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Antimicrobial, antioxidant and antibiofilm activities of polysaccharides extracted from *Moringa oleifera* leaves

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Abstract

Moringa oleifera is one of the world's most significant therapeutic plants, it is rich in many phytochemicals including polysaccharides. Natural polysaccharides have a wide range of beneficial therapeutic and health-promoting properties. The current study investigated the antimicrobial in a 96-well plate, antioxidant by DPPH scavenging assay and antibiofilm activities of both polysaccharides (MOLPs) extracted from *M. oleifera* leaves and crude extracts of leaves (MOLCEs) by different solvents (methanol, 70% ethanol, ethyl acetate, and hot distilled water (80°C)). Results revealed that the antimicrobial activity of MOLPs against *S. aureus*, *B. Subtilis*, *E. coli*, and *P. aeruginosa* at 500µg/ml was 72.7%, 73.1%, 75.2%, and 74.4%, respectively. The antimicrobial activity of hot water crude extract ranged from 67% to 71% while other crude extracts were less effective. DPPH scavenging activity of MOLPs and MOLCEs was concentration-dependent, at 500 µg/ml it was 76.56% and 81.87% for MOLPs and hot aqueous crude extract, respectively. Other crude extracts were less effective. The biofilm inhibitory activity of MOLPs against *S. aureus* and *E. coli* was 62.56% and 65.65%, respectively while was 55.5% and 57.42% for hot water crude extract. Other MOLCEs by organic solvents (methanol, 70% ethanol and ethyl acetate) showed low inhibitory activity. These results confirmed the importance of polysaccharides as a bioactive agent that can be used as a promising therapeutic agent.

Keywords: Plant polysaccharides, *Moringa oleifera*, antioxidant, antimicrobial, antibiofilm.

1 Introduction

Moringa oleifera is a member of the *Moringaceae* family. The "drumstick tree" and "horseradish tree" are other names for it. It can be grown in any tropical or subtropical

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region of the world where the temperature ranges between 25 and 35 °C. It needs loamy or sandy soil that ranges in pH from relatively acidic to little alkaline and net rainfall of 250–3000 mm. **[1, 2]**. Almost every part of this plant has been utilized in traditional medicine to treat a range of illnesses, including the bark, gum, root, leaves, flowers, pods, seeds, and seed oil **[3]**. It has been shown to have a significant number of bioactive components; as a result, various food supplements based on it are commercially accessible **[4]**. *M. oleifera* has several health advantages including antibacterial, antifungal, anti-hypertensive, antiulcer, antioxidant, anti-inflammatory, anticancer, antihyperglycemic and antiepileptic, characteristics **[5, 6]**. The leaves of *M. oleifera* are rich in carbohydrates, proteins, minerals, moisture, fats, crude fibers, flavonoids, β -carotenes, ash, vitamin C and alkaloids. Polysaccharides are carbohydrate molecules that have important roles in chemistry, biology, and materials science **[7, 8]**.

Polysaccharides are biopolymers made up of several monosaccharides connected by glycosidic linkages. They have been used to create safe substances for functional products in agriculture, cosmetics, medicine, and health food **[9]**. Polysaccharides have complicated structures with a variety of sugar residues, glycosidic linkages, and bonding locations, as a result of this complex intramolecular information, polysaccharides exhibited many conformations and structures that resulted in important biological activities such as antioxidant, antimicrobial, antiglycation, anticancer, hypolipidemic, and prebiotic activities**[10, 11]**.

This study aimed to investigate the antimicrobial, antioxidant and antibiofilm activities of polysaccharides (MOLPs) extracted from *M. oleifera* leaves by hot water extraction method and compare these activities with that of the crude extracts (MOLCEs) by different solvents (methanol, 70% ethanol, ethyl acetate, and hot dis.water).

2 Materials and methods

2.1 The used plant

Moringa oleifera leaves were collected from the garden of Botany Department, Faculty of Women for Arts, Education and Science, Ain Shams University, Cairo, Egypt.

2.2 The tested microorganisms.

Gram-positive bacteria (*Staphylococcus aureus* NRRLB-767 and *Bacillus Subtilis* ATCC6633), Gram-negative bacteria (*Escherichia coli* ATCC25922 and *Pseudomonas*

aeruginosa ATCC10145), yeast (*Candida albicans* ATCC10231), and fungi (*Aspergillus niger* NRRLA-326) were obtained from MERSEN, Agriculture Faculty, Ain Shams University, Cairo, Egypt.

2.3 Preparation of leaves powder

The freshly collected leaves of *M. oleifera* were carefully rinsed with running tap water to eliminate dirt particles then dried at room temperature. The dried sample was crushed using a grinder to obtain the powder **[12]**.

2.4 Preparation of *M. oleifera* leaves crude extracts (MOLCEs) using different solvents

Twenty grams of the dry powder of *M. oleifera* leaves were soaked in 100 ml of methanol, 70% ethanol, and ethyl acetate separately at room temperature for 24 hours **[12]** and by hot distilled water at 80˚C for an hour **[13]**. Extracts were filtered using Whatman No. 1 filter paper, The solvents were evaporated and extracts were collected, concentrated, and fully dried at room temperature before being placed in a refrigerator to be used later. **[12].**

2.5 Extraction of polysaccharides from *M. oleifera* leaves (MOLPs)

Polysaccharides from *M. oleifera* leaves were extracted using the method of **Dong *et al.,*** [**3**] with a few modifications. Briefly, powdered plant sample was defatted with petroleum ether [**14**]. Then was extracted twice with hot water at 90˚C, for 4 h and sample/water ratio was 1:20 (w/v). By a rotary evaporator operating at 45 °C under vacuum, the extract was concentrated to 1/4 volume then deproteinized by trichloroacetic acid (TCA) **[15]**. Finally, in order to precipitate polysaccharides, the extract was subjected to four volumes of absolute ethanol then kept overnight at 4°C. The formed precipitate was collected and lyophilized in vacuum freeze dryer. The dried *M. oleifera* leaves polysaccharides (MOLPs) were stored in the refrigerator till use **[16].** The polysaccharide yield was calculated as follows:

$$\text{Yield (\%)} = \frac{polysaccharides\ content\ of\ crude\ polysaccharide\ (g)\times 100}{weight\ of\ pretreated\ powder\ of\ Moringa\ oleifera\ leaves\ (g)} \quad \textbf{[17]}$$

2.6 Antimicrobial activity of MOLCEs and MOLPs

The antimicrobial activity of methanol, 70% ethanol, ethyl acetate and hot water crude extracts and MOLPs was performed in a 96-well flat polystyrene plate. Each well was filled with 180 µl of nutrient broth, 10 µl of tested extracts at a concentration of 500 µg/ml and

10 µl of culture suspension (0.5 McFarland) of (*S. aureus*, *B. Subtilis*, *E. coli*, *P. aeruginosa*, *C. albicans*, and *A. niger* separately) in triplicate. The plates were then incubated at 37°C overnight. Positive antibacterial effect appeared as clearance in the wells after incubation, while negative effect appeared opaque. The pathogen without any treatment was used as a control. Ciprofloxacin and Nystatin were the positive control for the tested bacteria and fungi respectively. After 20 h the absorbance was measured at 600 nm for bacteria while at 340 nm for fungi in a Spectro star Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrun, Germany) **[18]**.

2.7 Antioxidant activity of MOLCEs and MOLPs by DPPH scavenging assay

A mixture of 2.96 ml of (1, 1 Diphenyl 2- Picryl Hydra Zyl) DPPH solution (4mg of DPPH in 100ml of methanol) and 40µl of samples (at concentrations of 100, 200, 300, 400 and 500 µg/ml) were added. The mixture was shaken vigorously and left for 20 min in the dark, 3ml of DPPH solution was taken as control. UV/Visible spectrophotometer 2401 PC (Shimadzu, Kyoto, Japan) was used to measure the absorbance at 517nm **[19]**. Ascorbic acid was used as reference standard.

The scavenging activity was calculated as follows:

$$\text{Scavenging ability (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where, the absorbance of DPPH solution represents $A_{control}$, while the absorbance of DPPH solution + sample represents A_{sample}.

The IC_{50} values were derived via using the GraphPad 8.2.4® program.

2.8 Antibiofilm activity of MOLCEs and MOLPs by crystal violet assay

The biofilm inhibitory activity of MOLCEs and MOLPs was performed by microtiter plate assay (MTP) method. According to **[20, 21]** 180 µl of lysogeny broth (LB), 10 µl of overnight growing bacterial isolates (*S. aureus* and *E. coli*) 0.5 McFarland and 10 µl of MOLCEs and MOLPs at 500 µg/ml were added to each well of the 96 well plate, the negative control well was without extracts. Then the plate was incubated at 37°C for 24 h. All wells were washed with 200 µl of phosphate buffer saline pH 7.2 to eliminate non adherent bacteria. For staining, crystal violet (0.1%, w/v) was added to each well for 1 h, then 200 µl of dis. water was used for washing. The plate was dried under sterilized conditions, then the absorbance was measured at 570 nm using (SPECTRO star nano

absorbance plate reader – BMG LABTECH) after adding 200 µl of 95% ethanol to all wells.

2.9 Statistical analysis

One-way analysis of variance (ANOVA 1) was used. Each test was in triplicate, with the results shown as a mean and standard deviation with a p-value of 0.001 denoting statistical significance.

3 Results

3.1 Preparation of crude extracts and Extraction of polysaccharides

After collecting and processing of *M. oleifera* leaves in powder form, different crude extracts were made by different solvents (methanol, ethanol, ethyl acetate and hot dis. water). Also, polysaccharides present in the leaves were extracted by hot water extraction method and the yield represented 19% of the dry weight.

3.2 Antimicrobial activity

Crude plant extracts and polysaccharides were tested for their antimicrobial activity against bacterial and fungal strains as shown in **Table 1**. Among MOLCEs, hot aqueous extract showed higher antibacterial activity against both Gram-positive and Gram-negative bacteria compared to methanol, 70% ethanol, and ethyl acetate extracts. Moreover, polysaccharides showed higher antimicrobial activity against the tested bacteria compared to other MOLCEs by all solvents. On the other hand, all extracts had no antifungal effect against *C. albicans* and *A. niger*.

Results presented in **Table 1** indicated that there were highly significant differences between extracts and different pathogens, where p value was less than 0.001.

Table 1: Antimicrobial activity of MOLCEs and MOLPs.

	Antimicrobial activity (%)					
	Gram positive		Gram negative		Yeast	Fungi
Extract	*S. aureus*	*B. Subtilis*	*E. Coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
Ethyl acetate MOLCE	23.14±0.89^{e}	20.98±1.14^{f}	15.01±1.04^{f}	13.18±0.92^{f}	- ve	- ve
70% Ethanol MOLCE	49.24±1.23^{d}	53.08±0.57^{e}	18.22±1.70^{e}	21.99±1.09^{e}	- ve	- ve
Methanol MOLCE	44.94±1.10^{c}	50.71±1.24^{d}	20.44±0.89^{d}	17.33±1.03^{d}	- ve	- ve
Hot water MOLCE	71.17±1.66^{b}	69.55±1.21^{c}	67.58±1.11^{c}	68.09±0.97^{c}	- ve	- ve
Polysaccharides (MOLPs)	72.74±1.05^{b}	73.10±0.97^{b}	75.15±1.02^{b}	74.41±1.23^{b}	- ve	- ve
Ciprofloxacin	96.90 ±0.86^{a}	91.63 ±0.55^{a}	98.40±0.22^{a}	98.87±0.14^{a}	**ND**	**ND**
Nystatin	**ND**	**ND**	**ND**	**ND**	97.27±0.34^{a}	98.27 ±0.11^{a}
F-value	**2.762*** **	**3.571*** **	**4.178*** **	**5.386*** **	2.455***	2.394***

ND: Not detected, Ciprofloxacin: positive antibacterial control, Nystatin: positive antifungal control, ***significant difference at p<0.001 and the values in the same column with the same letter are not significant.

3.3 Antioxidant activity

Results in Error! Not a valid bookmark self-reference. showed that the scavenging activity was concentration dependent and hot water MOLCE exhibited the highest scavenging activity of DPPH compared with other MOLCEs, followed by MOLPs. And results presented in **Table 2** indicated that there were highly significant differences between extracts and concentrations, where p value was less than 0.001.

Table 2: Antioxidant activity of MOLCEs and MOLPs.

	Antioxidant activity (%)				
Conc.(µg/ml) Extract	**100**	**200**	**300**	**400**	**500**
Ethyl acetate MOLCE	19.29±1.88^{d}	24.89±1.02^{d}	29.89±1.14^{c}	30.39±1.09^{f}	33.51±1.34^{e}
70% Ethanol MOLCE	0.0^{e}	0.0^{e}	0.0^{d}	38.24±1.64^{d}	40.64±1.50^{d}

Methanol MOLCE	0.0^{e}	0.0^{e}	31.89±1.17^{c}	35.21±1.39^{e}	39.53±1.63^{d}
Hot water MOLCE	39.19±1.14^{c}	51.26±0.17^{c}	65.39±1.59^{b}	73.36±2.01^{b}	81.87±1.85^{b}
Polysaccharides	44.47±1.87^{b}	58.54±1.69^{b}	63.55±1.89^{b}	69.16±1.40^{c}	76.57±1.84^{c}
Ascorbic acid (+ve control)	89.74±0.25^{a}	93.55±0.63^{a}	96.34±0.58^{a}	98.13±0.49^{a}	99.45±0.14^{a}
F-value	2.466***	5.616***	2.282***	1.088***	1.006***

***significant difference at $p<0.001$ and the values in the same column with the same letter are not significant.

3.4 Antibiofilm activity

Polysaccharides exhibited moderate biofilm inhibitory activity against the tested strains as it inhibited *E.coli* and *S. aureus* by 65% and 62%, respectively at 500 µg/ml. as shown in **Figure 1** followed by hot aqueous MOLCE (80°C) that inhibited them by 57% and 55%, respectively. While other MOLCEs showed weak inhibitory activity against the tested strains.

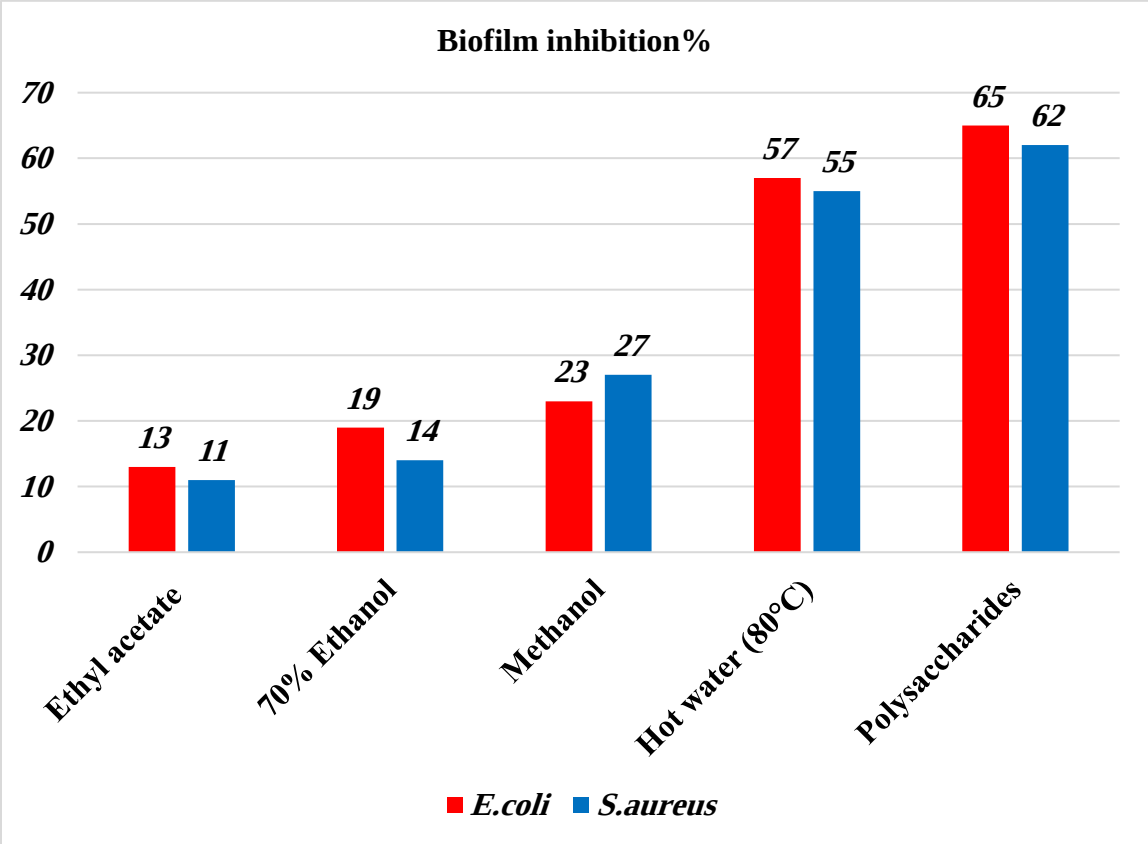

Figure 1: Biofilm inhibition of *E. Coli* and *S. aureus* by MOLCEs and MOLPs.

4 Discussion

Plant polysaccharides are one of the most important bioactive substances, they have immunomodulatory, anticancer, antioxidant, intestinal health protective, and antidiabetic properties, they are advantageous in boosting human health and physiological activities, so researchers have begun to focus on their biological roles **[22]**.

The current study revealed that MOLPs have significant high antibacterial activity against both Gram-positive and Gram-negative tested strains at 500µg/ml and was higher than that of MOLCEs by all solvents. These results are consistent with results obtained by **Gaafar *et al.*,** in which antibacterial activity of polysaccharides was higher than that of cold and hot water extracts at 200 and 400 µg/ml **[23]**. Bioactive polysaccharides have been reported to have antimicrobial activity via the interference process between the cell wall and membrane or a change in the permeability of cell membrane. Modifications in permeability may prevent the microorganism from absorbing nutrients and lead to releasing of helpful ions and bio-macromolecules which is destructive to bacterial growth **[24, 25, 26]**.

Oxidative stress is known by its role in causing a number of major illnesses, including nephritis, diabetes, cancer, and arteriosclerosis **[27]**. Thus, the need for natural antioxidants from plant sources is a global orientation. Our study revealed that MOLPs have relative potent and concentration dependent DPPH scavenging activity reached 76.57% at 500 µg/ml with $IC_{50\%}$.=136.54 µg/ml which is lower than MOLP extracted by **[28]** with $IC_{50\%}$= 0.31 mg/ml. The antioxidant process was based on the polysaccharides' ability to provide electrons and hydrogen. However, due to their complex physicochemical structure, the mechanism of this antioxidative activity is still not entirely understood **[26]**.

Biofilm is a microbial aggregate in which cells are attached to each other and to surface irreversibly and embedded in extracellular matrix **[29]**. Biofilm formation represents one of the indirect mechanisms through which bacteria become antibiotic resistant where 2/3rds of bacterial infections in humans are formed due to the biofilms **[30]**. Plants provide a renewable supply of antibiofilm agents with diverse modes of action and characteristics **[31]**. According to **[32]** the concentration (60 mg/ml) of *M. oleifera* leaves aqueous extract inhibited biofilm formation of *S. aureus* by 28.57%, compared to 4.96% inhibition in the presence of the same extract at 20 mg/ml.

In this study MOLPs inhibited biofilm formed by *S. aureus* and *E. coli* by 62.56% and 65.65% respectively at 500μg/ml. while it was 55.5% and 57.42% for hot aqueous MOLCE. The mode of action followed by polysaccharides as antibiofilm may include interference in quorum sensing pathways, adhesion mechanisms, destruction of extracellular DNA, protein, lipopolysaccharides, exopolysaccharides, and secondary messengers involved in numerous signaling pathways **[33]**.

5 Conclusion

This study confirmed the importance of polysaccharides extracted from *M. oleifera* leaves as a bioactive agent that can be used as a promising therapeutic agent. So further studies are required for fractionation of these crude polysaccharides, purification, characterization and studying their mode of action to employ it as part of functional food and as natural pharmaceutical agents.

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الملخص العربي

أنشطة مضادات الميكروبات ومضادات الأكسدة ومضادات الأغشية الحيوية لعديد السكريات المستخرج من أوراق المورينجا أوليفيرا

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الملخص العربى:

تعتبر المورينجا أوليفيرا واحدة من أهم النباتات الطبية في العالم، فهي غنية بالعديد من المواد الكيميائية النباتية بما في ذلك عديد السكريات. تشتهر عديد السكريات بخصائصها العلاجية المفيدة والمعززة للصحة؛ لذلك قامت الدراسة الحالية بدراسة أنشطة المضاد للميكروبات في لوح ٩٦ بئر ومضاد الأكسدة عن طريق فحص كسح DPPH وتثبيط الغشاء الحيوي لكل من عديد السكريات (MOLPs) المستخرج من أوراق المورينجا اوليفيرا والمستخلصات الخام (MOLCEs) للأوراق بواسطة مذيبات مختلفة تتمثل في (ميثانول، إيثانول ٧٠٪، إيثيل أسيتات، وماء مقطر ساخن).

وأوضحت النتائج أن النشاط المضاد للميكروبات لـــ MOLPs ضد *S. aureus* و*B. Subtilis* و *E. coli* و *P. aeruginosa* عند ٥٠٠ ميكروجرام / مل كان ٧٢,٧٪ و٧٣,١٪ و٧٥,٢٪ و٧٤,٤٪ على التوالي. بينما تراوح النشاط المضاد للميكروبات للمستخلص المائي الساخن من ٦٧٪ إلى ٧١٪ في حين كانت المستخلصات الخام الأخرى أقل فعالية. وكان نشاط كسح DPPH لـ MOLPs وMOLCEs معتمدًا على التركيز، فعند ٥٠٠ ميكروغرام / مل كان ٧٦,٥٦٪ و٨١,٨٧٪ لـ MOLPs والمستخلص المائي الساخن على التوالي. وكانت المستخلصات الخام الأخرى أقل فعالية. أما بالنسبة للنشاط التثبيطي للغشاء الحيوي لـــ MOLPs ضد *S. aureus* و*E. coli* فكان 62.56 ٪ و٦٥,٦٥٪ على التوالي، بينما كان ٥٥,٥٪ و٥٧,٤٢٪ للمستخلص المائي الساخن. في حين أظهرت المستخلصات الأخرى بالمذيبات العضوية (ميثانول، إيثانول ٧٠٪، وإيثيل أسيتات) نشاطًا مثبطًا منخفضًا؛ وبذلك أكدت هذه النتائج أهمية عديد السكريات كعامل نشط بيولوجيًا يمكن استخدامه كعامل علاجي واعد.