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Chemical Profiling and Pharmaceutical and Biological Activities of Methanolic Extract of *Citrullus colocynthis* L. Seeds Collected from the Arid Zone of Qassim, Saudi Arabia against *Aphis craccivora*

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Abstract

Background: *Citrullus colocynthis* L. (*C. colocynthis*) is a medicinal plant with a long history of traditional usage in Saudi Arabia, particularly for the treatment of digestive issues such as indigestion and stomach pain.

Methods: *C. colocynthis* seeds were examined using qualitative and quantitative phytochemical methods. Antioxidant testing was conducted using DPPH assay and the reducing power test. Antibacterial properties were evaluated using the well-diffusion test, minimum inhibitory concentration (MIC) test, and minimum bactericidal concentration (MBC) test. The effectiveness of the extract against *Aphis craccivora* (*A. craccivora*) was assessed using a leaf-dip bioassay, measuring generation time (GT), net reproduction rate (R_0), intrinsic rate of increase (rm), doubling time (DT), and finite rate of growth (λ).

Results: The methanolic extract of *C. colocynthis* seeds included diverse bioactive compounds, a good quantity of phenolic content (60.45 mg GAE/g) and flavonoid content (46.66 mg/g). The antibacterial tests showed that the extract was effective only against *Staphylococcus aureus* and *Proteus mirabilis*. The extract recorded inhibition zones of 14.5 ± 0.7 mm and 10.5 ± 0.5 mm for *Staphylococcus aureus* and *Proteus mirabilis*, respectively. *Staphylococcus aureus* showed the greatest sensitivity, with MIC of 6.25 mg/mL and MBC of 25.0 mg/mL. The extract was found to have a fatal concentration (LC_{50}) of 9.02% and a lethal concentration (LC_{95}) of 20.50% against *A. craccivora* aphid.

Conclusions: The current *vitro* study on *C. colocynthis* reported that the seeds are effective as antioxidants, antibacterial agents (only against Gram-positive bacteria), and aphicidal agents against aphids. More investigations are recommended to examine the possible toxicity and biochemical interactions *in vivo*.

Introduction

Aphis craccivora Koch (Hemiptera: Aphididae) is a widely distributed pest that feeds on a variety of plants. It has been documented to infest 50 different host plants belonging to 19 different families. This pest poses a significant danger to leguminous plants worldwide [1,2]. The nymphs and adults extract the sap from the leaves, flowers, and pods of cowpea plants using suction. In addition, the aphid serves as a vector for plant viruses and has a negative impact on crop productivity [3]. Plant extracts and their formulations are typically more environmentally friendly, cost-effective, and less persistent than synthetic pesticides. Also, plant compounds generally have fewer side effects for human consumption (except for toxic plants), and some plant secretions have shown noticeable insecticidal activity [4,5].

Citrullus colocynthis (L.), sometimes referred to as bitter apple, is a medicinal plant that is widely distributed in the Mediterranean area. It belongs to the Cucurbitaceae family. The fruits have been used in traditional medicine for a considerable period of time owing to their diverse therapeutic characteristics. This includes anti-inflammatory, analgesic, antibacterial, anticancer, and antidiabetic activities [6,7]. Cucurbitaceae family is mostly able to tolerate abiotic stresses such as drought [8]. *C. colocynthis* is an annual plant species, has a fleshy root and able to climb on other bushes. The individual plant produces about 15 to 30 fruits. The fruits of *C. colocynthis* are round with a diameter ranging from 7 to 10 cm. These fruits are greenish with yellow stripes, which become yellowish when dry [9,10]. The seeds of *C. colocynthis* are rich oil content, comprising around 50% of the total constituents. The seeds of *C. colocynthis* are abundant in essential nutrients like protein, carbs, ash, and fiber. The oil production of *C. colocynthis* seeds are similar to that of oilseeds such as sunflower and safflower, and it surpasses the oil production of soybean and cotton seeds [11]. *C. colocynthis* has some diverse bioactive chemicals, including cucurbitacins, flavonoids, and alkaloids, with various therapeutic benefits. Cucurbitacins are mostly found in the fruits of several plant species and are classified as triterpenoid chemicals. Empirical research has shown that these substances possess anti-inflammatory, analgesic, and anticancer characteristics [12,13]. Furthermore, *C. colocynthis* contains flavonoids that have been scientifically shown to have antioxidants, anti-inflammatory, and antidiabetic effects [14]. In addition, chemicals derived from *C. colocynthis* have shown antibacterial and anti-inflammatory properties [14]. *C. colocynthis* has significant anti-inflammatory properties. Studies have shown that the extract derived from the fruit has the ability to decrease the production of inflammatory cytokines [15]. The fruits of *C. colocynthis* have antitumor and anticancer properties by stimulating apoptosis, a mechanism of programmed cell death, in cancerous cells [16]. Antimicrobial drugs have a crucial role in preventing and treating infectious illnesses caused by bacteria and fungus. Antibiotic resistance threatens public health by causing ineffective treatments, lengthier hospital stays, and higher healthcare costs. Thus, innovative antimicrobials that fight drug-resistant bacteria are needed [17,18]. Altering known antibiotics has been the main strategy for developing new antimicrobials, resulting in multiple effective medicines. However, this technology has produced antimicrobial-resistant bacteria, a global problem. Thus, innovative antibacterial medication production strategies are needed [19,20]. Antioxidants are substances that have the capacity to directly neutralize or indirectly impede the creation of pro-oxidant molecules, which are mostly associated with reactive oxygen species (ROS) [4]. There is a significant amount of data suggesting that oxidative stress has a role, to different extents, in the development and/or advancement of several illnesses, including cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular diseases [5]. High antioxidant activity has been demonstrated by many medicinal plants and fruits. The consumption of natural antioxidants has been linked to reduced risks of cancer, cardiovascular disease, diabetes, and other age-associated diseases, but there is still considerable controversy in this area [6].

Traditional methods of pest control have relied heavily on the use of synthetic insecticides, but the overuse of these chemicals has led to the development of insecticide-resistant pests, environmental pollution, and health hazards to humans and non-target organisms. Several approaches have been employed to develop new natural antifeedants. These include the identification of novel compounds from natural sources, the modification of existing compounds to increase their potency, and the use of plant breeding techniques to develop insect-resistant cultivars [7,8]. In general, little is known about the biological activities of the flora of Qassim region, Saudi Arabia. Accordingly, the current study aimed to evaluate the phytochemical, antibacterial, antioxidant and the possible aphicidal activity against *A. craccivora* of the methanolic extract of *C. colocynthis* seeds grown in Wadi Ar Rumah, at Qassim area, Saudi Arabia.

Methods

Plant extract

The dry fruits of *C. colocynthis* were manually collected, and seeds were subsequently isolated from the fruits. The samples were obtained in August 2022 (during the

summer season) from Wadi Ar Rumah in the Qassim region, Saudi Arabia. This dry valley is the largest and longest dry river in the Arabian Peninsula, measuring over 2,000 kilometers in length [9] (Figure 1). The climate in this area is hot and dry during the summer, with temperatures ranging from 36 to 40 °C. The latitude and longitude coordinates for this area are approximately 26.5214° N and 43.9783° E. The seeds were ground into a fine powder using a Moulinex grinding machine from France. The powder obtained was stored in a dark glass bottle at a temperature of 30-35°C until ready for use. To prepare the extract, 50 grams of the powdered material were soaked in 500 mL of 80% methanol (Merck KGaA, Germany) in a tightly sealed opaque container. This mixture was left to macerate for 3 days at 40°C with periodic agitation. Subsequently, the solvent was evaporated to dryness under reduced pressure using a rotary evaporator, leaving behind a residue free of methanol [10]. The dried crude extract (stock extract) was weighed and dissolved into 80% methanol to create a stock concentration of 250 mg/mL. This solution was then refrigerated at 4°C until required.

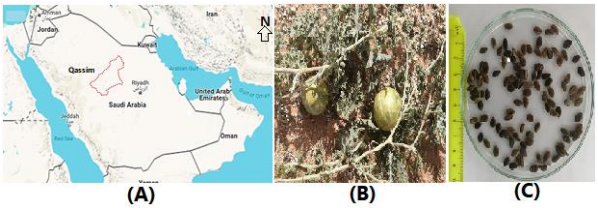

Figure 1: A map delineating the collection region of Citrullus colocynthis within Saudi Arabia. (A): Location of Qassim region, (B): Fruits of *C. colocynthis*, (C): Seeds of *C. colocynthis*. Source of the map: https://www.google.com/maps. Other photos were taken by the authors.

Qualitative phytochemical analysis

To identify the various phytochemical components, a solution of the crude methanolic extract (100 mg/mL) was utilized, and the colorimetric tests described below were employed, as previously documented [11] with minor modifications.

Flavonoids

A positive test for flavonoids was achieved by adding 1 mL of the methanolic extract to 1 mL of a 10% lead acetate solution, resulting in the observation of the formation of a yellow-colored precipitate.

Phenols (phenolic acids)

The presence of phenols (phenolic acids) was confirmed through the addition of 1 mL of the methanolic extract to 0.5 mL of a 10% ethanolic ferric chloride solution, leading to the observation of a blue-green to dark blue coloration for a positive test.

Saponins

For the identification of saponins, 5 mL of pure distilled water was added to a test tube, and then 1 mL of the methanolic extract was added. By stirring the liquid well, we noticed a continual release of gas bubbles, indicating a positive test.

Terpenoids

The terpenoids were identified by mixing 2.5 mL of the methanol extract of the plant with 1 mL of chloroform, then added 1.5 mL of a concentrated sulphuric acid solution. The positive test was confirmed by the visual detection of a reddish-brown color.

Tannins

In order to detect the presence of tannins, 2-3 drops of a 1% lead acetate solution was added to 1 mL of the methanolic extract. As a result, a noticeable shade of deep blue or greenish-grey was identified, suggesting the existence of tannins.

Alkaloids

The alkaloids were identified using a process of mixing 1 mL of the methanolic extract with 1 mL of a 1% hydrochloric acid solution, then gradually heating it until steam began to be released. Afterwards, six drops of Wagner's reagent were added to a one milliliter portion of the acidified extract, leading to the creation of a brownish-red precipitate that indicates a positive result in the test.

Bacterial strains

Five bacterial strains were utilized in this study, comprising one Gram-positive bacterium *Staphylococcus aureus* ATCC BAA-1026, and four Gram-negatives, namely *Escherichia coli* ATCC 9637, *Pseudomonas aeruginosa* ATCC 10145, *Klebsiella pneumoniae* ATCC 27736, and *Proteus mirabilis* (clinical isolate). The bacterial strains were obtained from our laboratory at Qassim University, Saudi Arabia.

Well-diffusion test

The antibacterial activity of the methanol extract of *C. colocynthis* seeds was evaluated using the agar-well diffusion test. The bacteria were loaded into a broth media and incubated overnight. In Sterile plates containing 25 mL of Nutrient Agar,100 microliters of bacterial solution were loaded over the plate, a sterile cotton swab was used to uniformly distribute the bacteria across the agar medium. Furthermore, three wells, each with a diameter of 6 mm, were created on every Petri plate using a sterile corn borer. Two wells were filled with a methanolic extract at a concentration of 200 mg/mL, while a third well was treated with Chloramphenicol at a concentration of 2.5 mg/mL as a reference for its antibacterial activity. Preliminary experiments demonstrated that the solvent, which contained 80% methanol, did not have any inhibitory

impact on the bacteria. The plates were thereafter put in an incubator set at a temperature of 35°C for a period of 18-24 hours. The evaluation of antibacterial activity included measuring the degree of inhibition in millimeters (mm), and the findings were presented as the mean ± standard deviation of three repeated evaluations [12].

Minimum inhibitory concentration test (MIC)

We identified the MIC of the methanol extract of *C. colocynthis* by using a micro-dilution method on 96-well microplates, similar to what Sulieman et al. [12] carried out, but with a few minor modifications. Two-fold dilutions of the extract were made in 5% DMSO. The concentrations of the dilutions ranged from 200 to 0.78125 mg/mL. In order to ensure consistency in the dilutions throughout all wells, a quantity of 100 µL was extracted from the preceding dilution, resulting in each well containing 100 µL. In addition, 95 µL of concentrated nutrient broth and 5 µL of the microorganism being examined were introduced to each well. The microplates were then incubated for 24 hours at 37°C. To evaluate bacterial growth, 40 µL of 0.2 g/mL 2,3,5-triphenyltetrazolium chloride (TTC) was added. This took 30 minutes at 37°C. TTC uses red dye to stain bacterial cells to identify wells with bacterial growth. The lowest extract concentration in microplate wells without bacterial growth was the minimum inhibitory concentration (MIC).

The determination of total phenolics

The Folin-Ciocalteu solution was used to measure the number of total phenols, following the procedure explained by Blainski [12]. Methanol was added to the sample until it reached a value of 10 mg/mL. Then, a method was used to mix 1 mL of the diluted methanol sample with 500 µL of the solution and 6 mL of pure water. A 5-minute incubation time with no changes was followed by the addition of 1.5 mL of 20% Na2CO3 and 1.9 mL of pure water. The mixture was then constantly shaken to ensure even dilution. Afterwards, the solution was placed in darkness and allowed to incubate for a duration of two hours. Subsequently, the material was transferred into plate wells in triplicate using a pipette. Quantitative data was collected at a wavelength of 420 nm using a microplate reader (Fluo Star Omega). The results were quantified in milligrams of gallic acid equivalent per milligram of the extract.

Determination of total flavonoids

The determination of total flavonoid content followed the calorimetric method of Sharma et al. [12] with some modifications. The standard used in this study was quercetin, dissolved in ethanol at concentrations ranging from 0.0625 to 1.0000 mg/mL. A mixture was prepared by combining 125 µL of standard solutions with 375 µL of 75% ethanol, followed by the addition of 25 µL of 10% aluminum chloride. Subsequently, 25 µL of potassium acetate (0.1M) and 700 µL of distilled water were added to the mixture. After vortexing, the solution with various standard concentrations was left undisturbed for 30 minutes at ambient temperature. Subsequently, a volume of 100 µL of the solution was carefully transferred to a 96-well plate, and the absorbance was quantified at 415 nm using a Multiskan GoThermo Fisher microplate reader. The aforementioned methods were duplicated for the specified specimen, and the overall flavonoid content (TFC) was quantified in quercetin equivalent (Q) units, specifically in milligrams per gram of dry weight (mg Q/g DW).

Free radical scavenging assessment

The extract's antioxidant activity was tested by neutralising DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals, per Elsharkawy et al. [13]. To conduct the experiment, 200 µL of extract was mixed with 2 mL of DPPH solution (125 µM in 100 mL of 80% methanol). The resultant combination was then subjected to a 60-minute reaction at room temperature, while being protected from light. Afterwards, the absorbance of the solution was measured at a wavelength of 520 nm. The IC50 values, representing the concentration of the extract needed to neutralize 50% of the radicals, were calculated by linear regression analysis. The readings were denoted in micrograms per milliliter (µg/mL). The below equation was used to compute the percentage of radical absorption:

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

$Abs_{control}$ refers to the absorbance of the reagent combination when no sample or standard is added, whereas Abs_{sample} indicates the absorbance of the mixture when the sample or standard reagent is included.

Reducing power assay

The ferric reduction capability of the extract was assessed using an assay outlined in the procedure described by Priyanka et al. [13] . The mixtures were treated with trichloroacetic acid (2.5 ml, 10%) (TCA) and then centrifuged for 10 minutes at 3000 revolutions per minute after preparation. Distinct concentrations of extracts (1 ml, in triplicate), phosphate buffer (2.5 ml, pH 6.6), and potassium ferricyanide (2.5 ml, 1%) were individually mixed and allowed to incubate at 50°C for 30 minutes. After diluting approximately 2.5 ml of the supernatant and 2.5 ml of water, 0.5 ml of 0.1% ferric chloride (freshly prepared) was added to the mixture. Then, the absorbance was then determined at 700 nm using a spectrophotometer.

Rearing the insect

A population of *Aphis craccivora* (*A. craccivora*) was initially collected from field of broad bean, *Vicia faba* (*V. faba*) in Assuit Governorate, Upper Egypt and transferred to Insect Research Laboratory at Assuit, Plant Protection Institute, Agricultural Research Center (ARC). The culture was consistently maintained on broad beans, *V. faba*, in a controlled environment at a temperature of 20 ± 1°C, with a photo period of 16 hours of light and 8 hours of darkness, as well as relative humidity of 65 ± 5%. This breeding was continued for multiple generations. In each experimental trial, the insects were introduced into freshly planted broad bean plants, which were grown in small pots with a diameter of 8 cm. Each plant was cultivated in its own pot. Following this, the insects were individually enclosed within glass cylinders measuring 10 cm in diameter and 22 cm in length. Muslin was utilized to cover the tops of the cylinders, which were secured in place with rubber bands.

Insecticidal activity

The insecticidal activity of *C. colocynthis* extract on adult *A. craccivora* was assessed using a leaf-dip bioassay. Five different concentrations of *C. colocynthis* extract (1%, 5%, 10%, 15%, and 20%) were prepared. Various quantities of *C. colocynthis* extract were prepared by dissolving it in distilled water. Thirty mature aphids were deliberately introduced onto a bean leaf disc placed in sterile petri dishes 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 *C. colocynthis* extract of different concentrations for a duration of 10 seconds. Subsequently, the leaf discs infested with aphids were allowed to air-dry for a duration of 10 minutes at an ambient temperature. Subsequently, a dual layer of muslin fabric was positioned on every petri dish and secured with a manually punctured lid to prevent asphyxiation and prevent the aphids from escaping. Distal water was used as a control. The petri dishes were placed into an incubator with a temperature of 20 ± 1°C, a photo period of 16:8 L: D, and a relative humidity of 65 ± 5%. The mortality of aphids was assessed after a 24-hour period. To identify dead aphids, one might gently strike the pest with a little brush and observe for any movements of legs or antennae. Three trials were conducted for each concentration. Percent mortality was calculated. The value of LC_{50} and LC_{95} and slopes were calculated by computerized Probit analysis program.

Life table (population performance) study

To examine the effects of *C. colocynthis* on the life cycle and survival rate of cowpea aphids, newly emerged apterous females (< 24 h) were tested. A group of adult aphids were subjected to LC_{50} (9.02%) of seed methanolic extract of *C. colocynths*, while another group served as a control and were treated with water using the leaf dipping technique. Every treatment commenced with a cohort of 30 wingless female insects, and the broad bean leaves were replenished as necessary every 2 days. The petri dishes were positioned in an incubator set at a temperature of 20.0± 1.0 °C, with a photo period of 16:8 L: D and a relative humidity of 65 ± 5%. Subsequently, treated aphid adults were examined daily throughout their entire lifespan to document the number of offspring produced per adult until the death of all adults. The acquired data were utilized to compute the subsequent life table parameters in accordance with Birch's (1948) methodology, viz. net reproduction rate (R_0), generation time (GT), intrinsic rate of increase (r_m), finite rate of increase (λ) and population doubling time (DT).

Statistical analysis

The study employed statistical analysis using Microsoft Excel 2010, and mean comparisons were conducted using one-way analysis of variance (ANOVA) where necessary. Several tests were performed in triplicate, and the results are reported as the mean value along with the standard deviation. To determine statistical significance, a significance criterion of $p < 0.05$ was applied.

Results

Phytochemical composition

The qualitative phytochemical analysis of the seeds of *C. colocynthis* methanol extract indicated the presence of the bioactive compounds, terpenoids, saponins, alkaloids, flavonoids, and phenolic acids in appreciable concentrations. Tannins, as per colorimetric analysis, were detected but in relatively low concentrations (Table 1). Moreover, the quantitative results of the total phenolics and total flavonoids content showed high phenolic content (60.45 mg GAE/g of extract), and high flavonoid content (46.66 mg/g of extract) as shown in Table 1.

Antioxidant activity

The antioxidant potential of the methanol extract of *C. colocynths* seeds was assessed through radical scavenging using DPPH methods (Figure 2). The extract exhibited notable antioxidant activity (73.55% at concentrations of 50 µg/ml) with an IC_{50} of 274.78 µg/ml, in comparison to the standard ascorbic acid. As illustrated in Figure 2, the reducing capacity of *C. colocynths* seeds extract FRAP test was significant and equivalent to the standard (ascorbic acid). This ability rose gradually with the rise in concentration. A higher reduction of power was demonstrated by the reaction's increased absorbance.

Phytochemicals	Methanol extract
Flavonoids	+++
Phenolic acids	+++
Saponins	+++
Terpenes	+++
Tannins	+
Alkaloids	++
Total phenolics	60.45 ± 3.40 GAE mg /g
Total Flavonoids	46.66 ± 2.88 Rutin mg/g

*80% v/v methanol extract of *C. colocynths* seeds, ** GAE: Gallic acid equivalents, +++ = Found in substantial quantities, ++ = Present to a moderate degree, + = Detected in small quantities, – = Not detected.

Table 1: The qualitative phytochemical examination of an 80% methanol extract (v/v) derived from *C. colocynthis* seeds.

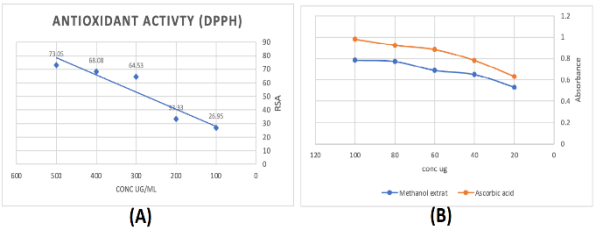

Figure 2: The antioxidant activity of *C. colocynths* seeds utilizing (A) the DPPH method and (B) the reducing power method.

Antibacterial activity

The current findings of the antibacterial evaluation are shown in Tables 2. The results revealed that based on the width of the inhibitory zones measured in millimeters (mm), the test findings were interpreted. The antibacterial activity of *C. colocynthis* seeds methanol extract at 200 mg/ml is classified as weak at 10 mm, moderate at >10 to 15 mm, and high at >15 mm [14]. Consequently, the methanol extract from *C. colocynthis* seeds exhibited limited to negligible antibacterial activity against the tested microorganisms. Specifically, among the five bacteria examined, only two demonstrated moderate susceptibilities: *Staphylococcus aureus* (14.5 ± 0.7 mm), a gram-positive bacterium, and *Proteus mirabilis* (10.5 ± 0.5 mm), a gram-negative one. No susceptibility was observed for the remaining bacteria. *S. aureus* and *P. mirabilis*, which among the other bacteria showed the greatest sensitivity to *C. colocynthis* seed methanol extract, were chosen for MIC, MBC, and MBC/MIC assays, are also represented in Table 2. The results revealed that the lowest MIC and MBC values (high susceptibility) were recorded for *S. aureus* (MIC=6.25, MBC= 25.0 mg/mL), followed by *P. mirabilis* (MIC=25.0, MBC= 50.0 mg/mL). The MBC/MIC ratios were 4 and 2, indicating that *C. colocynthis* seed methanol extract has a bactericidal mechanism on these two bacteria.

Insecticidal activity

The findings presented in Table 3 demonstrates the aphicidal activity of the *C. colocynthis* extract against adult *A. craccivora*. The results demonstrated a range of death percentages across the various concentrations. The mortality percentage was concentration dependent. Table 3 indicates that the LC_{50} and LC_{95} values were 9.02% and 20.50%, respectively.

Effect of *C. colocynthis* on Reproductive Ability, Adult Longevity and Fecundity

The duration of adult lifespan is defined by three main stages: pre-reproductive, reproductive, and post-reproductive. The pre-reproductive and post-reproductive stages of *A. craccivora* exhibited negligible disparities in the data. The mean reproductive period was dramatically reduced after treatment with *C. colocynthis* compared to the control group (Table S1). The average lifespan of adult *A. craccivora* was 17.47 ± 6.01 days in the control group. However, after treatment with the tested compound, the lifespan reduced to 4.50 ± 2.44 days. The application of *C. colocynthis* had a significant impact on the reproductive period and total lifetime of *A. craccivora*. There were substantial changes in the number of progenies per female of *A. craccivora* between the control treatment and the application of LC_{50} of *C. colocynthis*. The control treatment yielded the largest average number of offspring per female of *A. craccivora*, with a value of 53.13 ± 16.79. The number exhibited a significant drop (5.25 ± 3.2) upon treatment with the tested substance (Table S1).

Effect of *C. colocynthis* on Age-Specific Survival Rate (lx) and Age-Specific Fecundity Rate (mx)

The survival rate (lx) and fecundity per day (mx) at different ages were compared between individuals exposed to the LC_{50} of *C. colocynthis* and those in the control treatment. The survivorship (lx) for female *A. craccivora* was higher on the control treatment compared to the application of the plant extract. The survival rates of *C. colocynthis* decreased to 50% within approximately 4 days, but it took around 19 days for the population on the control treatment to reach the same level. The age-specific fecundity per day (mx) of cowpea aphids reached its peak in the control group, with a value of 4.67 females per female per day, on the 7th day. However, when in treated *C. colocynthis*, the maximum reproduction rate per female per day (mx) reached 1.54 female/female/day on the second day. Furthermore, the age-specific fecundity curves (mx) indicated that the reproductive phase of *A. craccivora* persisted for 23 days under normal conditions, whereas it lasted for just 8 days under sublethal concentration.

Effect of *C. colocynthis* on life table parameters

The impact of *C. colocynthis* on the life table parameters of *A. craccivora* was compared to a control group (Table S2). The net reproductive rate (R_0) of the cowpea aphid was significantly lowered by *C. colocynthis*, with just (1.62 nymphs/ female), compared to the control group

Clinical bacterial strains	*C. colocynthis* (200 mg/ml)	Chloramphenicol (2.5 mg/ml)	Methanol (80 % v/v)	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC ratio
Gram-positive bacteria						
Staphylococcus aureus	14.5 ± 0.7	34.5 ± 0.7	0.0 ± 0.0	6.25	25.0	4.0
Gram-negative bacteria						
Escherichia coli	6.0 ± 0.0	30.0 ± 1.4	0.0 ± 0.0	NA	NA	NA
Pseudomonas aeruginosa	6.0 ± 0.0	16.5 ± 0.5	0.0 ± 0.0	NA	NA	NA
Klebsiella pneumoniae	6.0 ± 0.0	28.0 ± 0.7	0.0 ± 0.0	NA	NA	NA
Proteus mirabilis	10.5 ± 0.5	14.0 ± 0.0	0.0 ± 0.0	25.0	50.0	2.0

Data expressed as mean± standard deviations, diameter of well is 6 mm. NA: Not tested.
Table 2: Preliminary antimicrobial activity, MIC, MBC and MBC/MIC ratios of *C. colocynthis* seeds methanol extract (80%v/v).

which had (52.96 nymphs/ female). Furthermore, it was shown that the mean generation time (GT), referring to the duration between the birth of the parents and the birth of the offspring, was greater in control treatment compared to the treatment including *C. colocynthis*. The administration of plant extract affects the doubling time (DT) of the *A. craccivora* population. The doubling duration (DT) of female subjects treated with the plant extract of *C. colocynthis* exhibited a greater value compared to those in the control treatment. The intrinsic rate of rise (r_m), which is indicative of the interplay between fecundity, generation, and survival, was observed to decrease to (0.0449 nymphs/female/day) compared to the control value of (0.2369 nymphs/female/day). Conversely, when the value of (r_m) was converted into the finite rate of increase (λ), it was revealed that the population of *A. craccivora* had the capacity to multiply approximately 1.0469 and 1.2673 times per aptera per day in groups of aphids treated with *A. colocynthis* extract and control groups, respectively. This indicates that a population of ten wingless cowpea aphids might grow to approximately 13 and 52 individuals in groups treated with *A. colocynthis* extract and control groups, respectively, within a one-week period.

Tested concentrations (%)	Number of individuals		Mortality (%)	Number of dead (Individuals)
1	32		1	3.12
5	41		7	17.07
10	37		15	40.54
15	33		22	66.67
20	35		33	94.28
control	80		0	0
Tested compound	**Lethal concentration**		**Slope ± SE**	**Regression equation**
C. colocynthis	LC_{95}	LC_{50}	3.84± 0.36	Y=-3.91+3.84X
	20.50	9.02		

Table 3: Mortality (%) and toxicity of *A. craccivora* treated with *C. colocynthis* extract after 24 h.

The findings demonstrated a clear influence of *C. colocynthis* on multiple biological factors of *A. craccivora*, suggesting that *C. colocynthis* has potential as an option in integrated pest management. Nevertheless, additional field tests are required to examine the impact of *C. colocynthis* on aphids and the predators that are linked to them.

Discussion

Our study revealed the presence of many bioactive phytochemicals. The results were in partial agreement with previous studies; The methanolic extract obtained from the seeds of *C. colocynthis* in Algeria exhibited the presence of tannins, flavonoids, and terpenoids, while no alkaloids were detected [15]. Another study reported that the seeds of *C. colocynthis* from Nigeria exhibited the presence of alkaloids, steroid glycosides, and flavonoids [16]. The Agro climatic conditions, together with the ratio of rainfall and temperature, are considered to have a significant influence on the composition of phyto-constituents and antioxidant capabilities of plants [17]. Furthermore, our investigation reveals a significant abundance of phenolic acids, despite the level of tannins is rather low. Many factors related to the botanical specimen and extraction technique may have affected the result. Thus, this study found different amounts of phenolic acids and tannins than previous research due to extraction methods, plant genetics, environmental conditions, and *C. colocynthis* seed chemical composition. To completely understand the results, these aspects need to be thoroughly investigated and assessed [18–20]. Total flavonoids and total phenolics were also identified in large levels in *C. colocynthis* seeds. Research on *C. colocynthis* seeds has been validated by this investigation. Listed are the milligrams of gallic acid equivalent per gramme, concentrations of catechin (flavonoids) or gallic acid (polyphenols) per gramme of extract, or both. The methanolic extract showed the greatest polyphenol and flavonoids levels, 238.80 and 147.76 mg/100 ml, respectively [15]. The *C. colocynthis* methanolic extract also has high total phenolics and total flavonoids in its leaves and roots. [21]. The biological efficiency of *C. colocynthis* comes from these substances. The *C. colocynthis* seed methanol extract showed significant antioxidant activity utilizing different radical-scavenging methods. Ethyl acetate, hydroethanolic, and aqueous *C. colocynthis* extracts showed antioxidant activities, as previously described. The ethyl acetate extract reduced 88.8%, the hydroethanolic 74.5%, and the aqueous 66.2%. The IC50 values for these extracts were 350, 580, and 500 µg/ml [22]. Our findings are substantiated by numerous prior studies, which have highlighted that, among various

fruit components of *C. colocynths* (such as rinds, pulps, and seeds), the seeds consistently exhibit the highest antioxidant activity. The generation of phenolic compounds and antioxidant activity across different fruit parts is contingent upon both environmental and genetic factors [18]. Roots and leaves of *C. colocynths* also showed good antioxidant activity [19].

The antibacterial testing showed the methanol extract of the seeds of *C. colocynths* was active only against two of five bacteria, namely, *Staphylococcus aureus* and *Proteus mirabilis*. Our findings are in agreement with previous studies, it was mentioned that the methanol extract of *C. colocynthis* recorded good, weak and no inhibition zones using disc diffusion test, amongst these microorganisms were Salmonella typhi (good activity: 13±0.65 mm), *Staphylococcus aureus* (weak activity: 6±0.25 mm), *Escherichia coli* (weak activity: 3±0.26 mm), *Klebsiella pneumoniae* (No activity) [41]. Perhaps the methanol that used for the extraction may not be the ideal solvent that traps the antimicrobial molecules. It was published that the ethanol extract of the fruit of *C. colocynthis* has better antibacterial activity against gram-positive bacteria, and seeds showed weak or no activity [42]. Interestingly, the whole plant (without fruits) showed good antibacterial activity as well as antifungal activity against many gram-positive, gram-negative and fungal strains [43]. Our investigation also uncovered that, based on the MIC, MBC, and MBC/MIC tests, the methanolic extract from *C. colocynthis* seeds possesses bactericidal properties. However, it is possible that the antibacterial agents present in the methanolic extract are in relatively low quantities. Consequently, further studies are recommended to isolate and identify these unidentified antibacterial agents. These current findings are consistent to some degree with previous studies that have reported the antimicrobial activity of *C. colocynthis* methanol extracts against various bacterial strains. For example, a study by Keikhaie et al. [20], found that, based on MIC and MBC tests, the doses of *C. colocynthis* fruit ethanol extract at which 100% of *Staphylococcus aureus* were eliminated that was between 10 and 20 mg/ml showed the greatest sensitivity. Also, it was stated that the methanol extract of *C. colocynthis* fruit shown significant effectiveness against drug-sensitive and drug- resistant Mycobacterium tuberculosis (MIC≤62.5 µg/mL) [21]. Antimicrobial activity could be attributed to the phytochemical constituents and its nature. Anyhow, our study did not include antifungal examination, while a previous study stated that the aqueous extract of this plant possessed antifungal activity with low MIC and minimum fungicidal concentration (MFC) values (MIC and MFC = 0.10 mg/mL) [22].

Researchers have become interested in *C. colocynthis* as a potential alternative botanical insecticide. They have tested the extracts and isolated components of this plant against insect pest species that are commercially significant, in order to determine their efficiency. This plant exhibits antifeedant, deterrent, and infertile properties against many pests [23]. Our research indicates that the death rate of *A. craccivora* significantly rose as the content of the plant extract increased. Significantly more intriguing outcomes were achieved when examining the sublethal impacts of *A. colocynthis* extract on the demographic aspects of *A. craccivora*. Exposed *A. colocynthis* to feeding resulted in decreased adult longevity and fecundity relative to the control group. This finding aligns with the observations made by Kamel and El-Gengaihi [24], who reported that the lifespan and reproductive capacity of the cabbage aphid decreased dramatically when subjected to higher levels of cucurbitacin B. Our study found that the plant extract of *C. colocynthis* induced alterations in the life table characteristics of *A. craccivora*, when we compared it to the control group. The presence of a sublethal quantity of *C. colocynthis* resulted in a reduction in generation time (GT), net reproduction rate (R_0), intrinsic rate of increase (rm), and finite rate of increase (λ). In spite of this, the groups that got the plant extract had a significantly greater increase in doubling time (DT) than the control group. When Yousaf et al. [25] did their research, they looked at how two different amounts of cucurbitacin B affected the bodies of fully grown melon aphids, especially *Aphis gossypii*. A lot of biological factors went down because of the substance. These included the intrinsic rate of growth (r) (day−1), generation time T (day), finite rate of rise λ (day−1), and net reproductive rate R_0 (offspring/individual). There is strong evidence that a large amount of cucurbitacin B is harmful to melon bugs. This result suggests that cucurbitacin B could be used to choose plant types that are naturally resistant to melon bugs and are often harmed by them. Ibrahim [25], has demonstrated the efficacy of several solvent extracts from *C. colocynthis* fruits against adult cowpea aphids. The methanol extract had the greatest effectiveness against adult organisms, followed by the ethyl acetate extract, and finally the petroleum ether extract. The methanolic extract exhibited an LC_{50} value of 639.85 ppm and an LC_{90} value of 2472.40 ppm. Sayeda et al. [26] found that the methanol extract of *C. colocynthis* exhibited the highest efficacy, with an LC_{50} value of 621.94 ppm. In their study, Soliman et al. [27] found that hexane, diethyl ether, ethyl acetate, acetone, and ethanol extracts of *C. colocynthis* exhibited greater toxicity against adult cotton aphids (*Aphis gossypii* Glover). Torkey et al. [28] observed that the Ethanol extracts derived from the fruit of *C. colocynthis* contained cucurbitacin E glycoside and induced mortality in the cowpea aphid, *A. craccivora*. In their study, Soam et al.

[29] observed that when the aerial portions of *C. colocynthis* were applied at concentrations of 250µg/ml and 500µg/ml, it resulted in the complete death of mustard aphids (*Lipaphis erysimi*) within 24 hours. The ethanol-based seed extract of *C. colocynthis* exhibits antifeedant and toxic effects against mites, as demonstrated by Mansour et al. [30]. The water extract of *C. colocynthis* showed toxicity against *Rhopalosiphum padi*, as reported by Khalid [31]. Multiple investigations have been carried out to examine the effectiveness of insecticides derived from *C. colocynthis* against various insect pests. This research has consistently demonstrated the poisonous properties of *C. colocynthis* against *A. craccivora*, which was also observed in the present study. Gulzar et al., [32] conducted laboratory research to assess the impact of *C. colocynthis* extract in various solvents on second instar larvae of the Cotton bollworm, *Helicoverpa armigera* (Hubner). The study aimed to analyze the toxicity, sublethal effects, and antifeedant properties of the extract. In their study, Mullai and Jebanesan [33] discovered that the larval stage of the filarial vector *Culex quinquefasciatus* (Say) (Diptera: Culicidae) experienced complete mortality when exposed to a 450-ppm concentration of *C. colocynthis* leaf extract.

In conclusion, the current study provides valuable insights into potential therapeutic applications of this medicinal plant collected from arid zones. The presence of several bioactive elements makes *C. colocynthis* seeds a potential organic reservoir of antioxidants and antibacterial compounds with medicinal benefits. The antibacterial assessment showed effectiveness against Gram-positive and Gram-negative bacteria, with significant MIC and MBC values, especially against S. aureus, suggesting therapeutic uses. The extract of *C. colocynthis* can also kill adult Aphis craccivora, which is a common pest in agriculture. This shows that it can be used as a natural herbicide. So, our work shows that *C. colocynthis* seeds are good for both health and the environment. In addition, it gives scientists a way to study their health and environmental benefits. These results help researchers learn more about medical plants and show how they can be used in many ways in medicine and healthy farming. More research needs to be done to fully understand and confirm the safety of *C. colocynthis* before it can be used effectively for medical and farming purposes.

Author Contributions

Conceptualization: Emad M. Abdallah, Ahmed M. H. Ali, Adil Mujawah; Data Curation: Emad M. Abdallah; Formal Analysis: Emad M. Abdallah, Ahmed M. H. Ali, Eman R. Elsharkawy; Methodology: Emad M. Abdallah, Ahmed M. H. Ali, Hanaa F. Hashem, Adil Mujawah, Eman R. Elsharkawy; Funding Acquisition: None; Project Administration: Emad M. Abdallah, Ahmed M. H. Ali; Software: Ahmed M. H. Ali; Supervision: Emad M. Abdallah; Validation: Emad M. Abdallah, Ahmed M. H. Ali; Writing – Original Draft Preparation: Emad M. Abdallah, Ahmed M. H. Ali, Eman R. Elsharkawy; Writing – Review & Editing: Emad M. Abdallah.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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