The Antioxidant and Antibacterial Activity of *Moringa oleifera* Extracts against some Foodborne Pathogens

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Abstract

The aim of this study is to determine the antioxidant and antibacterial activity of *Moringa oleifera* extracts. Maceration and Soxhlet apparatus were used to prepare aqueous and methanolic extracts respectively, while petroleum ether was used to extract seed oil. Many tests were conducted include, phytochemical detection, evaluation of antioxidant activity utilizing 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay, total phenolic content. The extracts of *Moringa oleifera* were investigated against some foodborne pathogens include *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Klebsiella pneumonia.* The results of phytochemical test showed the presence of alkaloids, phenols, flavonoids, tannins, saponins and glycosides in aqueous and methanolic leaves extracts. The antioxidant activity showed that the aqueous and methanolic leaves extract was more effective than the seed's husk extract. The radical scavenging capacity (EC_{50}) of methanolic and aqueous leaves extract were found to be (1.6 and 1.9 mg/ml) respectively, while the EC50 of methanolic and aqueous seed's husk were (5.1 and 10 mg/ml) respectively. The antibacterial activity of *Moringa oleifera* extracts showed the best effect was seen in the aqueous and methanolic leaves extract on *Staph. aureus* in 200 mg/ml with inhibition zone 14.83 and 22.6 mm respectively. The results of the minimum inhibitory concentration (MIC) showed that the methanolic leaves extract was 16 mg/ml for both *Staph. aureus* and *E. coli*, and 32 mg/ml for *B. cereus* and *K. pneumonia*, while the MIC of aqueous leaves extract was 64 mg/ml for all bacterial isolates.

Keywords: *Moringa oleifera extracts, antibacterial activity, MIC, DPPH, Total phenol.*

Introduction

Moringa oleifera is a plant belongs to the family moringaceae which consists of one genus moringa [1]. It is a tree with a high value, distributed in many countries of the tropics and subtropics. The tree has different argot names such as marango, moringa, horseradish tree, drumstick tree, miracle tree, tree of life [2]. Almost all parts of the plant are used culturally for its nutritional value, purported medicinal properties and for taste and flavor as a vegetable and seed. The leaves of moringa oleifera can be eaten fresh, cooked or stored as dried powder for many months without any major loss of its nutritional value. Epidemiological studies have indicated that *moringa oleifera* leaves are a good source of nutrition and exhibit anti-tumor, anti-inflammatory, anti-ulcer, anti-atherosclerotic and anti-convulsant activities [3]. The leaves of *moringa oleifera* are a good source of natural antioxidants due to the presence of different compounds such as ascorbic acid, flavonoids, phenolics and carotinoids. These compounds have the ability to do numerous functions including acting as free radicals scavengers, enzyme inhibitors, reduce damage caused by free radical activity and oxidation [4]. Medicinal plants are a source for a wide variety of natural active compounds and are used for the treatment of diseases

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throughout the world. Several types of plant extracts or plant-derived molecules have been investigated for their potential as antibacterial and antioxidant sources against several diseases [5, 6, 7]. Thus, main purpose of this research is detection of the active compounds in *Moringa oleifera* cultivated in Iraq and evaluate the antibacterial activity on some foodborne pathogens.

Materials and Methods

Collection of *Moringa oleifera*

Moringa oleifera seeds and leaves were obtained from the plantation of Al-Diwaniyah city, Iraq. The plant materials were botanically identified by the Laboratory of College of Science of the University of Baghdad. The leaves were washed with water and dried at room temperature, and ground using a grinder, and then stored at 4ºC for further analysis. The seeds were shelled by using mortar and pestle. The husk and kernel were ground separately to a fine powder and stored at 4ºC for further analysis.

Preparation of aqueous extract

The aqueous extract was prepared according to [8]. Macerated 100 gram of *Moringa oleifera* leaves in 700 ml of distilled water for 72 hours, after extraction, the mixture was vacuum filtered through Whitman No. 1 paper. The filtrate evaporated to dryness under vacuum at 50°C by a rotary evaporator to eliminate water. The resulting extract stored in amber glass vials at 4 °C until analyzed.

Preparation of methanolic extract

Methanolic extract was prepared according to [9], using Soxhelt apparatus. 100 gram of *Moringa oleifera* leaves was put in a thimble and 350 ml of 70% methanol was added within 40-60 °C for 6 hours. The solution was filtered through a filter paper Whitman No.1 and evaporated to dryness under vacuum at 40°C by a rotary evaporator to get rid of methanol; the extract was stored in amber glass vials at 4 °C until analyzed.

Phytochemical screening

The phytochemical screening of the aqueous and methanolic leaves and seed extracts has been done according to [10, 11, 12, 13, 14].

Evaluation of the Antioxidant activity DPPH assay

The radical scavenging activity of samples was determined according to [15]. 5ml of a freshly prepared 0.004% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was mixed with 100 µl of different concentrations (2, 4, 6, 8 and 10) mg/ml of the methanolic and aqueous leaves and seeds extracts, and (20, 40, 60, 80 and 100) µl \ml of the seed oil extract, then the mixture was left to stand for 30 min. The absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) (artificial antioxidant) and vitamin C (natural antioxidant) were used as positive control. All tests were performed in triplicate. The percentage of DPPH reduction was calculated as:

% Reduction = (Abs DPPH – Abs Dil.) / Abs DPPH × 100

Where:

Abs DPPH = average absorption of the DPPH solution

Abs Dil. = average absorption of the three absorption values of each dilution.

With the obtained values, a graphic was made using Microsoft Excel. The EC_{50} of each extract (concentration of extract or compound at which reduced 50% of DPPH) was taken from the graphic.

Determination of total phenolic contents

Total phenolic content of *Moringa oleifera* extracts were determined spectrophotometrically using the Folin-Ciocalteu method described by [16]. 2 ml of Folin-Ciocalteu reagent (diluted 10 times) was mixed with 1.6 ml of 7.5% sodium carbonate solution and 0.4 ml of *Moringa oleifera* extracts. The volume was completed to 5 ml by adding distilled water. The tubes were covered with parafilm for 30 min. at room temperature, and then the absorbance was read at 760 nm spectrophotometrically.

Bacterial isolates

Bacillus cereus, *Staphylococcus aureus*, *Escherichia*

coli and *Klebsiella peneumonia*e isolated from food, obtained from the Department of Food Sciences- College of Agricultural Engineering Sciences- University of Baghdad, and emphasize diagnoses by using VITEK-2 System.

Agar well diffusion method

Agar well diffusion method was employed for the determination of this study. Muller- Hinton agar plates were swabbed (sterile cotton swabs) with broth culture of respective bacteria. Wells 6 mm diameter was made in each of these plates using a sterile cork borer. 100 µl from each concentration (50 , 100 and 200 mg/ml) of the methanolic and aqueous leaves and seeds extracts and (125 , 250 and 500 µl/ ml) of the seed oil extract were put in each hole by using micropipette and allowed to diffuse at room temperature for 30 min. The plates were incubated at 37 ^{o}C for 18-24 hours. All tests were performed in triplicate. The diameter of any resulting zone of inhibition was measured in millimeters [17].

Determination of the minimum inhibitory concentration (MIC) of the *Moringa oleifera* extracts

Broth Microdilution method was used to determine the MIC of *Moringa oleifera* extracts using the 96-well microtiter plate. The extracts were prepared in a double concentration, the desired final concentration as it will be diluted with an equal amount of bacteria in broth. 200 µl of the prepared methanolic and aqueous extracts (for leaves and seeds) were introduced into the first wells in columns 1-4 (in row A). Rows B-H in columns 1-4 had 100µl of broth alone while rows A-H in column 5 had 100 µl of the broth only, and 100µl of broth was in A-H in column 6. Twofold serial dilutions using micropipette was done systematically down the columns 1-4 (from rows B-H). 100 µl was removed from the starting concentrations (columns 1-4 in row A) and transferred to the next row with the 100µl broth, properly mixed, and the procedure was repeated up to the last row (H) where the last 100µl was discarded. This brings the final volume in all the test wells with the extracts to 100 µl except the 6th column which had 200 µl of the broth that served as sterility control. 100µl of the 1×10^{6} CFU/ ml bacterial inoculum was transferred into all the wells except the 6th column to give us the desired final inoculum load of 5×10^{5} CFU/ml. Column 5 served as positive control (bacteria- free). Microtiter plates were incubated at 37^{o}C for 18-20 hrs. After incubation, 20 µl of resazurin dye was added to all the wells and incubated for some minutes to observe any color changes. The Minimum Inhibitory Concentrations were determined visually in broth microdilution as the lowest concentrations of the extracts at which color changed from blue to pink in the resazurin broth assay [18].

Statistical Analysis

The Statistical Analysis System was used to detect the effect of difference factors in study parameters. Least significant difference-LSD test was used to significant compare between means in this study [19].

Results and Discussion

Phytochemical characterizations of methanolic and aqueous (seed's husk and leaves) of *Moringa oleifera* extracts were subjected to different chemical tests for the detection of different phyto-constituents. Table 1 show the phytochemicals presence in *Moringa oleifera* extracts. These results agreed with Shanmugavel *et al.* [20] they reported the presence of alkaloids, tannins saponins, flavonoids and glycosides in methanolic extract of *Moringa oleifera* leaves.

Table 1: Phytochemical screening of *moringa oleifera* leaves and seed's husk extracts

Phytochemical compound		leaves extracts		seed's husk extracts	
		Aqueous Extract	Methanolic Extract	Aqueous Extract	Methanolic Extract
Alkaloids	Meyer's test	+	+	+	+
	Wagner's reagent	+	+	+	+
Tannins	Lead acetate	+	+	+	-
	Ferric chloride	+	+	+	-
phenols	Lead acetate	+	+	+	+
	Ferric chloride	+	+	+	+
Saponins		+	+	-	-
Flavonoids		+	+	-	-
Glycosides		+	+	-	-

DPPH assay

Moringa oleifera extracts had free radical scavenging activity; this was evident in a concentration-dependent manner with significant differences (P≤0.05) between concentrations, and the results revealed that the leaves extracts were more effective than seed's husk extracts extract in free radical scavenging activity as shown in Table 2.

Charoensin [21] study the antioxidant activity of *Moringa oleifera* leaves using (DPPH) assay. Fitriana *et al*. [22] revealed that the methanolic extract of *Moringa oleifera* leaves had the highest free radical scavenging activity compared to ethyl acetate, n-hexane and dichloromethane extracts. Furthermore, El-Hadary and Ramadan [23] in their study of antioxidant traits of *Moringa oleifera* leaves extracts referred to the scavenging activity of aqueous leaves extract which was 79.13 ± 0.28.

Table 2: Radical scavenging activity of *moringa oleifera* leaves extracts

Concentration mg\ml	Leaves extracts		seed's husk extracts		BHT	V.C.
	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract		
2	56.10 ± 0.08	64.27 ± 0.06	17.25 ± 0.16	37.83 ± 0.03	88.48 ± 0.02	93.44 ± 0.04
4	66.17 ± 0.14	86.95 ± 0.06	18.10 ± 0.01	43.57 ± 0.03	89.14 ± 0.01	93.57 ± 0.01
6	83.40 ± 0.01	90.83 ± 0.02	28.33 ± 0.01	54.29 ± 0.18	91.40 ± 0.01	96.08 ± 0.02
8	86.26 ± 0.19	91.40 ± 0.01	40.59 ± 0.03	57.98 ± 0.27	91.47 ± 0.01	96.27 ± 0.02
10	87.33 ± 0.03	92.88 ± 0.07	50.25 ± 0.06	63.47 ± 0.06	92.76 ± 0.03	96.34 ± 0.02
LSD value	0.353 *	0.154 *	0.254 *	0.472 *	0.053 *	0.067 *

*** (P≤0.05)**

Total phenolic content of *Moringa oleifera* extracts

The results of the total phenolic content of *Moringa oleifera* extracts as shown in Table 3. The methanolic extract in both leaves and seed's husk had the highest total phenolic content which was (73.71 and 63.30) in 50 mg/ml respectively. The results were in agreement with Vyas *et al*. [24] which referred that the highest total phenolic content of *Moringa oleifera* was for leaves extracted by methanol, compared to other parts. Furthermore, Karim

et al. [25] mention, the low total phenolic content of aqueous leaves extract, which was 5.57 mg/g.

Table 3: Total phenolic content of *moringa oleifera* leaves extracts

Concentration (mg\ml)	Moringa oleifera leaves extracts		seed's husk extracts	
	Methanolic extract (mg\g)	Aqueous extract (mg\g)	Methanolic extract (mg\g)	Aqueous extract (mg\g)
10	42.34 ± 0.24	25.31 ± 0.35	18.11 ± 0.11	13.67 ± 0.03
25	62.89 ± 0.12	50.11 ± 0.12	42.77 ± 0.15	26.92 ± 0.26
50	73.71 ± 0.14	61.59 ± 0.18	63.30 ± 0.2	47.34 ± 0..02
LSD value	0.613 *	0.827 *	0.571 *	0.529 *

* (P≤0.05)

Antibacterial activity of *Moringa oleifera* extracts

Agar well diffusion method was used to evaluate the antibacterial activity of *Moringa oleifera* extracts against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and two gram negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*). The results were listed in Tables 4 and 5. For the methanolic leaves extract, all concentrations were found to be active against all tested bacteria. The maximum antibacterial activity was observed on *Staph. aureus* with inhibition zone 22.66 ± 0.66 mm in concentration (200 mg/ml), while the methanolic seed's husk extract showed there was no inhibition zone at concentration 50 mg/ml in all tested isolates. The highest effect was seen on *Staph. aureus* with the inhibition zone (9.83±0.16 mm and 14.83±0.16 mm) in concentrations (100 mg/ml and 200 mg/ml) respectively, followed by *E. coli* and *K. pneumonia* (12.66 ± 0.33 and 14.33±0.33 mm) in concentrations (200 mg/ml) respectively.

The result was agreement with a study by Abdallah *et al* [26] which referred to the high inhibitory effects of methanolic leaves extracts of *Moringa oleifera* on *S. aureus* and *K. pneumonia* and the water extract of leaves at concentration 200 mg/ml had the lowest effect on the gram negative bacteria. Furthermore Yetunde and Comfort [27] mentioned that the aqueous extract of *Moringa oleifera* leaves at concentrations 50, 100 and 200 mg/ml did not show any inhibitory effect on *B. cereus*, *E. coli* and *Staph. aureus*. In contrast, Singh and Tafida [28] refer to the significant high antibacterial activity of aqueous and methanolic extracts of *Moringa oleifera* leaves on *E. coli* which were 7.33 + 0.57 mm and 8.67 + 0.57 mm respectively compared to *Staph. aureus* and *Pseudomonas aeruginosa.*

Table 4: Antibacterial activity of *moringa oleifera* leaves extract

Methanolic leaves extract				
Concentration (mg/ml)	*Staph. aureus*	*Bacillus cereus*	*E. coli*	*K. pneumoniae*
50	7.83 ± 0.16	6.16 ± 0.16	7.33 ± 0.33	8.83 ± 0.16
100	15.66 ± 0.33	12.66 ± 0.33	14.16 ± 0.16	14.66 ± 0.33
200	22.66 ± 0.66	17.66 ± 0.33	20.00 ± 0.57	20.33 ± 0.33
LSD value	1.338 *	1.095 *	2.066 *	1.169 *
Aqueous leaves extract				
50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
100	8.83 ± 0.16	5.66 ± 0.33	5.83 ± 0.16	8.66 ± 0.33
200	14.83 ± 0.16	12.66 ± 0.66	13.00 ± 0.57	14.66 ± 0.33
LSD value	1.239 *	0.966 *	0.902 *	1.317 *

* (P≤0.05)

Table 5: Antibacterial activity of *moringa oleifera* seed's husk extract

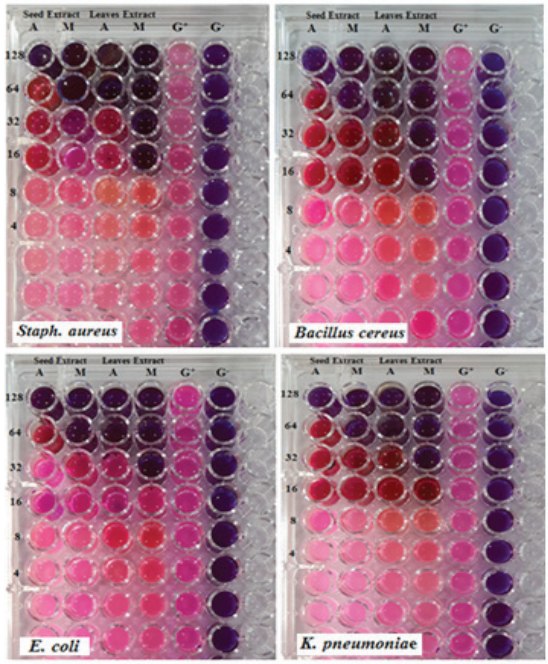
Concentration (mg/ml)	*Staph. aureus*	*Bacillus cereus*	*E. coli*	*K. pneumoniae*
Methanolic seed's husk extract				
50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
100	9.83 ± 0.16	6.83 ± 0.16	8.16 ± 0.16	8.66 ± 0.33
200	14.83 ± 0.16	9.66 ± 0.33	12.66 ± 0.33	14.33 ± 0.33
LSD value	1.255 *	0.863 *	1.147 *	2.168 *
Aqueous seed's husk extract				
50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
100	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
200	9.50 ± 0.28	7.16 ± 0.16	8.16 ± 0.16	9.16 ± 0.16
LSD value	0.855 *	0.729 *	0.894 *	0.862 *

*** (P≤0.05)**

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of *Moringa oleifera* extracts was shown in Figure 1. A method using the oxidation-reduction colorimetric indicator resazurin has been proposed for the determination of drug resistance and MIC of antimicrobial agents against pathogenic organisms [29]. The results of leaves extract showed that the MIC values of methanolic extract were 16 mg/ml for both *Staph. aureus* and *E. coli*, and 32 mg/ml for *B. cereus* and *K. pneumonia*, while the MIC of aqueous extract was 64 mg/ml for all bacterial isolates. For seed's husk extracts, the result showed that the MIC of methanolic and aqueous extracts was 64 and 128 mg/ml respectively for each bacterial isolate.

Phenolic compounds of plants are of noticeable interest due to their antioxidant and antibacterial properties [30]. The means by which microorganisms are inhibited by phenolic compounds involves a sensitization of the phospholipids bilayer of the cell membrane, causing an increase in permeability and leakage of vital intracellular constituents, or impairment of bacterial enzyme systems. Phenolic compounds act by inhibiting the amino acid decarboxylase in target bacteria [31].



(A): Aqueous extract, (M): Methanolic extract, (G^+): Growth positive (Bacteria + Media), (G^-): Growth negative (Media only)

Figure 1: MIC of *moringa oleifera* leaves and seed's husk extracts

Conclusion

Moringa oleifera extracts have a wide variety of phytochemicals that show effectiveness against different diseases and The methanolic leaves extract of *Moringa oleifera* shows relatively a higher amount of phenolic compounds and have equal antioxidant activity to the synthetic antioxidant (BHT) moreover Different *Moringa oleifera* extracts have a potential antimicrobial

agent against both gram positive bacteria (*Staph. aureus* and *B. cereus*) and gram negative bacteria (*E. coli* and *K. pneumonia*). The methanolic leaves extract has the largest effects against foodborne pathogen used in this study.

Conflict of Interest: The authors declared that present study was performed in absence of any conflict of interest.

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