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Original article

Efficiency of Cynara Cornigera Fruits on Antibacterial, Antifungal and Its Phytochemical, Anti-Oxidant Screening

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ABSTRACT

The Cynara Cornigera is one of the most plants growing naturally in many of Libyan areas. Its fruits were widely consumed due to interesting taste, so the local population used it in a variety of medical applications. In this the Cynara Cornigera fruits were selected to investigate the efficiency of some extracts (aqueous and alcohol) against Gram-positive bacteria of (Staphylococcus aureus, Bacillus cereus, and Streptococcus pneumonia) and Gram-negative (Escherichia coli and Proteus vulgaris), besides some fungal specie including Aspergilla's niger., also the phytochemical, total phenols and antioxidant activities were detected. The results recorded that the plant fruits containing many natural product constituents such as carbohydrates and/or glycosides, flavonoids, sterols and/or triterpenes, and cardiac glycoside, there are small variations of their contents in aqueous and alcoholic extracts. For the antibacterial activities, higher concentrations of extracts of (0.1 – 0.8 g/ 100 ml) showed high effects compared with lower ones of (0.0001 – 0.1 g/ 100 ml) against the selected bacteria in this study. The contents of total phenols were 648.61 and 178.5 mg/ml in Alcohol and aqueous extracts, respectively, while the anti- oxidant capacity was 45.20 mg/ml. The study concluded that the presence of natural product compounds mainly the main reason for the inhibition of bacteria and the different effects were attributed to the effect of solvent polarizes during the extraction.

Introduction

People from every continent have a long-standing custom of using plants as medicine to treat a variety of illnesses, which dates back to prehistoric times. Long before the existence of bacteria was recognized, people were looking for ways to treat infectious diseases. Many of these herbal medicines were successful in their early attempts, which used natural components, typically native plants or their extracts. A vast array of bioactive chemicals, most likely developed as chemical defenses against infection or predators, as well as antioxidant substances, make plants a rich source of medicinal products [1]. The antibacterial properties of many plants make them useful for treating a variety of ailments and activities [2]. The Mediterranean region is the origin of the ancient herbaceous plant known as artichokes, which is now grown extensively worldwide and is a member of the Asteraceae family. In addition to being consumed as a vegetable, its flower head is used to make a variety of high-value goods, including salad, jam, concentrate, and canned drinks [3]. Flavones, their glycosides, coumarins, sterols, caffeoylquinic acids, and triterpenoid saponins are the most potent substances present in Cynara species [4].

Gaamool is the popular name for Cynara cornigera L. (Asteraceae) in Libya. This shrub is commonly cultivated in Mediterranean nations. The plant family Asteraceae is regarded as one of the significant groups of plants with strong hypoglycemic properties [5]. Originally from the Mediterranean region, wild artichokes (Cynara cornigera) are a member of the Asteraceae family and are now grown extensively throughout [6]. Interesting evidence of protection against degenerative diseases including cancer has been found in artichokes (Cynare sp.). The medicinal properties of the plant, particularly in the management of hepato-biliary dysfunction and digestive disorders, as well as its efficacy in treating hyperlipoproteinemia and irritable bowel syndrome, as well as its choleretic and antioxidant properties, have been confirmed by the widespread use of artichoke parts in folk medicine as astringents, blood cleansers, cardiotonic, detoxifiers, diuretics, hypoglycemic, and hypocholesterolemia [7–8].

Traditional folk medicine has utilized artichoke extracts for their diuretic, choleretic, spasmolytic, and hepatoprotective properties. In light of these biological processes that have been documented. This study aims to Phytochemical screening of *Cynara cornigera* which growing in Al-Gabal AL-Akhder region Libya.

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Also to determine the total phenolic and anti-oxidant activity of the studied plant and to evaluate the antimicrobial activity of methanol extracts from fruits of *Cynara cornigera* used against a variety of micro-organisms causing infectious diseases in humans.

Methods

Selection of medicinal plants for this study

The fruits of *Cynara* were collected from Al gabal Akhder region, Libya. The samples were repeatedly cleaned using tap water and then distilled water. The frits were then cut into little pieces and allowed to dry outdoors, as shown in Figure (1). Omar Al Mukhtar University's Seliphium Herbarium, Botany Department, Faculty of Science, is where the collected samples were identified.

Figure 1. *Cynara cornigera* L fruits

Phytochemical screening

All the Phytochemical screening tests were carried out according to standard methods [9 &10].

Test for sterols and/or triterpines

Libermann-Burchad's test

One ml of the chloroform extract of each sample, 0.3 ml of acetic anhydride were added then followed by a few drops of concentrated sulphuric acid along the side of the dry test tube. 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 was rendered alkaline where a faint yellow color is produced in case of 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 process of making the reagent: Solution A: 10 ml of acetic acid and 40 ml of water are used to dissolve 0.85 g of basic bismuth nitrate. 8 g of potassium iodide in 20 ml of water is solution b. Equal amounts of solutions A and B are combined to create the stock solution. A few drops of chloroformic extract were applied to filter paper, allowed to dry and sprayed with the reagent. 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%, filter, and the hydro-alcoholic clear solution was subjected to the following test: 1ml of the reagent (1% $FeCl_3$) is added to the hydro-alcoholic solution. Blue color develops in cases of the presence of tannins.

Test for carbohydrates and /or glycosides

Water was added to the extracts of the examined herbal plants, and the resulting aqueous extract was then put through the Molish test in the manner described below: On the side of the dry test tube, two ml of concentrated sulphuric acid was added after two ml of the extract and 0.2 milliliters of ethanolic α-naphthol

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(20%) have been combined. When carbs and/or glycosides are present, a violet ring is seen where the two layers converge.

Tests for cardiac glycosides
Using a pipette, one ml of each extract of the tested herbal preparations was dissolved in glacial acetic acid that contained traces of ferric chloride. Concentrated sulphuric acid that contained the same amount of ferric chloride was then placed at the bottom of the test tube. In the presence of deoxy-sugars, the intense blue colour at the surface between the reagents developed for two to five minutes before gradually spreading into the acetic acid layer.

Test for anthraquinones
After adding 1 ml of each extract, shake with either caustic soda or aqueous ammonia. When anthraquinone glycosides are present, the aqueous layer turns rose-red.

Test for saponins
Five ml of tape 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 indicating the presence of saponins.

Estimating total phenols using the Folin-Ciacalteu Method
In a 10-ml flask, aliquots of the extracts were mixed with three ml of distilled water. Next, 0.5 ml of folin ciocalteu reagent (1:1 with water) and 2 ml of Na2CO3 (2%) were added. Following a minute of warming and chilling, the absorbance of the test solutions at 650 nm was measured and compared to the reagent used as a blank. A standard calibration plot was created at 650 nm using known tannic acid values between 4 and 20µg/ml [11].

Antioxidant power determination using the Prussian blue technique
Petroleum ether was utilised to defatten one gm of powder. Then, using 10 ml of methanol and 10 ml of 1% hydrochloric acid: methanol (v/v), the defatted powder was agitated twice in order to extract it. The residue from the vacuum-evaporation of the three combined extracts was dissolved in ten ml of methanol. Three ml of distilled water, three ml of 0.008M K3Fe (CN)6, three ml of 0.1M HCl, and one ml of 1% FeCl3 were added to half a ml of the solution. After five minutes for the blue color to develop, the absorbance at 720 nm is measured in comparison to the blank A [12].

Antimicrobial activity
Preparation of extracts
The whole aerial part of the plants collected were 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 a rotary evaporator and stored at 4^{0C} until further use [13,14].

Microorganisms
The following bacteria and fungus were investigated to determine whether the extracts were effective against them.

Bacterial strains
Gram-positive bacteria
The Department of Microbiology at El-Bayda Hospital provided the three Gram-positive bacteria that were used: Staphylococcus aureus, Bacillus cereus, and Streptococcus pneumoniae.

Gram-negative bacteria
The species of selected bacteria Obtained from the Department of Microbiology 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 has been used to evaluate the antibacterial 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]. Following the addition of 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 investigation to each well, the plates were incubated for 24–48 hours at 37°C for bacteria

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and 48–72 hours at 28°C for fungi. The findings are displayed as the average of the three inhibitory zone measurements. The minimal inhibitory concentration (MIC) values were evaluated by recognized techniques [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 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

Antimicrobial susceptibility to nine antibiotics in vitro (Table 1). Isolated colonies of the microorganism from an overnight nutrient agar plate were added to 2 ml of tryptone soy broth (TSB) to create the inoculums. They dipped a sterile cotton swab into the modified suspension. Excess inoculums were removed from the swab by rotating it multiple times and applying pressure to the tube's inner wall above the fluid level. The sterile Mueller Hinton Agar plate was streaked with the swab all over its surface. Two more streaking were performed, with the plate being rotated roughly each time to guarantee an even dispersion of inoculums.
The antimicrobial discs were applied to the surface of inoculated agar plates using an antibiotic after the plates had been let to dry for five minutes. Following that, the plates were incubated at 37°C for 18 to 22 hours. 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) following NCCLS (2005) [18].

Results

Phytochemical screening studies

The dried powdered plants 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 (1) 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 plants species. The flavonoids not found in the *Cynara cornigera* plant, The anthraquinones were found in high concentration in aqueous extracts compared with alcoholic extracts. The tannins recorded high concentrations in alcoholic extracts in comparing with aqueous extracts. The carbohydrates and/or glycosides are found in high concentrations in alcoholic extracts. The alkaloids were found equal concentrations in aqueous and alcoholic extracts. The cardiac glycosides were found in equal concentrations in aqueous and alcoholic extracts concentration in alcoholic extracts. the cardiac glycosides were found equal concentration in aqueous extracts, and the cardiac glycosides were found equal concentration in aqueous and alcoholic extracts in *Cynara cornigera* fruit plant. The difference in the contents of detected compounds is mainly due to the different polarities of Alcohol and water solvents which mainly directly affect the extraction of active compounds.

Table 1. Phytochemical screening of the studied each plant:

Plant/Chemical Test	*Cynara cornigera*	
	Extract	
	Aq	Al
Saponins	_	_
Tannines	++	+++
Carbhydrate and/or Glycosides	++	+++
Alkaloids	+	+
Flavonoids	_	_
Anthraquinones	++	+
Steroids and/or Triterpenoids	/	++
Cardiac Glycosides	+	+

(+) Present, (-) Absent, (/) Not done.

Total phenols

The concentrations of total phenols contents in plant extracts were determined using Folin Ciacalteu Method the obtained observations recorded that the total phenols content was found to be in methanol extracts of *Cynara cornigera* was high of value 648.61mg/ml comparing to its content of aqueous extracts 278.5 mg/l. It has been approved that the antioxidant properties of medicinal plants and other botanical materials are due to phenolic and flavonoid components [19,20].

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Antioxidant
Evaluation of the antioxidant activity by using prussian blue method. The obtained observations were found to have Antioxidant potential of fruits *cynara cornigera* 45.207 mg/ml immense antioxidant potential which can be correlated to its diverse medicinal values.

Antimicrobial activity
The antimicrobial activity studies were carried out on solvent 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 (1&2)**.**

Gram positive bacteria
Table 2 demonstrated the impact of varying quantities of the plant extracts under investigation on the gram-positive bacteria (Streptococcus pneumonia, Bacillus cereus, and Staphylococcus aureus) used for this investigation. Except for Cynara cornigera fruits, the results indicated that the inhibition zone and MIC in all extracts were 0.01 g/ml. Similar results were observed by [21]. By applying different concentrations of (0.0001 -0.8 g/ml). The values of minimum inhibition concentration showed different values of effect, for the *Staphylococcus aure*us bacteria the values of MIC ranged between (0 –30 mm) whereas, for the bacteria, the values of MIC ranged between (0 – 25 mm) and the MIC were (0 –35 mm). The current findings support a study by certain authors [22] that found that the extract affected both gram-positive and gram-negative bacteria. The investigated plant extract's various concentrations against Streptococcus pneumonia are displayed in Table (2). Results showed that inhibition zone and MIC at 0.1 g/ml for *c.colocynthis* seed, while for fruits of *Cynara cornigera*, 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 observed by study [23].

Table 2. Antimicrobial activities of different concentrations of studied plants extract against staphylococcus aureus

Bacteria Concentration	***staphylococcus aureus***	***Bacillus cereus***	***Streptococcus pneumoniae***
0.8 g/ml	30	25	35
0.4g/ml	28	20	32
0.2 g/ml	26	16	30
0.1 g/ml	24	10	26
0.01 g/ml	3	3	5
0.001g/ml	N. A	N. A	N. A
0.0001g/ml	N. A	N. A	N. A
0.00001g/ml	N. A	NA	N. A

Gram negative bacteria
Escherichia coli
Table (3) different concentrations of the studied plant extract against *Escherichia coli* were tested. The results showed that the inhibition zone at 0.1 g/ml for seeds C*itrullus colocynthis*, while found for fruits of C*itrullus colocynthis*, the inhibition zone of 0.01g/ml was found for fruits of *Cynara cornigera*, and MIC was 0.001 g/ml. Similar results were observed by [21]. Table (3) showed different concentrations of the studied plant extract against *Proteus vulgaris* were tested. The results showed that the inhibition zone and MIC of all extracts 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 indicating that it kills bacteria according to the structure of their cell walls, However, some studies found that either gram-positive bacteria are more susceptible than gram-negative bacteria, or the inverse is true [24].

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Table 3. Antimicrobial activities of different concentrations of the studied plant extract against Escherichia coli and Proteus vulgaris

Bacteria concentration	*Escherichia coli*	*Proteus vulgaris*
0.8 g/ml	32	20
0.4g/ml	30	18
0.2 g/ml	26	16
0.1 g/ml	25	11
0.01 g/ml	3	NA
0.001g/ml	2	NA
0.0001g/ml	N. A	NA
0.00001g/ml	N. A	NA

Antifungal activity

Extracts from all of the plants under study did not affect fungi; only Aspergilla's niger is affected. According to certain research, volatile chemicals found in plants, particularly essential oils, have antibacterial, fungicidal, and insecticidal properties.

Antibiotic Sensitivity

Table 4 displays the sensitivity rates of both Gram-positive and Gram-negative bacteria. The findings indicated that S. aureus's sensitivity pattern was resistant to CTX and TIC and sensitive to K, CN, C, MEZ, SXT, TE, and OFX. Figures 2–6 show that B. cereus was resistant to CTX, TIC, MEZ, SXT, TE, and OFX, but sensitive to K and CN.C. K, CTX, TIC, SXT, TE, and OFX were ineffective against S. pneumoniae, while CN, C, and MEZ were effective against it. CN, CTX, TIC, SXT, TE, and OFX did not affect E. coli, but K, C, and MEZ did. Additionally, P. vulgaris showed resistance to all antibiotics, including K, CN, C, CTX, TIC, MEZ, SXT, TE, and OFX. The international medical and research community acknowledges that antimicrobial resistance, which is caused by bacteria that develop resistance to antibiotics, is a critical issue that sparks intense discussions. 25

Table 4. Antibiotic Sensitivity testing

Antibiotic	Symbol	Concentration	Organism				
			S.aureus	*B.cereus*	*S.pneumoniae*	*E.coli*	*p.vulgaris*
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.

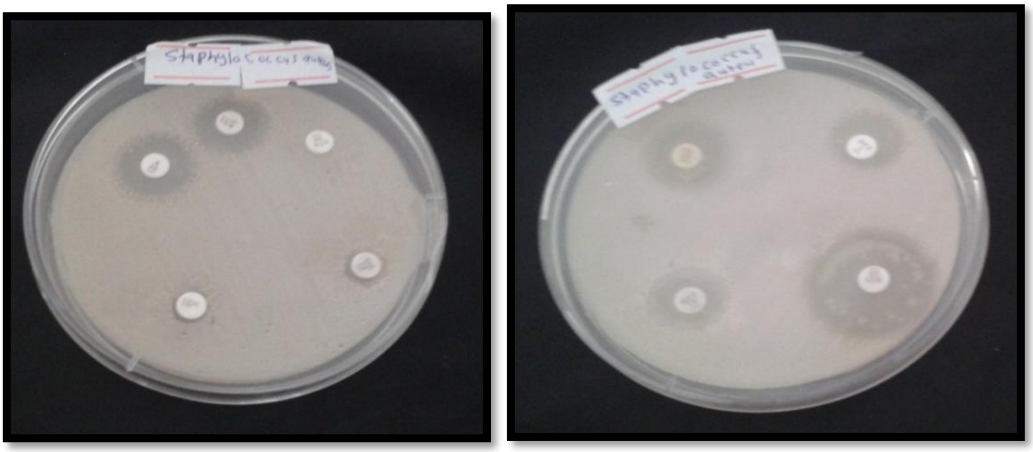

Figure 2. Antibiotic Sensitivity testing of Staphylococcus aureus.

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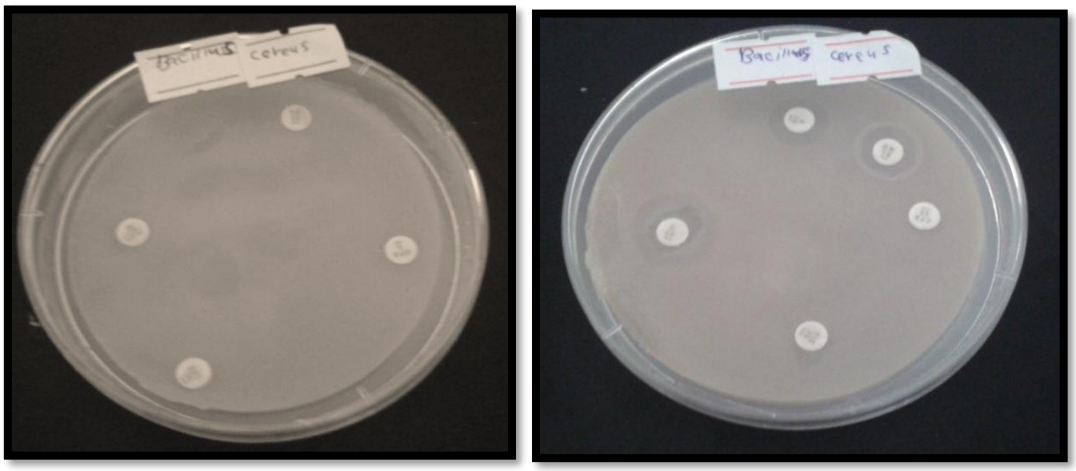

Figure 3. Antibiotic Sensitivity testing of Bacillus cereus.

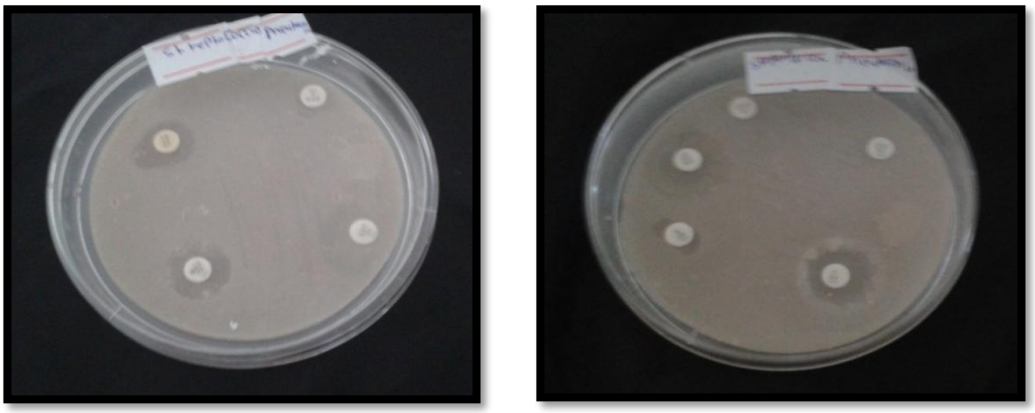

Figure 4. Antibiotic Sensitivity testing of Streptococcus pneumoniae.

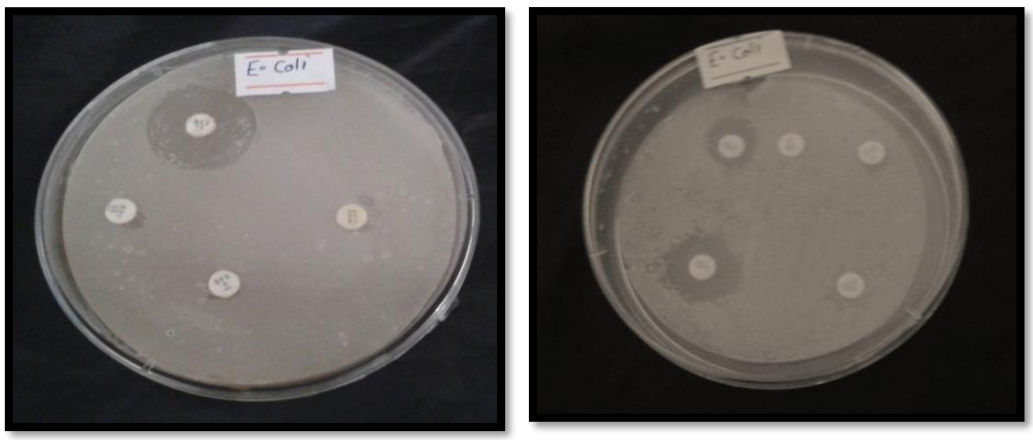

Figure 5. Antibiotic Sensitivity testing of Escherichia coli.

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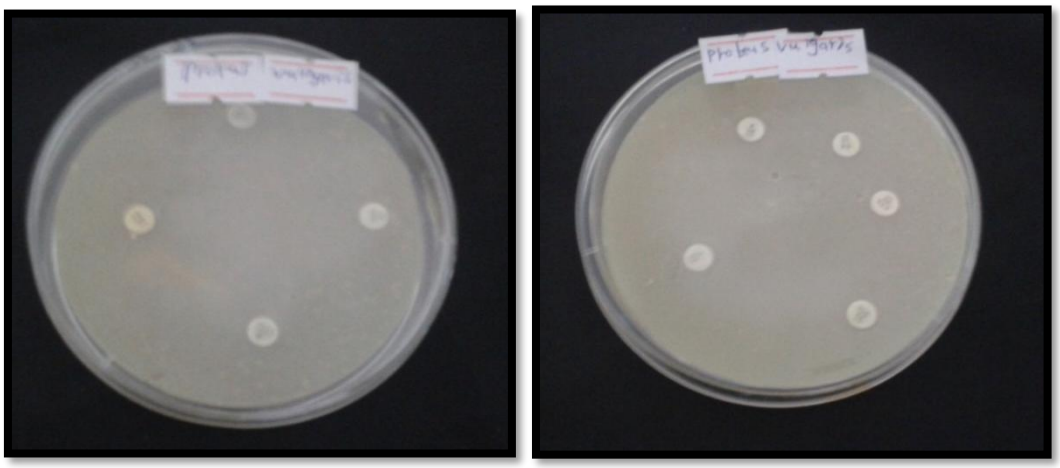

Figure 6. Antibiotic Sensitivity testing of Proteus vulgaris.

Discussion

In this study the fruits were utilized as antibacterial and antifungal inhibitors, also the antioxidant, total phenols, and phytochemical properties were investigated. The results showed that there are very important compounds, such as carbohydrates and/or glycosides, sterols and/or triterpenes, and cardiac glycoside which give high health value for this fruit, in addition presence of important values of antioxidant and phenol compounds. The results indicated that the extracts of *Cynara Cornigera* showed antibacterial activity at a high concentration of (0.1-0.8 mg/ml) compared to lower concentrations of extracts (0.00001- 0.1). this effect is attributed to the effect of natural product compounds observed in the extracts, our finding was stated in many studies [26-30] which stated that the presence of chemical compounds in plant extracts gave antibacterial activities. The main effect is due to these compounds attacking the membrane of the bacteria wall, this also depends on the concentration of active material in the extracts. In this study, the lower concentration does not have any effect, whereas the higher concentrations showed an antibacterial effect due to the presence of high quantities of active material such as phenols.

Conclusion

According to the obtained results, the fruits of *Cynara Cornigera* plant grown in wide areas of Libya have antibacterial activities against a wide of pathogenic bacteria. Also, very important compounds have anti-oxidant capacity and phenols were recorded in the studied fruits.

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يُعد نبات القنطريون من أكثر النباتات نموًا طبيعيًا في العديد من المناطق الليبية. وقد استُهلكت ثماره على نطاق واسع نظرًا لمذاقه اللذيذ، مما دفع السكان المحليين إلى استخدامه في تطبيقات طبية متنوعة. وفي هذا الصدد، تم اختيار ثمار القنطريون لدراسة فعالية بعض المستخلصات (المائية والكحولية) ضد البكتيريا موجبة الجرام (المكورات العنقودية الذهبية، والعصوية الشمعية، والعقدية الرئوية)، وسالبة الجرام (الإشريكية القولونية والبروتيوس الشائع)، بالإضافة إلى بعض أنواع الفطريات، بما في ذلك الرشاشيات السوداء. كما تم الكشف عن النشاط الكيميائي النباتي، والفينولات الكلية، ومضادات الأكسدة. سجلت النتائج أن ثمار النبات تحتوي على العديد من مكونات المنتج الطبيعي مثل الكربوهيدرات و/أو الجليكوسيدات والفلافونويدات والستيرولات و/أو التربينات والجليكوسيدات القلبية، وهناك اختلافات طفيفة في محتوياتها في المستخلصات المائية والكحولية. بالنسبة للأنشطة المضادة للبكتيريا، أظهرت التركيزات الأعلى من المستخلصات (0.1 - 0.8 جم / 100 مل) تأثيرات عالية مقارنة بالتركيزات المنخفضة (0.0001 - 0.1 جم / 100 مل) ضد البكتيريا المختارة في هذه الدراسة. كان محتوى الفينولات الكلية 648.61 و 178.5 ملجم / مل في الكحول والمستخلصات المائية على التوالي، بينما كانت السعة المضادة للأكسدة 45.20 ملجم / مل. وخلصت الدراسة إلى أن وجود مركبات المنتج الطبيعي هو السبب الرئيسي في تثبيط البكتيريا وأن التأثيرات المختلفة تُعزى إلى تأثير استقطاب المذيبات أثناء الاستخلاص.

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