

Original article

## Phytochemical Investigation and Exploring the *Citrullus Colocynthis* Extracts as Antibacterial Agents Against Some Gram and Negative Bacteria Species

Hamad Hasan<sup>1\*</sup>, Zuhir Akrim<sup>2</sup>, Farag Shuib<sup>3</sup>, Dala Abdraba<sup>3</sup><sup>1</sup>Department of Chemistry, Faculty of Science, Omar Al-Mukhtar University, El-Bayda, Libya<sup>2</sup>Department of Chemistry Pharmacology and Toxicity, Faculty of Pharmacy, Omar Al Mukhtar University, El-Bayda, Libya<sup>3</sup>Department of Botany, Faculty of Science, Omar Al -Mukhtar University, El-Bayda, LibyaCorresponding Email. [hamad.dr@omu.edu.ly](mailto:hamad.dr@omu.edu.ly)

### Abstract

The *Citrullus colocyn* plant grows in some Libyan regions, especially at Al Gabal Al Akhder, and was considered as medicinal plant. In this study, the extracts of the seed and fruits of *Citrullus colocyn*. This plant was used as an antibacterial agent against gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, and *Streptococcus pneumonia*) and gram-negative bacteria (*Escherichia coli* and *Proteus vulgaris*). Also, the phytochemical, total phenols, and antioxidant activities were detected. The results showed some natural product compounds such as carbohydrates and/or glycosides, flavonoids, sterols and/or triterpenes, cardiac glycoside, and small variations of their contents in aqueous and alcoholic extracts. For the antibacterial activities, higher concentrations of extracts (0.1 -0.8 g/100 ml) showed high effects compared with lower ones (0.0001 -0.1 g/100 ml) against the selected bacteria in this study. There is a relative increase in total phenols and antioxidant contents in fruit extracts compared with seeds. The study concluded that the presence of natural product compounds was the main reason for the inhibition of bacteria, and the difference of the effects was attributed to the effect of solvent polarizes during the extraction

**Key words.** Phytochemical, Antibacterial, *Citrullus Colocynthis*, Extracts.

### Introduction

World Health Organization survey indicated that about 70-80% of the world's population rely on nonconventional medicine, mainly of herbal sources, in their primary healthcare. This is especially the case in developing countries where the cost of consulting a Western-style doctor and the price of medication is beyond the means of most people [1].

Medicinal plants (MP) have been used in folk medicine in Libyan rural areas at relatively cheaper expenses than modern medicine. They have been widely used as diuretics, topical anti-inflammatories, and haemostatics [2]. Plants generally produce several secondary metabolites like phenols, flavonoids, quinones, tannins, alkaloids, saponins, and sterols which are important sources of biocides and many other pharmaceutical drugs [3]. Medicinal plants are important in pharmacological research and drug development [4]. *Citrullus colocynthis* (cucurbitaceae) is a native of arid soils. It is commonly found in Saudi Arabia, Syria, Jordan, Egypt, Iran, India, and Pakistan. It is commonly known as Hanzal, Indrian, Tumma, or Bitter apple. It is a large creeping herb with deeply dissected lobulate leaves. Flowers are solitary, monoecious of yellow colour. Fruits rounded 7-9cm in diameter, green and white, striped become yellow when ripe. The *Citrullus colocynthis* of family Cucurbitaceae is useful against fever, intestinal parasites, hepatic and abdominal diseases, visceral and cerebral congestions.

Fruit juice with sugar is a household remedy in dropsy. Seeds are diuretic. Fruits are used against tumors of the gastrointestinal tract. It is more pronouncedly used in anticancerous drugs. It is effective in leukemia and joint pains. *C. colocynthis* is widely used by rural inhabitants as a purgative, anti-diabetic, anti-neoplastic, anti-rheumatic, and anti-allergic agent. They are found mainly in plants belonging to the Cucurbitaceae family but have also been found in several other families of the plant kingdom [5]. Although, the whole fruit is often used for the treatment of the aforementioned diseases, but some particular parts of the fruit are also used for specific purposes. One such example is the traditional application of the dried pulp and seed extract of *C. colocynthis* for the treatment of constipation and diabetes [6,7]. The use of colocynth as a drug has been documented in ancient times and religious books [8].

*C. colocynthis* is traditionally used as an antidiabetic medication in tropical and subtropical countries [9]. This study was conducted to investigate for the following targets: Phytochemical screening of *Citrullus colocynthis*) which is growing at Al-Gabal AL-Akhder region Libya. Determination of the total phenolic compounds of the selected plant. Measured the antioxidant activity of the studied plants and evaluated the antimicrobial activity of methanol extracts from fruits and seeds of *Citrullus colocynthis* uses against a variety of microorganisms causing infectious diseases in humans.

## Methods

### **Selection of medicinal plants for this study**

Due to the important many plants which used at AL-Gabal AL-Khder region This study was designed to select one of medicine plant used in traditional thereby collected from Al-Gabel Al –Kadar Region during spring 2024, the samples include; *Citrullus colocynthis* L Fruits and seeds of Plant were separated and washed with distilled water several times, then dried in open air (Figure 1).

### **Taxonomical investigation**

The collected samples were identified in Seliphium herbarium, Botany Department, Faculty of Science, Omar Al Mukhtar University.



**Figure 1. *Citrullus colocynthis* L Fruits**

### **Phytochemical screening**

All the Phytochemical screening tests were carried out according to standard methods [10].

#### **Test for sterols and/or triterpenes**

##### **Libermann-Burchad's test**

One ml of the chloroform extract of each sample, 0.3 ml of acetic anhydride was added then followed by few drops of concentrated sulphuric acid along the side of the dry test tube. A reddish-violet colour is produced at the junction of the two layers, and chloroformic solution acquires a green colour in case of the presence of sterols and/or triterpenes

#### **Test for flavonoids**

The extracts (alcohol and aqueous) of the tested herbal plants were further extracted with 1% hydrochloric acid; each extract was subjected to the following test: 10 ml of each extract is rendered alkline where a faint yellow colour is produced in case of the presence of flavonoids.

#### **Test for alkaloids**

The extracts of the tested herbal plants were further extracted with 20ml of dilute hydrochloric acid, cooled and rendered alkaline with dilute ammonium hydroxide solution, then extracted with chloroform. The chloroform extract is subjected to the following test: The preparation of the reagent: Solution a: 0.85 g of basic bismuth nitrate is dissolved in a mixture of 10 ml acetic acid and 40 ml water. Solution b: 8 g potassium iodide in 20 ml water. Stock solution: Equal volumes of solutions a and b are mixed. A few drops of chloroformic extract were applied to filter paper, allowed to dry, and sprayed with the reagent. An orange color is observed in cases of the presence of alkaloids.

#### **Test for tannins**

The extracts (alcohol and aqueous) of the tested herbal plants were further extracted with ethanol 50%, filtered, the hydro-alcoholic clear solution is subjected to the following test: 1ml of the reagent (1%  $\text{FeCl}_3$ ) is added to the hydro-alcoholic solution. Blue colour develops in cases of the presence of tannins.

#### **Test for carbohydrates and /or glycosides**

The extracts of the tested herbal plants were further extracted with water; the produced aqueous extract was subjected to Molish test as follows: Two ml of the extract was mixed with 0.2 ml ethanolic  $\alpha$ -naphthol (20%), and 2ml of concentrated sulphuric acid was added on the side of the dry test tube. Violet ring is observed at the junction of the two layers cases of the presence of carbohydrates and/or glycosides.

### **Tests for cardiac glycosides**

One ml of each extract of the tested herbal preparations was dissolved in glacial acetic acid containing traces of ferric chloride; concentrated sulphuric acid containing the same amount of ferric chloride is placed at the bottom of the test tube with a pipette where intense blue colour at the surface between the reagents develops for 2-5 minutes, spreading gradually into acetic acid layer, in cases of the presence of deoxy- sugars.

### **Test for anthraquinones**

One ml of each of the successive extracts of aqueous ammonia or caustic soda is added and shaken. The rose-red color in the aqueous layer develops as a result of the presence of anthraquinone glycosides.

### **Test for saponins**

#### **Chemical studies**

Five ml of tap water is added to 1 ml of each extract, then shaken vigorously for five minutes, froth develop having 1cm high and persists for 15 minutes indicates the presence of saponins.

### **Determination of total phenols by Folin Ciocalteu Method**

Aliquots of the extracts were taken in a 10 ml flask and made up to a volume of 3ml with distilled water. Then 0.5 ml folin ciocalteu reagent (1:1 with water) and 2ml Na<sub>2</sub>CO<sub>3</sub> (2 %) were added. The test solutions were warmed for 1 minute, cooled, and absorbance was measured at 650 nm against the reagent used as a blank. A standard calibration plot was generated at 650 nm using known concentrations of tannic acid from 4 to 20µg/ml [11].

### **Determination of antioxidant power by prussian blue method**

One gram of powder was defatted with petroleum ether. The defatted powder was then extracted sequentially by stirring with 10 ml methanol twice, then with 10 ml 1% hydrochloric acid: methanol(v/v). The three combined extracts were evaporated under a vacuum, and the residue was dissolved in 10 ml methanol. Half ml of the solution was diluted with 3 ml distilled water, 3 ml 0.008M K<sub>3</sub>Fe(CN)<sub>6</sub> was added, 3 ml 0.1M HCl, and 1 ml 1% FeCl<sub>3</sub>. The blue color is allowed to develop for 5 min, and the absorbance is measured at 720 nm against the blank [12].

### **Antimicrobial activity**

#### **Preparation of extracts**

The whole aerial part of plants collected was identified were dried shade and reduced to coarse powder using a mechanical grinder. The powdered plant (50g) was extracted for 72h with methanol 80 % using rotary evaporator and stored at 4 °C until further use [13&14].

#### **Microorganisms**

The following bacteria and fungus were investigated in order to determine whether the extracts were effective against them:

#### **Bacterial strains**

##### **Gram positive bacteria**

Three species of Gram positive (*Staphylococcus aureus* and *Bacillus cereus* *Streptococcus pneumoniae*) were used and obtained from Department of microbiology El-Bayda Hospital.

##### **Gram-negative bacteria**

The species of selected bacteria was obtained from the Department of Microbiology at El-Bayda Hospital. Obtained from Department of microbiology of Faculty Veterinary Medicine Omar Al- Mukhtar University El-Bayda, Libya.

#### **The minimal inhibition concentration determination**

The agar well diffusion method was used to assess the antimicrobial activity of the plant extracts. A sterile standard borer was used to create wells on each plate after bacterial and fungal strains were seeded on Mueller-Hinton (MH) agar plates and potato Dextrose Agar (PDA) plates, respectively [15]. The plates were incubated for 24–48 hours at 37°C for bacteria and 48–72 hours at 28°C for fungi after 30µl of the various quantities (0.8, 0.4, 0.2, 0.1, 0.01, 0.001, 0.0001, 0.00001 g/ml) of the plant extracts under study were added to each well. The results are shown as the mean of the three measurements of the inhibitory zones. Following established protocols, the minimal inhibitory concentration (MIC) values were assessed [16 & 17]. Only microorganisms exhibiting inhibitory zones were used to calculate the least inhibitory concentration (MIC). Plant extracts were diluted, and 50µl of each dilution was pipetted into wells containing extracts ranging in concentration from 0.8 to 0.00001 g/ml in order to calculate the minimum inhibitory

concentration (MIC). The lowest concentration that prevented apparent microbial growth was known as the minimum inhibitory concentration (MIC) [18].

### Antibiotic sensitivity tests

*In vitro* antimicrobial susceptibility to nine antibiotics (Table 1). The inoculums were prepared by adding isolated colonies of the microorganism from an overnight nutrient agar plate into 2ml tryptone soya broth (TSB). A sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab. The swab was streaked over the entire surface of the sterile Mueller Hinton Agar plate. This procedure was repeated by streaking two more times, rotating the plate approximately each time to ensure an even distribution of inoculums. Plates were allowed to dry for 5 minutes, and then the antimicrobial disks were dispensed onto the surface of inoculated agar plates using an antibiotic. After that, the plates were incubated for 18 to 22 hours at 37°C. Using venier calipers (Junior), the diameters of the zones of inhibition are measured to the closest millimeter. The zones' diameters were evaluated as either resistant (R) or susceptible intermediate (I) by NCCLS (2005) [18].

**Table 1. Antibiotic Sensitivity testing**

Antibiotic	Symbol	Concentration
Kanamycin	K	30mg/ml
Gentamicin	CN	10mg/ml
Chloramphenicol	C	30mg/ml
Cefotaxime	CTX	30mg/ml
Ticarcillin	TIC	75mg/ml
Mezlocillin	MEZ	73mg/ml
Sulphamethoxazole/trimethoprim	SXT	25mg/ml
Tetracycline	TE	30mg/ml
Ofloxacin	OFX	5mg/ml

## Results

### Phytochemical screening studies

The samples were screened for the following constituents: carbohydrates and/or glycosides, tannins, flavonoids, sterols and/or triterpenes, saponins, and anthraquinone. The obtained results were recorded in table (2) and revealed the presence of carbohydrates and/or glycosides, sterols and/or triterpenes and cardiac glycosides and alkaloids in all plants, while saponins were absent in all studied plant. The flavonoids found in *Citrullus colocynthis* plant have a high concentration in alcoholic extracts of fruits, while found low concentration in aqueous extracts. while found equal concentration in aqueous and alcoholic extracts in *Citrullus colocynthis* seeds plant. The anthraquinones were not found in *Citrullus colocynthis*. Tannins are not found in *Citrullus colocynthis*. The carbohydrates and/or glycosides were found in equal concentration in aqueous and alcoholic extracts in *Citrullus colocynthis* seeds plant while found in high concentration in alcoholic extracts in *Citrullus colocynthis* fruit. The carbohydrates and/or glycosides were found in high concentration in alcoholic extracts in *Cynara cornigera* fruit plant while found low concentration in aqueous extracts. The alkaloids were found in high concentration in alcoholic extracts in *Citrullus colocynthis* seed plant and low concentration in aqueous extracts while found in high concentration in alcoholic extracts in *Citrullus colocynthis* fruit plant and low concentration in aqueous extracts.

The cardiac glycosides were found in equal concentration in aqueous and alcoholic extracts in *Citrullus colocynthis* seeds plant, while found low concentration in aqueous extracts, the Cardiac glycosides. Phytochemical screening revealed that *C. colocynthis* extract contains triterpene, flavonoids, tertiary and quaternary alkaloids, glycosides, and saponin compounds [19,20]. Confirmed that; three phytochemical constituents were identified in *C. colocyn*. This Shard seed extracts as alkaloid, steroid, glycosides and flavonoids [21].

### Chemical studies

#### Determination of total phenols:

The concentrations of total phenol contents in plant extracts was determined using Folin Ciocalteu Method the obtained observations are mentioned in table 3, total phenols content was found to be in methanol extracts of *Citrullus colocynthis* fruit plant 146.84 mg/ml while total phenols content was found to be in aqueous extracts of *Citrullus colocynthis* fruit plant 219.92mg/ml. Total phenols contents were found to be in methanol extracts of *Citrullus colocynthis* seed plant 108.79 mg/ml while total phenols content was found to be in aqueous extracts of *Citrullus colocynthis* seed plant 145.83mg/ml (Table 3).

**Table 2. Phytochemical screening of the studied each plant**

Plant Chemical Test	<b>Citrullus Colocynthis</b>			
	Seeds		Fruit	
	Aq	Al	Aq	Al
Saponins	–	–	–	–
Tannines	–	–	–	–
Carbohydrate and/or Glycosides	+	+	++	+++
Alkaloids	+	++	++	+++
Flavonoids	+	++	+	++
Anthraquinones	–	–	–	–
Steroids and/or Triterpenoids	/	+	/	+
Cardiac Glycosides	+	+	++	+++

(+) Present (-) Absent (/) Not Detected

Total phenols contents were found to be in methanol extracts of *Cynara cornigera* fruit plant 648.61mg/ml while total phenols content was found to be in aqueous extracts of *Cynara cornigera* fruit 278.5in total phenols content. Among the studied plants, *Cynara cornigera* contained the highest amount of total phenols, followed by *Citrullus colocynthis*. The Folin-Ciocalteu method is a rapid and widely-used assay to detect the total phenolic content, but it is known that different phenolic compounds have different responses in the Folin-Ciocalteu method. Phenolic and flavonoid compounds have been reported to be responsible for the antioxidant activities of medicinal plants and other botanical materials [22].

**Table 3. Total phenols contents in Citrullus colocynthis:**

Plant Total phenols (mg/g)	<b>Citrullus colocynthis</b>	
	Seeds	Fruits
Extract water	145.83	219.92
Extract methanol	108.79	146.84

#### Determination of antioxidant

Evaluation of the antioxidant activity by using the prussian blue method. The obtained observations mentioned in table 4 were found to have different levels of Antioxidant potential of the fruit *Citrullus colocynthis*, which was observed at a maximum 48.74Omg/ml while found at seeds *citrullus colocynthis*. This plant has antioxidant activity. The Fruit part and Fruit pulp of *Citrullus colocynthis* plant have been explored for antioxidant activity [23].

**Table 4. Antioxidant in Citrullus colocynthis**

Plant Antioxidant(mg/g)	<b>Citrullus colocynthis</b>	
	Seeds	Fruits
	38.074	48.740

#### Antimicrobial activity

The antimicrobial activity studies were carried out on solvents extracts for all studied plants in both, seeds and fruits, against the selected bacteria and fungi species. The results of antimicrobial tests are shown in Tables (5-9) and were described in Table 5, showed different concentrations of studied plants extract against *Staphylococcus aureus*. The results showed that the inhibition zone and MIC in all extracts recorded at 0.1 g/ml. Similar results were observed by some studies [24].

**Table 5. Antimicrobial activities of different concentrations of studied plant extract against Staphylococcus aureus**

Samples Concentration	Fruits	seed
0.8 g/ml	19	28
0.4g/ml	17	26
0.2 g/ml	15	24
0.1 g/ml	10	19
0.01 g/ml	N.A	N.A
0.001g/ml	N.A	N.A
0.0001g/ml	N.A	N.A
0.00001g/ml	N.A	N.A

**Bacillus cereus**

By applying different concentrations of studied plant extract against *Bacillus cereus* the results showed that the inhibition zone and MIC of all extracts was recorded at 0.1 g/ml except for *Cynara cornigera* fruits, where the inhibition zone was 0.01g/ml (Table 6). The extract effect on both gram-positive and gram-negative bacteria, the present result agrees with some of studies [25] (Table 6).

**Table 6. Antimicrobial activities of different concentrations of studied plant extract against *Bacillus cereus***

Samples Concentration	Fruits	Seed
0.8 g/ml	33	31
0.4g/ml	27	28
0.2 g/ml	25	23
0.1 g/ml	22	20
0.01 g/ml	N.A	N.A
0.001g/ml	N.A	N.A
0.0001g/ml	N.A	N.A
0.00001g/ml	N.A	N.A

**Streptococcus pneumoniae**

Table 7 shows different concentrations of the studied plant extract against *Streptococcus pneumoniae*. Results showed that inhibition zone and MIC at 0.1 g/ml for *c. colocynthis* seed, where the inhibition zone was 0.01g/ml except for fruits of *Citrullus colocynthis* no zones of inhibition did not show any effect on the bacterial growth. Similar results were observed in a previous study [26].

**Table 7. Antimicrobial activities of different concentrations of studied plant extract against *Streptococcus pneumoniae***

Samples Concentration	Fruits	Seed
0.8 g/ml	33	31
0.4g/ml	27	28
0.2 g/ml	25	23
0.1 g/ml	22	20
0.01 g/ml	N.A	N.A
0.001g/ml	N.A	N.A
0.0001g/ml	N.A	N.A
0.00001g/ml	N.A	N.A

**Gram-negative bacteria*****Escherichia coli*:**

Table 8 shows the different concentrations of the studied plant extract against *Escherichia coli* that were tested. The results showed the inhibition zone at 0.1 g/ml for seeds *Citrullus colocynthis*. Similar results observed were recorded previously [23].

**Table 8. Antimicrobial activities of different concentrations of the studied plants extract against *Escherichia coli***

Samples Conc.	<i>C. Colocynthis</i>	
	Seed	Fruits
0.8 g/ml	30	21
0.4 g/ml	25	19
0.2 g/ml	19	17
0.1 g/ml	17	10
0.01g/ml	N.A	3
0.001g/ml	N.A	N.A
0.0001g/ml	N.A	N.A
0.00001g/ml	N.A	N.A

***Proteus vulgaris***

Table 9 shows different concentrations of the studied plant extract against *Proteus vulgaris* that were tested. The results showed that the inhibition zone and MIC of all extract recorded at 0.1 g/ml, except for fruits of

*Citrullus colocynthis* no zones of inhibition did not show any effect on the bacterial growth, which showed that it kills bacteria in respective to their cell wall structure, however other studies showed that gram-positive bacteria are more sensitive than gram negative bacteria or vice versa [27].

**Table 9. Antimicrobial activities of different concentrations of the studied plants extract against *Proteus vulgaris***

Samples Concentration	Seeds	Fruits
0.8 g/ml	16	NA
0.4g/ml	13	NA
0.2 g/ml	11	NA
0.1 g/ml	10	NA
0.01 g/ml	NA	NA
0.001g/ml	NA	NA
0.0001g/ml	NA	NA
0.00001g/ml	NA	NA

### Antibiotic Sensitivity

Table 10 shows the rates of sensitivity of Gram-negative and Gram-positive bacteria results showed that the sensitivity pattern of *S. aureus* was sensitive to K, CN, C, MEZ, SXT, TE, OFX and resistant to CTX, TIC. However, *B. cereus* was sensitive to K, CN, and C resistant to CTX, TIC, MEZ, SXT, TE, OFX. Whereas *S. pneumoniae* was sensitive to CN, C, MEZ and resistant to K, CTX, TIC, SXT, TE, OFX. *E. coli* was sensitive to K, C, and MEZ, resistant to CN, CTX, TIC, SXT, TE, and OFX. Moreover, *p. vulgaris* was resistant to all antibiotics K, CN, C, CTX, TIC, MEZ, SXT, TE, and OFX. Antimicrobial resistance developed by microbes against antibiotics opens serious debates on this issue and is recognized as a serious problem by the global medicinal and research community [28].

**Table 10. Antibiotic Sensitivity testing**

Antibiotic	Symbol	Concentration	Organism				
			<i>S.aureus</i>	<i>B.cereus</i>	<i>S.pneumoniae</i>	<i>E.coli</i>	<i>p.vulgaris</i>
Kanamycin	K	30mg/ml	S	S	R	S	R
Gentamicin	CN	10mg/ml	S	S	S	R	R
Chloramphenicol	C	30mg/ml	S	S	S	S	R
Cefotaxime	CTX	30mg/ml	R	R	R	R	R
Ticarcillin	TIC	75mg/ml	R	R	R	R	R
Mezlocillin	MEZ	73mg/ml	S	R	S	S	R
Sulphamethoxazole/trimethoprim	SXT	25mg/ml	S	R	R	R	R
Tetracycline	TE	30mg/ml	S	R	R	R	R
Ofloxacin	OFX	5mg/ml	S	R	R	R	R

*S-Sensitive; R-Resistant.*

### Discussion

In this study, the extracts of seeds and fruits of *Citrullus colocynthis* plant showed anti-bacterial activities of the studied bacteria species of negative gram and positive gram bacteria. The difference of the effect of extracts on the bacteria is mainly due to the presence of amounts of natural compounds as phenols and flavonoids and other compounds [23-26]. The main mechanism of the damage bacteria is mainly due to the attack extracts or compounds the wall of bacteria. Many studies showed the same results after using plant extracts against different species of bacteria, they attributed that to compounds' presence in the extracts [15 -20]. Also, the antibacterial observations of *Citrullus colocynthis* extract at different concentrations have that able to inhibit the growth of the selected bacteria species that agreement with the results of [29], who studied guava extracts against food born pathogen and spoilage bacteria that due to the phenolic components which make them effective against the tested microorganisms. This result was confirmed by [30], and these observations also matched with that of the findings of [31], who showed the antibacterial activity of guava leaf extracts based on how the phenolic components act, particularly flavonoids. These results are also in harmony with [32], who exhibited the antibacterial activity of guava against food-borne pathogens. According to the study, the extracts of *Ziziphusspina christi* of all parts act as antibacterial agents against the tested pathogenic bacterial strains. This inhibition is attributed to existing tannins, as well as other active components like saponins, flavonoids, steroids, and glycosides [33]. This result is recorded by those results stated in some antibacterial investigations [34], which found the seed extracts were effective only against three bacterial strains. and also confirmed by a study recorded that the most active extracts observed by *M. alba* against Gram-positive and Gram-negative bacteria [35]. That activity may be due to the plant being rich in phyto constituents slike as tannins, phytosterols, sitosterols, saponins, anthroquinones, glycosides, and Oleanolic Compounds [36].

## Conclusion

According to the results obtained in this study, different natural compounds were detected in the studied extracts of *Citrullus colocynthis* plant. Also, the results of the antibacterial investigation showed that the higher concentrations of extracts gave higher activity compared with lower ones against some selected bacteria species. The study revealed that the effect of natural compounds' presence in the extracts investigated in this study mainly gave the anti-bacterial activates.

## Acknowledgment

The authors were grateful to the chemistry laboratory of the chemistry department for their help during the chemical analysis. They also appreciate the support of the microbiological investigation of the botany department, Faculty of Science, Omar Al –Muktar University, Libya.

## References

1. Chan, K. Some aspects of toxic contamination in herbal medicines, *Chemosphere*. 2000; 5(2):1361-71.
2. Burt S. Essential oils: Their antibacterial properties and potential applications in foods, *International Journal of Food Microbiology*. 2004; (4): 223-253.
3. Naili M, Alghazeer R, Saleh N, Al-Najjar A. Evaluation of antibacterial and antioxidant active-ties of *Artemisia Campestris* (Astraceae) and *Ziziphus lotus* (Rhamnaceae). *Arabian Journal of Chemistry*. 2010; (3): 73-134.
4. El-Mokasabi F. Survey of wild trees and shrubs in eastern region of Libya and their economical value. *AlQalam Journal of Medical and Applied Sciences*. 2022 Jan 11:48-55.
5. Tannin-Spitz TS, Grossman S, Dovrat H E, Gottlieb M, Bergman M. Growth inhibitory activity of Cucurbitacin glucosides isolated from *Citrullus colocynthis* on human breast cancer cells," *Biochemistry Pharmacology*. 2007; (3):56-67.
6. Kumar S, Kumar D, Saroha K, Singh N, Vashishta B. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. Methanolic fruit extract, *Acta Pharm*. 2008; 58(2):215-220.
7. Nmila R, Gross R, Rchid H, Roye M, Manteghetti M, Petit P. Tropic effect of *Citrullus colocynthis* fruit extracts", *Planta Medica*. 2000; 66(5):418-423 pp.
8. Khafagi I, Zakaria A, Dewedar A, El-Zahdany K. A voyage in the world of plants as mentioned in the Holy Quran, *International Journal of Botany*. 2006. 2(3):242-251.
9. Diwan F, Abdel-Hassan I, Mohammed ST. Effect of saponin on mortality and histopathological changes in mice. *Journal Eastern Mediterranean Health*. 2000; 6:345-351.
10. Mahmoud S A, Hasan H M, Abdul Salam N A. Phytochemical screening, total phenolic, antioxidant activity, metals and mineral contents in some parts of *Plantago albicans* grown in Libya. *World Journal of Pharmaceutical Research*. 2024; 13(3):91-103.
11. Kaur C, Kapoor H C. Antioxidant and total phenolic contents of some Asian vegetables", *International Journal of Food Sciences and Technology*. 2000; 37: 153-161.
12. Elsalhin H, Abobaker H A, Hasan H M, El -Dayek G. Antioxidant capacity and Total phenolic compounds of some algae species (*Anabaena* and *Spirulina platensis*). *Scholars Academic Journal of Biosciences (SAJB)*. 2016.4(10):782-786.
13. Nora N B, Hamid K, Snouci M, Boumediene M, Abdellah M. Phytochemical and antibacterial screening of *Citrullus colocynthis* of South-west Algeria. *J Chem Pharm Res*. 2015; 7(5):1344-1348.
14. Eltawaty S, Abdalkader G, Hasan H, Hussein M. Antibacterial activity and GC-MS analysis of chloroform extract of bark of the Libyan *Salvia fruticosa* Mill. *International Journal of Multidisciplinary Sciences and Advanced Technology*. 2021;(1): 715-721.
15. Aljamal MA, Hasan H M, Al Sonosy HA. Antibacterial Activity Investigation and Anti-Biotic Sensitive for Different Solvents (Ethanol, propanol, DMSO and di Ethel ether) Extracts of Seeds, Leafs and Stems of (*Laurus azorica* and *Avena sterilis*) Plants. *Int.J.Curr.Microbiol.App.Sci*. 2024. 13(11): 175-190.
16. Iscan G, Demirci F, Kirimer N, Ku"rkcü"oglu, MB, Aser KH. Antimicrobial screening: *Mentha piperita* essential oil", *Journal of Agricultural Food and Chemistry*. 2002. 50: 3943-3946.
17. Guven K, Celik S, Uysal L. Antimicrobial activity of *Entaurea species*", *Pharmaceutical Biology*. 2005.4(3): 67-71.
18. NCCLS. Performance Standards for Antimicrobial Susceptibility Testing. MIC Testing Document. 2005;(22): 82-112.
19. Hasan H, Ibrahim H, Gonaid M, Mojahidul Islam. Comparative phytochemical and antimicrobial investigation of some plants growing in Al Jabal Al-Akhdar. *J. Nat. Prod. Plant Resour*. 2011; 1 (1):15-23.
20. Alaila AK, El Salhin H E, Ali R F, Hasan , HM. Phytochemical screening of some herbal plants (*Menthe*, *Origanum* and *Salvia*) growing at al-gabal al-akhder region- Libya. *International Journal of Pharmacy & Life Sciences*. 2017; (8):4 -11.
21. Ambi A, Abdurrahman E, Sule M, Pateh U, Abdurrahman Y, Ibrahim N. Phytochemical screening and histopathological studies on the seeds of *Citrullus colocynthis* in albino rats. *Nigerian Journal of Pharma Science*. 2007;6(2):7-13.
22. Mohamed A, Amal K, EL-Bettagi H. Antioxidant and antimicrobial properties of kaff maryam (*Anastatica hierochuntica*) and doum palm (*Hyphaene thebaica*). *Grasas Aceites*. 2010; 6(1): 67-75.
23. Gonaid M, Ibrahim H, Al-Arefy H. Comparative chemical and biological studies of *Salvia fruticosa*, *Ocimum basillicum* and *Pelargonium graveolans* cultivated in Al-Jabal Al- Akhdar. *J. Nat. Prod. Plant Resour*. 2012;6(2): 705 -710.



24. Memon U, Brohi H A, Syed WA, Iqbal A, Husan B. Antibacterial screening of *Citrullus colocynthis*”, Pakistan Journal of Pharmaceutical Sciences.2003;16(1):1-6.
25. Sokmen A, Gulluce M, Akpulat H, Daferera D, Tepe B, Polission M. The In vitro antimicrobial and antioxidant activities of the essential oil and methanol extracts of endemic *Thymus spathulifollzs*”, Food Control. 2004;1(5):627-634.
26. Mahsa P, Bokaeian M, Gholamreza K, Malihe R. Evaluation of antioxidant and antibacterial activity on *Citrullus colocynthis* seed extract Bulletin of Environment”, Pharmacology and Life Sciences.2014;3: 59-62.
27. Karaman I, Sahin F, Gulluce M, Oguton H, Sengul M, Adiguzel A. Antimicrobial activity of aqueous and methanol extracts of *Juniperus otycedrus* L, Journal of Ethnopharmacology.2003;8(5):231 -235.
28. Finch RG. Antibiotic resistance a view from the prescriber”, Nature Review Microbiology. 2004;2(12):989-994.
29. Concalves F, Andrade N M, Bezerra J N, Macrae A, Sousa O, FontelesFilho A, Vierira R. Antibacterial activity of guava, *Pisidium guajva* Linnaeus, Leaf extracts on diarrhea – causing enteric bacteria isolated from Seabob shrimps. *Xiphopenaenskroyeri* (Heller). Rev. Inst. Med. Trop. S. Paulo. 2008;50(1):11-15
30. Malaviya A, Mishra N. Antimicrobial activity of tropical fruits. Biological forum. Int. J. 2011;3(1):1-4.
31. Hogue M, Bari M, Inatsu Y, Vijay K, Kawamoto S. Antibacterial activity of Guava (*Pisidiumguajava* L.) and Neem (*Azadirachtaidaica* A Juss.) extracts Against Foodborne Pathogens and Spoilage Bacteria. 2007;4(4):481-488.
32. Ismail M, Minhas PS, Khanum F, Sahnun VM, Sowmya C. Antibacterial (Activity of leaves extracts of Guava (*PisidiumGuajava*) Int. J. Res. Pharmaceut. Biomed. Sci. 2012;3(1):1-2.
33. Lee C. Who’s in the business of saving lives? J. Med. Philos. 2006;31:465-482.
34. El-Kamali H, Mahjoub S A. Antibacterial activity of *francoeuriacrispa*, *pulicariaundulatat*, *ziziphus spina-Christi* and *cucurbitapepo* against seven standard pathogenic bacteria. Ethnobot. Leaflets. 2009;13:722-733.
35. Mohamed Z, Hussein M, Yousry A, Abdel-EL Wahab G. Biological activity of extracts from *Morus alba* L., *Albizialebbeck* (L) Benth. and *CasuarinaglaucaSieber* against the growth of some pathogenic bacteria. Int. J. Agric. Food Res. 2013;2(1):9-22.
36. Chen C, Liu L, Hsu J, Huang H, Yang M, Wang C. Mulberry extract inhibits the development of atherosclerosis in cholesterol fed rabbits. Food Chem. 2005;9(1):601-607.

### المستخلص

ينمو نبات الحنظل في العديد من المناطق الليبية وخاصة الجبل الاخضر ويتم تصنيفه من النباتات الطبية المهمة . في هذه الدراسة تم استخدام مستخلصات بذور وثمار الحنظل كمثبطات لأنواع مختلفة من البكتريا (موجبه جرام) مثل انواع بكتريا (*Staphylococcus aureus* , *Bacillus cereus* and *Streptococcus pneumonia*) وسالبه جرام مثل (*Escherichia coli* and *Proteus vulgaris*). وتم اجراء التحليل الفيتوكيميائي و تقدير محتوى الفينولات الكلية ومضادات الاكسدة في كلا من البذور والثمار لنبات الحنظل. و قد بينت نتائج الدراسة وجود العديد من المركبات الكيميائية في المستخلصات مثل الكربوهيدرات او الجليكوسيدات والفلافونيدات والسيروتويدات والترينينات زالجليكوسيدات القلبية وبنسب متفاوتة في المستخلصات ، كما بينت نتائج الدراسة ان التراكيز المرتفعة من المستخلصات والتي تراوحت ما بين ( 0.1 - 0.8 جرام / 100 مل ) كانت اكثر تثبيط من التراكيز الاقل من المستخلصات والتي تراوحت ما بين ( 0.0001 - 0.1 جرام / 100 مل)، وبينت النتائج ان الثمار احتوت على تراكيز اعلى من الفينولات الكلية ومضادات الاكسدة بالمقارنة مع محتوياتها في البذور. وخلصت الدراسة الي ان وجود مركبات النواتج الطبيعية في عينات نبات الحنظل قيد الدراسة قد تكون هي المسببة في التأثير لمستخلصاتها على انواع البكتريا قيد الدراسة.