

Evaluation of the Antimicrobial Activity of the Methanolic Extract of *Citrullus colocynthis* Fruit Against Pathogenic Bacterial and Fungal Strains

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Abstract:

This study aimed to evaluate the antimicrobial activity of the ethanolic extract of *Citrullus colocynthis* against selected bacterial and fungal strains. The plant extract was obtained using ethanol as a solvent to extract the bioactive compounds, and its effect on the tested microorganisms was evaluated using agar diffusion technique. The results obtained revealed a significant inhibitory activity against the tested bacteria and fungi, suggesting its potential as a natural source of antimicrobial agents. These results were interpreted by analyzing the chemical composition of the extract by GC-MAS analysis, highlighting its potential applications in the medical and agricultural fields.

Keywords: *Citrullus colocynthis*, active compounds, GC-MAS analysis

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INTRODUCTION

Finding natural alternatives with antimicrobial properties is becoming more popular and widespread, due to the rapid development of antibiotic and antifungal resistance. Bitter gourd has been used for centuries as an herbal remedy as an anti-worm and anti-diuretic. *Colocynthis* has antifungal and antibacterial properties. Research proving the antibacterial efficacy of bitter gourd is still insufficient. Many countries use bitter gourd as a remedy in their traditional medicine. Several studies have shown that bitter gourd fruits contain biological activity due to the presence of phenolic, triterpenoid, steroidal and flavonoid chemicals directly related to it (1,2). *Colocynthis* is a plant grown and found as a natural desert plant in many countries around the world. There are many theoretical foundations and cultural motivations that justify the study and exploitation of the potential medicinal properties of the plant, in addition to historical evidence that confirms its therapeutic benefits. A number of studies conducted on the *colocynthis* plant have proven through chemical analysis that it contains chemical compounds, including flavonoids in large quantities, which

have antibacterial and antifungal properties. Other compounds contained in the plant include alcohol, tannins, and other compounds that may act as an anti-inflammatory and antibacterial product, which has made it possible to use it in traditional medicine. (3,4,5,6)

Bitter gourd is a perennial plant belonging to the Cucurbitaceae family and grows and thrives in sandy and rocky soils in the Mediterranean basin regions including Egypt, Turkey, Tunisia, Palestine, and also grows in the deserts of Saudi Arabia, Yemen, and Iran. Its dried leaves contain steroidal compounds and diterpenes that have biological properties, which helps in its traditional use in the treatment of fever, general weakness, asthma, hemorrhoids, wound healing, joint and muscle pain, and in the treatment of bacterial infections. Ethanol and methanolic extracts of bitter gourd, in addition to its steroidal compounds, have shown antibacterial and antifungal activity, and their effectiveness against various pathogens has been tested. A difference in the biological effect of the active compounds has been observed according to the mechanism of action of each compound (7,8,9). Bitter melon is a valuable medicinal plant due to its high nutritional value and the large number of active compounds with biological effects. Recently, there has been great interest from consumers around the world due to their knowledge of the unique biological properties of this plant, which has created great opportunities in the field of treatment with medicinal plants, in addition to its use in the food industry, where it plays an important role in food technology. (10).

Citrullus colocynthis seeds contain about 23-25% golden yellow oil, with 70% unsaturated fatty acids, including 51% polyunsaturated fatty acids (11). Ripe fruits have high moisture content exceeding 90% of their total weight, *C. colocynthis* contains essential amino acids, with glutamic acid and arginine identified at concentrations of 19.8 g/100 g and 15.9 g/100 g of protein, respectively, along with aspartic acids, serine, and glycine (12). According to another study, the moisture content of the seeds was 4.91 g/100 g, while the protein and ash were recorded as 13.19 g/100 g and 2.00 g/100 g, respectively, It is also rich in minerals, with the seeds being a major source of potassium (569 mg/100 g) and calcium (465 mg/100 g), in addition to high levels of magnesium and phosphorus, while lower levels of iron and zinc are found these nutrients contribute to the electrolyte balance and alkalinity of the body, enhancing its nutritional value for consumers (13).

(14) reported the effect of ethanolic extract of bitter apple fruits investigated using simple diffusion technique as well as disk diffusion technique. The results showed that it had antimicrobial effects on *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella pneumoniae*. The results also showed that the ethanolic extract taken from the pulp was more active against certain strains of Gram-positive bacteria. The seed extract showed slightly lower activity against Gram-positive and Gram-negative bacteria. (15) reported high antimicrobial effect in fully ripe bitter apple seeds and fruits. *C. colocynthis* extracts prepared in different solvents were tested for antimicrobial effects against *Salmonella*, *Staphylococcus aureus*, *Bacillus* spp., *Proteus vulgaris*, and *Pseudomonas* spp. This study aimed to know the extent of benefiting from *colocynthis* to investigate the effect of its components on various microorganisms, such as bacteria and fungi

MATERIALS AND METHODS

1. Collection of plant samples:

The *colocynthis* fruits were collected, washed with distilled water and dried in an oven at 40°C until the weight was constant, then ground in an electric grinder and converted into powder.

2. Extract preparation:

The alcoholic extract was obtained by weighing 50 grams of the powder obtained from the previous step and placing it in a 1000 ml beaker, adding 500 ml of 70% ethyl alcohol, closing the beaker tightly, shaking the mixture well, then placing it in an electric shaker at room temperature for 48 hours. After that, the mixture was filtered through several layers of gauze, and the filtrate was taken and filtered in filter paper. The resulting liquid was filtered in a centrifuge, and the liquid was collected in opaque glass containers and stored in the refrigerator. (16)

3. Microorganisms tested: Bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

Fungi: *Candida albicans*, *Aspergillus niger*.

4. Antimicrobial testing: The agar well diffusion method was used, where holes were created in the agar plates inoculated with the test organisms using a cork piercer, and then plant extracts were added at different concentrations (25, 50, and 100 mg/ml). Ampicillin was used as a positive control for bacteria, while fluconazole was used for fungi, and ethanol was the negative control. The plates were incubated at 37°C for 24 h for bacteria, and 28°C for 48 h for fungi, to evaluate the antimicrobial activity (17)

5. Identification of active compounds in plant samples using GC-MS technology: The concentration of active compounds in the leaves was estimated using gas chromatography coupled to mass spectrometry (GC-MS). Compounds were identified through the National Institute of Standards and Technology (NIST) database by comparing the resulting spectra of unknown compounds with reference spectra stored in the NIST library.

6. Estimation of some chemical components in the plant: The phenols, flavonoids, alkaloids, terpenes, saponins, tannins, DPPH Radical Scavenging, ABTS Radical Scavenging, proteins, FRAP (Ferric Reducing Power), carbohydrates, Ash and moisture content were determined according to the methods used in (18,19,20,21,22,23,24,25,26,27,28,29) each of them, respectively.

7. Data analysis: Statistical analysis was performed using Genstat software. (30)

RESULTS:

Table 1 shows the variation in the inhibitory activity of the plant extract against the tested bacterial and fungal strains, where a gradual increase in the inhibition diameters was observed with increasing extract concentration. *Staphylococcus aureus* showed remarkable sensitivity to the extract, with an inhibition diameter of 18 mm at the highest concentration, while *Pseudomonas aeruginosa* was the least responsive, with an inhibition diameter of no more than 8 mm.

With regard to fungi, *Candida albicans* recorded the highest response, with an inhibition diameter of 20 mm at 100 mg/ml, indicating high efficacy against it, while *Aspergillus niger* showed a lower response, indicating its low susceptibility to the extract.

When compared to standard antibiotics such as ampicillin and fluconazole, the extract showed less activity, but was still effective in inhibiting microbial growth to varying degrees, enhancing its potential as an antimicrobial agent. The passive solvent (ethanol) did not record any inhibitory effect, confirming that the observed efficacy is due to the active compounds in the extract and not to the solvent used.

Table 1: Effect of different concentrations of alcoholic extract of colocynth fruits on the inhibition diameters (mm) of microorganisms

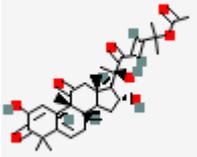
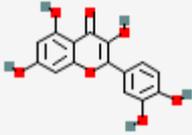
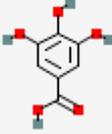
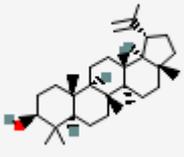
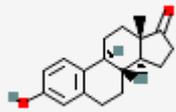
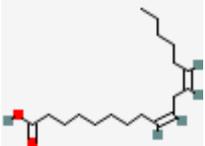
Con. / Microorganism	25 mg/mL	50 mg/mL	100 mg/mL	positive Control (mm)	Negative Control (mm)	micr.averg
bacteria				Ampicillin	Ethanol	
<i>Escherichia coli</i>	8	10	12	20	0	9.93
<i>Staphylococcus aureus</i>	12	15	18	25	0	14.00
<i>Pseudomonas aeruginosa</i>	6	7	8	22	0	8.60
fungi				Fluconazole		
<i>Candida albicans</i>	15	18	20	28	0	16.20
<i>Aspergillus niger</i>	10	12	15	24	0	12.20
Con. averg	10.20	12.40	14.53	23.80	0	
L.S.D.	C:0.1694	F:0.1694	F*C:0.3787			

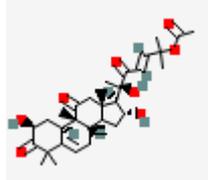
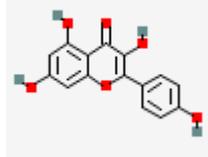
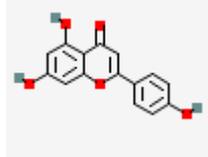
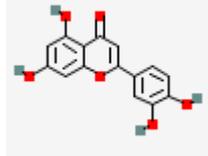
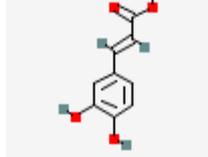
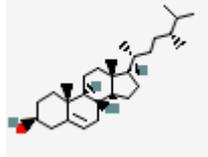
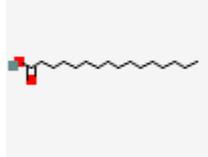
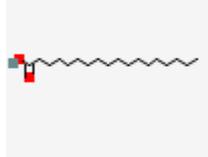
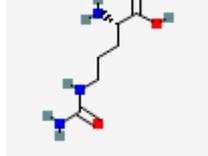
Qualitative analysis of chemical compounds in the alcoholic extract of colocynth fruits using GC-MS technique

Chemical analyses in Table (2) showed the presence of a variety of bioactive compounds, which belong to a wide chemical group, such as phenols, alkaloids, phenolic acids, flavonoids, triterpenoids, fatty acids and steroids, reflecting the chemical diversity of the alcoholic extract of colocynth fruits.

A number of chemical compounds present in the alcoholic extract, as the table shows the presence of 21 chemical compounds and that the highest peak area of the alcoholic extract was for the Cucurbitacin E compound with an area of 15.2 at minute 5.23 and the lowest peak area was for the Stearic Acid compound with an area of 1.1 at minute 12.45 and that the compounds varied in their time of appearance and that there were compounds that appeared repeatedly at different time periods

Table 2: GC-MS Analysis of Alcohol Extract of colocynth fruits

Peak No.	Area	Retention Time (min)	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Chemical Structure
1	15.2	5.23	Cucurbitacin E	$C_{32}H_{44}O_8$	556.70	
2	12.8	7.45	(Quercetin) Flavonoid	$C_{15}H_{10}O_7$	302.24	
3	10.5	9.12	(Colocynthin) Alkaloid	$C_{38}H_{54}O_{13}$	718.8	
4	8.7	11.34	(Gallic Acid) Phenolic Acid	$C_7H_6O_5$	170.12	
5	7.3	13.56	(Lupeol) Triterpenoid	$C_{30}H_{50}O$	426.72	
6	6.9	15.78	(Estrone) Steroid	$C_{18}H_{22}O_2$	270.4	
7	5.4	18.23	(Linoleic Acid) Fatty Acid	$C_{18}H_{32}O_2$	280.45	

8	3	10.4	(Cucurbitacin B) Triterpenoid	$C_{32}H_{46}O_8$	558.70	
9	4.5	5.67	(Kaempferol) Flavonoid	$C_{15}H_{10}O_6$	286.24	
10	5.8	12.7	(Apigenin) Flavonoid	$C_{15}H_{10}O_5$	270.24	
11	1.3	8.34	(Luteolin) Flavonoid	$C_{15}H_{10}O_6$	286.24	
12	1.2	4.97	(Caffeic Acid) Phenolic Acid	$C_9H_8O_4$	180.16	
14	1.3	11.82	(Campesterol) Steroid	$C_{28}H_{48}O$	400.69	
15	1.6	18.54	(Palmitic Acid) Fatty Acid	$C_{16}H_{32}O_2$	256.43	
16	1.1	12.45	(Stearic Acid) Fatty Acid	$C_{18}H_{36}O_2$	284.48	
17	2.1	18.21	(Citrulline) Amino Acid	$C_6H_{13}N_3O_3$	175.19	

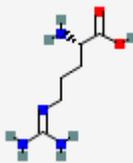
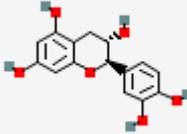
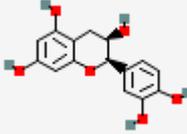
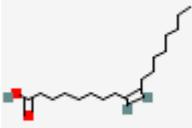
18	4.2	15.72	(Arginine) Amino Acid	C₆H₁₄N₄O₂	174.20	
19	1.6	14.3	(Catechin) Flavonoid	C₁₅H₁₄O₆	290.27	
20	3.3	12.6	(Epicatechin) Flavonoid	C₁₅H₁₄O₆	290.27	
21	2.2	6.48	(Oleic Acid) Fatty Acid	C₁₈H₃₄O₂	282.47	

Table 3 shows the results of the chemical analysis of the alcoholic extract of colocynth showed that it contains a high percentage of phenolic compounds (45.6 mg GAE/g) and flavonoids (22.3 mg QE/g), indicating that these compounds have antioxidant activity, which was also confirmed by the results of the DPPH test (85.3%) and ABTS (78.9%). The analysis also revealed the presence of percentages of alkaloids (12.8 mg/g), terpenoids (18.4 mg/g), saponins (9.7 mg/g) and tannins (7.2 mg/g). On the other hand, the extract showed a high reducing capacity according to the FRAP test (62.7 μ M Fe²⁺/g), while the protein content reached (5.4 mg/g) and carbohydrates (14.2 mg/g), and the ash and moisture content recorded a percentage of (3.8%) and (6.5%), respectively.

Table 3: Chemical components in alcoholic extract of colocynth fruits

Parameter	Result (mg/g)	Reference standard	importance
Total Phenolic Content	45.6 mg GAE/g	Gallic Acid	High phenolic content is associated with antioxidant activity.
Total Flavonoid Content	22.3 mg QE/g	Aluminum Quercetin	Flavonoids have antimicrobial properties.
Total Alkaloid Content	12.8 mg/g	Atropine	Alkaloids are antimicrobial compounds.
Total Triterpenoid Content	18.4 mg/g	Ursolic Acid	Terpenoids have anti-inflammatory activity.
Total Saponin Content	9.7 mg/g	Diosgenin	Saponins have antifungal and antibacterial properties.
Total Tannin Content	7.2 mg/g	Tannic Acid	Tannins are astringents and have antibacterial properties.
DPPH Radical Scavenging	85.3%	Ascorbic Acid	Possesses strong antioxidant properties.

ABTS Radical Scavenging	78.9%	Trolox	Possesses antioxidant activity.
Total Protein Content	5.4 mg/g	BSA	Biological activity is related to the presence of proteins.
FRAP (Ferric Reducing Power)	62.7 $\mu\text{M Fe}^{2+}/\text{g}$	Ascorbic Acid	The extract has the ability to reduce.
Total Carbohydrate Content	14.2 mg/g	Glucose	Carbohydrates enhance bioavailability.
Ash Content	3.8%	-	An indicator of mineral content in the extract.
Moisture Content	6.5%	-	The amount of water in the extract and its dryness percentage.

DISCUSSION:

From the results of table (1) The study showed that the plant extract had an inhibitory effect on the growth of a group of pathogenic bacteria and fungi, with its effectiveness increasing with increasing concentration used. *Staphylococcus aureus* was the most responsive among the bacteria, indicating that the active compounds in the extract may be more effective on Gram-positive bacteria than Gram-negative bacteria, which is in line with previous studies that indicated that the thick cell wall of Gram-positive bacteria may be more permeable to the active compounds in the alcoholic extract (31). On the other hand, the extract showed activity against fungi, especially *Candida albicans*, indicating the possibility that the extract contains compounds with an antifungal effect, such as flavonoids or terpenoids, which have previously been recorded to be effective against pathogenic fungi and are known to disrupt microbial cell membranes and inhibit growth. (32). *Aspergillus niger* showed a lower response, which may be related to the ability of these fungi to produce enzymes that degrade some of the active compounds. The antimicrobial activity may be due to disruption of cell wall synthesis, interference with protein synthesis, or inhibition of enzymatic activity in the microorganism (33).

The compounds in Table (2) have antioxidant properties such as quercetin, catechin, epicatechin and gallic acid, indicating the ability of the extract to reduce oxidative stress. Some compounds such as cucurbitacin B have also shown anticancer activity, which may make them important in future pharmacological studies (34). Other compounds such as luteolin, beta-sitosterol and caffeic acid have antimicrobial activity and may contribute to inhibiting the growth of bacteria and fungi, which contributes to the possibility of using them in natural anti-infective treatments. Compounds such as fatty acids such as linoleic acid and oleic acid have anti-inflammatory properties, which enhances their importance in anti-inflammatory therapeutic applications (35). The results of the chemical analysis of the extract showed that it contains a high percentage of phenolic compounds and flavonoids, which reflects an antioxidant activity test, which is also confirmed by the results of the DPPH and ABTS tests, as these compounds contribute to the elimination of free radicals and the reduction of oxidative stress (36).

The analysis also revealed the presence of moderate concentrations of alkaloids, terpenoids, and saponins, which are associated with antimicrobial and anti-inflammatory properties. Tannins contribute to the astringent and antibacterial effects, which supports the traditional uses of the plant in topical treatments. (37). The protein and carbohydrate content also indicate the possibility of enhancing the bioavailability of the active compounds. As for the ash and moisture content, they reflect the percentage of minerals and dryness in the extract, which may affect its stability and storage (38).

Phytochemical analysis revealed biologically active components in the plant, with aqueous extracts being rich in tannins/phenolic compounds, while methanolic extracts were rich in flavonoids/terpenoids. Antifungal activity was

observed against *Candida albicans* (17.3 ± 0.7 mm) and *Aspergillus niger* (14.4 ± 0.8 mm) at a concentration of 10 mg/ml (39). Colocynthis extract demonstrated broad-spectrum antibacterial activity against all five tested strains (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Escherichia coli*), with inhibition zones ranging from 19.0 mm (*Staphylococcus aureus*) to 31.0 mm (*Bacillus subtilis*), highlighting its broad-spectrum potential. These multi-targeted results strongly support the use of *C. colocynthis* seed oil in the development of novel therapeutic agents (40).

CONCLUSION:

We conclude from the results of the study that the plant extract has promising antimicrobial activity, as it showed remarkable efficacy against some bacterial and fungal strains, especially *Staphylococcus aureus* and *Candida albicans*. Its presence of active compounds, such as flavonoids and terpenoids, enhances its potential in medical and pharmaceutical applications. However, further studies are still needed to isolate the active compounds, understand their mechanism of action, and evaluate their safety and efficacy in living models. This extract may contribute to the development of natural antimicrobial preparations, whether for medical, agricultural or food preservation use, opening new horizons for future research.

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