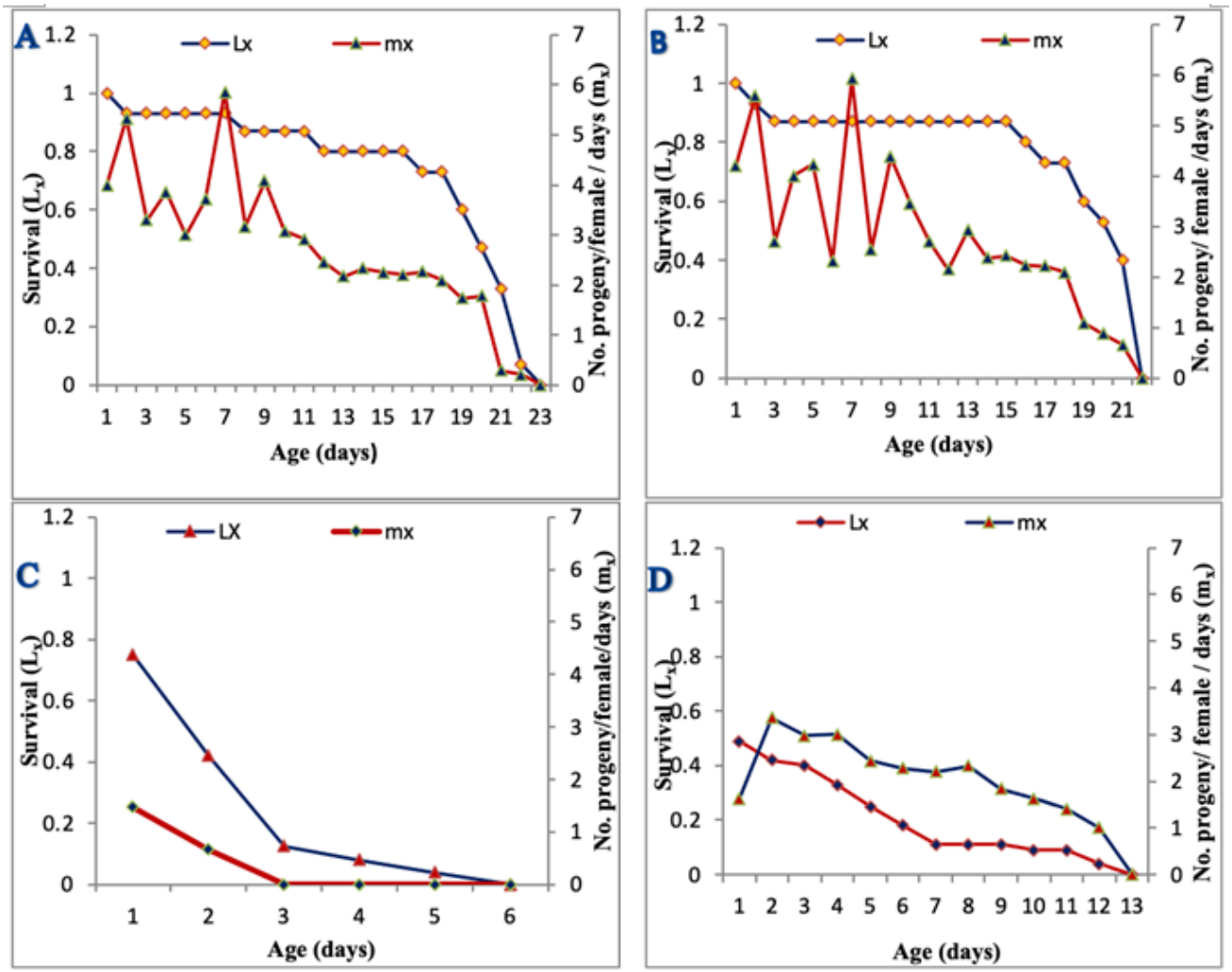

Figure 4. Population age-specific survival rate (l_x) and age-specific fecundity (m_x) of *A. craccivora* in the control (+ and −) populations (A and B) and exposed to sublethal concentrations of *J. procera* (C) and *C. camphora* (D).

Table 3. Life table statistics of *A. craccivora* treated with *J. procera* and *C. camphora* essential oils compared with control treatments.

Tested oils	(GT)	(DT)	(R_0)	Rate of increase	
				Intrinsic (r_m)	Finite (λ)
Juniper	9.20	11.40	1.75	0.0608	1.0627
Camphor	12.36	6.81	3.52	0.1018	1.1072
Control (+)	17.31	2.92	60.40	0.2369	1.2673
Control (-)	16.94	2.97	52.35	0.2336	1.2631

(GT) = Mean generation time, (DT) = doubling time, (R_0) = net reproductive rate, (r_m) = intrinsic rate of increase, and (λ) = finite rate of increase.

Table 4. Chemical compositions of the essential oils of *Juniperus procera* and *Cinnamomum camphora.*

Chemical compounds	RT	RI	*J. procera* %	*C. camphora* %
α-pinene	4.73	931	9.08	16.56
Camphene	4.97	935	0.47	1.00
o-Cymene	6.59	1025	0.9	1.32
β -Myrcene	6.52	979	14.60	-
1,8-cineole	6.67	1032	14.40	34.40
Fenchol	8.80	1100	0.2	1.30
trans-Pinocarveol	9.42	1152	2.5	11.54
Pinocarvone	9.79	1164	-	2.29
endo-Borneol	10.15	1151	16.42	2.23
4-Thujanol	10.51	1090	0.91	1.09
Alpha- Terpinen-4-ol	10.86	1261	2.91	3.05
p-Mentha-1,4(8)-dien-7-ol	11.2	1283	0.02	0.30
α -Copaene	15.51	1397	-	0.33
Caryophyllene	17.17	1419	4.5	2.16
Isoaromadendrene	17.68	1590	1.80	2.39
Humulene	17.99	1667	-	0.63
Muurolene	18.55	1642	3.5	1.18
beta-Cubebene	18.86	1545	-	2.15
Epicubenol	18.97	1623	2.88	2.84
cis-Calamenene	19.52	1537	-	2.73
Cadina-1(10), 4-diene	19.95	1524	-	1.74
Epiglobulol	20.44	1530	1.3	2.25
(-) Spathulenol	20.73	1569	2.80	-
Caryophyllene oxide	20.82	1576	0.04	2.5
α-Acorenol	22.98	1577	3.76	0.46

Table 5. IC50 (μg/mL) and EC50 (μg/mL) values of essential oils of *J. procera* and *C. camphora*.

Essential oil	*J. procera*	*C. camphora*	Torolox*
DPPH IC50 (μg/mL)	44.46 ± 0.68	64.78 ± 0.98	21.14 ± 0.89
FRAB EC50 (μg/mL)	61.64 ± 2.6	71.65 ± 2.1	24.42 ± 0.8

3.7. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is a straightforward assay that may be used to determine the antioxidant activity of a sample. The FRAP method was used to test the antioxidant capacity of the tested essential oils by reducing the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}). The results obtained showed that *J. procera* presented the highest reduction capacity, with an EC50 of 61.64 ± 2.6 μg/mL, followed by *C. camphora*, with an EC50 of 71.65 ± 2.1 μg/mL. However, the reducing capacity of Torolox was 24.42 ± 0.8 μg/mL (Table 5). All essential oils exhibited a certain level of Fe^{3+} reduction ability, and this increased as the essential oil concentration increased.

4. Discussion

J. procera and *C. camphora* are two widely distributed plants with a variety of biological properties, such as antibacterial, anticancer, antioxidant, and insecticidal effects [33,34]. In this work, essential oils were extracted using hydrodistillation methods and identified by gas chromatography–mass spectrometry (GC–MS). This analysis showed the prominence of important chemicals, including α-pinene, myrcene, α-terpineol, endo-Borneol, and 1,8-cineole. The essential oils of *J. procera* plants obtained from Kenya were also found to contain α-pinene, myrcene, camphor, trans-geraniol, eugenol, and α-terpineol [35]. On the other hand, camphor, linalool, and alpha-terpinen-4-ol were the main constituents of the essential oils of *C. camphora*, and their concentrations were not significantly different from those of compounds isolated from the leaves of the same plant grown in Chania (*D*-camphor and cineole); *D*-camphor and linalool were also isolated from the same plant [36]. In summary, mono- and sesquiterpene hydrocarbons make up the majority of the essential oil's constituents. This is in line with previous findings where the major compounds were camphor, α-pinene, and limonene [37]. Camphor exhibits several biological properties, including insecticidal, antibacterial, antiviral, anticoccidial, antinociceptive, anticancer, and antitussive effects [38]. The study of the antimicrobial and larvicidal activity of the essential oils was conducted to evaluate the potential role of essential oils as antimicrobial and larvicidal and to correlate the role of specific chemical constituents with these activities.

The antimicrobial efficacy of essential oils is attributed to their abundance of active phytochemical molecules, prompting extensive exploration in recent years for antibacterial applications. Many essential oils have demonstrated significant antibacterial potential, leading to recommendations for their use as food additives. Moreover, these oils have shown minimal or no side effects upon consumption [39]. Our findings were consistent with the results reported in earlier studies; *Juniperus excelsa*, recognized as a synonymous term for *J. procera*, demonstrated robust antibacterial efficacy against *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus durans*, as well as three strains of *Mycobacterium* spp. The observed results were comparable to the antibacterial effects exhibited by chloramphenicol and streptomycin [40]. The essential oils derived from *Juniperus procera* (syn. *Juniperus excelsa*) were assessed for their antibacterial activity against thirteen bacterial species, demonstrating noteworthy efficacy primarily against Gram-positive bacteria in comparison to Gram-negative species. The observed antibacterial activity of these essential oils may be attributed to the presence of α-pinene, limonene, and sabinene, known for their antibacterial properties [41]. The variation in sensitivity observed between Gram-positive and Gram-negative bacteria can be attributed to structural differences, particularly in the cell walls. Gram-positive bacteria, in contrast to Gram-negative bacteria, exhibit greater sensitivity to numerous antimicrobial phytochemical compounds present in plants. The relative resistance of Gram-negative bacteria can be attributed to the presence of the lipopolysaccharide layer and periplasmic space in their cell structure [42]. Our results regarding the antibacterial capacity of *C. camphora* are in agreement with previous reports, which found good antibacterial activity against *Staphylococcus aureus, MRSA, Bacillus subtilis, Enterococcus faecalis, Salmonella gallinarum*, and *Escherichia coli* [43]. Significant antibacterial activity against various food-related bacteria, including *Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, Staphylococcus aureus,* and *Bacillus subtilis*, has also been demonstrated [44]. Moreover, essential oils derived from the blend of leaves, branches, and wood of *C. camphora* exhibited potent antibacterial effects against *Serratia marcescens* and

demonstrated significant antifungal activity against *Aspergillus niger* and *Aspergillus fumigatus* [45]. Hence, the essential oils of these two plants are suggested for subsequent comprehensive microbiological and pharmacological examinations in both in vitro and in vivo settings, potentially resulting in the development of new natural antibacterial agents.

Botanical insecticides are natural compounds derived from plants that possess insecticidal capabilities, serving as a superior alternative to synthetic pesticides for crop protection, while mitigating the adverse effects associated with chemical insecticides. Botanical pesticides, including essential oils, flavonoids, alkaloids, glycosides, esters, and fatty acids, possess diverse chemical properties and mechanisms of action, impacting insects through various means such as repellents, feeding deterrents, toxicants, growth inhibitors, chemosterilants, and attractants [46]. These pesticides are deemed safe for pest control due to their minimal or nonexistent pesticide residue, rendering them safe for humans, the environment, and the ecosystem [47]. Essential oils exhibit notable larvicidal effects on *Limantria dispar* larvae, insecticidal activity, repelling properties against ants, cockroaches, bedbugs, and moths, and toxicity to termites [48].

The growing interest in plant-derived essential oils as insecticidal agents arises from the demand for environmentally acceptable, sustainable, and non-toxic alternatives to synthetic pesticides. Essential oils from *J. procera* and *C. camphora* are notably advantageous owing to their robust bioactive constituents, extensive insecticidal efficacy, and minimal environmental persistence [11]. Both *J. procera* and *C. camphora* essential oils comprise monoterpenes (e.g., α-pinene, camphor, eucalyptol) and sesquiterpenes, which demonstrate neurotoxic effects (inhibiting acetylcholinesterase) [49], repellent and antifeedant characteristics (inhibiting insect consumption), and growth inhibition activity (impacting molting and reproduction) [50]. Aromatic plant-derived essential oils have emerged as viable, environmentally acceptable substitutes for synthetic pesticides, owing to their extensive bioactivity, low environmental persistence, and reduced toxicity to mammals. Essential oils from *Laurus nobilis* and *Rosmarinus officinalis* showed significant fumigant toxicity against *Tribolium castaneum* (red flour beetle) [51]. Several investigations have evidenced their efficacy against diverse insect pests, encompassing stored-product beetles, agricultural nuisances, and disease vectors. Essential oils from *Cinnamomum zeylanicum* and *Syzygium aromaticum* have shown larvicidal efficacy against *Aedes aegypti* (dengue mosquito) [52], and stevia leaf extract exhibits aphicidal action against *Spodoptera frugiperda* [53]

Our research indicates that the death rate of *A. craccivora* increased as the concentration of essential oils increased. This result agrees with Karunamoorthi et al., 2014 [54], who evaluated the larvicidal activity of *J. procera* against late third-instar larvae of *Anopheles arabiensis*. They found that larval mortality increased as the concentration of essential oil increased. In another work, the essential oils of four *Juniperus* species had notable repellent and insecticidal effects on aphid species *Rhopalosiphum padi* (bird cherry-oat aphid) and *Sitobion avenae* [55]. Almadiy and Nenaah (2022) showed that *J. procera* has remarkable pest control potential against the khapra beetle [56]. Sobhi et al. (2020) [57] investigated camphor's essential oils against fourth-instar larvae of *Spodoptera littoralis*, showing considerable toxicity with an LC_{50} value of 20,000 ppm [58]. Moreover, Fergani et al. [59] recorded that *C. camphora* expressed noticeable toxicity against the fifth-instar larvae of *S. littoralis* after 48 hours. Both lethal and sublethal concentrations of essential oils greatly decrease the proportion of surviving larvae, as well as their ability to produce offspring [60]. Esmaeily et al. (2017) [61] showed that sublethal concentrations of essential oils had a negative effect on biological characteristics of *Tetranychus urticae* Koch (Acari: Tetranychidae), including development time,

fecundity, mortalities at different life stages, and adult emergence. The data support our findings that the sublethal effects of *J. procera* and *C. camphora* decreased the lifespan and reproductive capacity of the adult insects, in comparison to the control group. Furthermore, our investigation determined that the EOs induced alterations in the life table characteristics of *A. craccivora* when compared to the control group. A sublethal concentration of the tested oils led to a decrease in generation time (GT), net reproductive rate (R_0), intrinsic rate of increase (r_m), and finite rate of increase (λ). However, the doubling time (DT) increased in groups treated with essential oils more than in the control groups. These results indicated that sublethal concentrations of *J. procera* and *C. camphora*'s essential oils could disturb biological indices of *A. craccivora*. This result agrees with those of Esmaeily et al. [62], who showed that plant oils decrease the net reproductive rate, intrinsic rate of increase, and finite rate of increase and significantly increase the doubling time of *T. urticae* in comparison with a control.

Elsheikh et al. [63] assessed the pesticidal efficacy of *J. procera* stem extracts on the first-instar larvae of *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) to mitigate their proliferation. These extracts demonstrated significant impacts on the fecundity, fertility, and sterility of adult female *C. albiceps* as a result of larval therapy

The majority of pesticides employed in agriculture, whether synthetic or biological, depend on the neurotoxic effects of various chemicals on the target organisms. Essential oils function as insecticides via multiple mechanisms, including the overproduction of reactive oxygen and nitrogen species (RONS), inhibition of acetylcholinesterase (AChE), and alterations in the enzymatic activity of catalase (CAT) and glutathione S-transferase (GST) [64]. Acetylcholinesterase (AChE) is an essential enzyme for the neurological system of most animals, including insects [65]. The constituents of essential oils, such as terpenes, terpenoids, and phenylpropanoids, influence AChE activity. Their insecticidal impact is demonstrated through the suppression of enzyme activity. This inhibition results in the buildup of ACh at the synapses [66], leading to heightened neural excitation and finally culminating in the insect's demise. The essential oil of *Artemisia nakaii* Pamp. (Asteraceae) exhibited AChE inhibition potential on *Spodoptera litura* [67].

5. Conclusions

The current study sought to confirm the traditional uses of the essential oils of *J. procera* and *C. camphora* collected from the Qasuim region by examining their antioxidant, antimicrobial, and aphicidal properties. Both essential oils demonstrated high levels of antioxidant activity using two different methods: DPPH and FRAB. The main components (α-pinene, myrcene, α-terpineol, endo-borneol, and 1,8-cineole) are associated with significant potential for antioxidant activity. Both essential oils showed significant antibacterial effectiveness against all examined species, according to an antimicrobial analysis. Our results suggest that *C. camphora* and *J. procera* may be useful aphicidal agents against *A. craccivora*. However, further field research is needed to determine the effects of these essential oils on aphids.

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Author contributions

All authors searched in the literature, wrote, reviewed, and approved the final version of the manuscript equally.

Conflicts of interest

The authors declare no conflict of interest.

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