ISSN: 2755-0125

Journal of Virology Research & Reports

Research Article

Open Access

Antimicrobial Activities of Polysaccharides Isolated from Some Plant Leaves

Sorial A Moharib[1]* and Rimon S Adly[2]

[1]Biochemistry Department, National Research Centre, Cairo, Egypt

[2]Lecturer of anesthesia, Faculty of Medicine, Kasr-El Ainy, Cairo University, Cairo, Egypt

ABSTRACT

Plant leaves were found to have nutritional quality suitable for food, pharmaceutical and biomedical uses as therapy in medicine. Staphylococcus aureus (S.aureus) and Escherichia coli (E.coli) are responsible for infectious diseases in humans and animals. The present study aims to investigate antimicrobial activity (AMA) of polysaccharides isolated from Parsley (Petroselinum sativum), Spinach (Spinocia oleracea), Watercress (Eruca sativa), Radish (Raphanus sativus) and Lettuce (Lactuca sativa) leaves against Staphylococcus aureus (S.aureus), Escherichia coli (E.coli), Bacillus subtilis (B.subtilis) and (MRSA) at different concentrations using agar diffusion method in vitro. Polysaccharides (PS) were extracted, isolated and purified from different plant leave samples separately. Chromatographic analysis of the purified PS, revealed the presence of different percentages of monosaccharides constituents per each isolated PS from different plants. In vitro study was done to estimate the activities of the obtained PS separately against 4 microbial strains (S. aureus, E. coli, B subtilis and MRSA) using agar diffusion method. PS showed AMA against S. aureus and E. coli more than that of B subtilis and MRSA at 10% concentration and inhibition zones were estimated. Minimal inhibitory concentrations (MIC) of PS against S. aureus, E. coli, B subtilis and MRS were found in the range of 1-5 mg/ml. The result demonstrates the killing effect of PS against S. aureus, E. coli, B subtilis and MRSA at 10% PS within 24 hours. Inhibition zone diameters exhibited different levels of decreases with the PS concentrations decreases against S.aureus, E.coli, B.subtilis and MRSA. Inhibition effects of PS against different microbial strains were found to be depending on PS concentration. However, the lowest concentration of PS produced lower inhibitory activity against S.aureus B.subtilis E.coli and MRSA. Results of all the obtained PS showed higher AMA against S. aureus and E.coli than the other two microbial strains used in the present study. Results indicated that these PS have AMA against both Gram-positive and Gram-negative bacterium at 10% concentration. According to these results the obtained PS consider important sources of antimicrobial agents for treatment of infectious diseases with minimal concentrations (100-500µg/ml).

***Corresponding author**

Sorial A Moharib, Biochemistry Department, National Research Centre, Cairo, Egypt.

Received: March 06, 2025; **Accepted:** March 11, 2025; **Published:** March 18, 2025

Keywords: Antimicrobial, Polysaccarides, Plant leaves, Microorganisms, InVitro

Introduction

Plant leaves consider one of the most important sources of proteins, lipids, carbohydrates other phyovhemicals for human nutrition and treatment of diseases. Plants in general have a long history as a part of human culture associated with people from ancient time as food in many regions of the world and consumed due to its nutritive values, health benefits, pharmaceutical, biomedical and biological uses reported plant have medicinal therapeutic properties, used as therapeutic agent for protection against diseases[1-4]. Plant leave extracts have many active therapeutic compounds represent a source of their chemical constituents, including polysaccharides, tannins, phenolic, flavonoids, saponins, triterpenoids and alkaloids possess antibacterial properties were investigated these compounds as antimicrobial in vitro [5-7]. Carbohydrates consider the largest group of natural biopolymers produced in the world used in food and medicine, Polysaccharides represent majority of carbohydrate used in pharmaceutical and drug agents due to their biological functions [8-10]. Other investigators reported the majority of plant polysaccharides have health benefits, pharmaceutical and therapy in medicine [7,11,12]. Polysaccharides are water soluble and non-toxic suitable for different pharmaceutical and biomedical uses and play important roles in physiological and pathological conditions [13,14]. Polysaccharides represent natural biopolymers consisting of a large number of monosaccharide with various degree of polymerization have variety of branched or linear structures play an important role for their biological activities [15,16]. Polysaccharide are considered as natural sources play an important role in human growth and development reported polysaccharide has interest of some healthy food for patients with cardiovascular diseases as its nutritional and medicinal properties [12,13]. Recent study stated that some polysaccharides intake cause improve biochemical parameters and reduce the risk of most diseases [7,17]. Different types of natural polysaccharides used as hypoglycemic, hypolipidemic and anticancer agents [16-20]. Polysaccharides have anticancer therapeutic, antiviral, antiproliferative and antibacterial activities [7,8,14,21-26]. Polysaccharides are pharmacologically studied for its antifungal, anti-inflammatory, antioxidant and antimicrobial properties [5]. Water polysaccharide extracts have been shown to prevent tumor growth in rats [20]. Moreover, polysaccharides consumption results in treated and protection against colon cancer [27]. Bacteria, Salmonella sp., Pseudomonas sp., Escherichia coli and Staphylococcus aureus are responsible for several diseases in humans and animals reported these bacteria are the major agents cause damage in some fields including food industry [28-30]. Staphylococcus aureus, Clostridium perfringens and Staphylococcus epidermidis are producing toxic symptoms in humans and cause some diseases they reported many gram-negative bacteria are difficult food contaminants and pathogenesis of infections against antibiotics [31-34]. Other investigators reported S. aureus and E. coli causes hospital infections [30]. Bacteria species are responsible for upper respiratory, eye, ear, skin and urinary tract infections in general populations [34,35]. Antibiotic used in treatment of infectious diseases are failing to treat various infectious diseases due to pathogenic bacterial resistant and side effects of traditional antibiotics [30,31,33,36,37]. The high cost and ineffective of the conventional chemotherapy drugs used for treatment infectious diseases of bacteria need several researches to develop and production of novel antimicrobial drugs containing efficient natural compounds to overcome the resistance and side effects of the conventional antimicrobial agents [11,28,34,38-40]. In last decades, chemotherapy stimulates many scientists to developed natural bioactive agents with antibacterial and anticancer properties without side effects for treatment of different diseases, including microbial infections, cancer and other diseases [27,33,41]. Several researches on going to identifying naturally occurring active compounds, capable of inhibiting and controlling some infectious bacterial diseases [7,33]. Antimicrobial drugs growing rapidly to produce inexpensive antimicrobial agents from natural sources for treatment of infectious diseases without side effects [26,42,43]. Concentrated searches are still needed for production of new antimicrobial agents from natural sources due to human pathogenic microorganisms resistant to antibiotics and failing in treatment of different types of infectious diseases particularly in developing countries [16,36,40,43-45]. Many investigators, indicates the antibacterial activity is due to different chemical compounds that recognized as active antimicrobial agents [46-47]. Moreover, polysaccharides component has indirect antimicrobial activity through stimulate phagocytic leukocytes, they reported the medicinal importance of plants come from the presence of bioactive polysaccharides in different plants [5,26,48]. Other researches obtained new natural antimicrobial agents have lower incidence of adverse reactions compared to synthetic pharmaceuticals and the reduced costs of preparations as natural therapeutics [6,34,46]. Antibiotics used in medicine are derived from natural sources of fruits and vegetables [39,41]. Ant infectious and antitumor drugs either under clinical trials or in the market are of natural origin [35,36,49]. Different compounds were used in medicine as anti-spasmodic and antidiuretic reported some antibiotics were used for treated of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal and abdominal infection including gram negative and gram positive bacteria [32,50]. Moreover, different plant extracts are widely used as antidiabetic, antimicrobial, antibacterial, antidiuretic and anticancer they found specific plant extracted compounds such as saponins, polysaccharides, anthraquinones and dihhydroxyanthraquinones have direct antimicrobial activities [5,11,17,18,34,35,50,51]. Polysaccharides are known to exhibit antimicrobial activities against clinical, food-borned pathogens and food spoilage bacteria. Moreover, Parsley (Petroselinum sativum), Spinach (Spinocia oleracea), Watercress (Eruca sativa), Radish (Raphanus sativus) and Lettuce (Lactuca sativa) leaves are commonly used as food or in medicine in many regions of the world.

Aim of the Study

Thie present study aims to isolate and purify polysaccharides from Parsley (Petroselinum sativum), Spinach (Spinocia oleracea), Watercress (Eruca sativa), Radish (Raphanus sativus) and Lettuce (Lactuca sativa) leaves Antimicrobial activities of polysaccharides (PS) against bacterial strains in vitro were determined. Four bacterial strains were used in the present study including Staphylococcus aureus (S. aureus), Escherichia coli (E.coli), Bacillus subtilis (B. subtilis) and Methicillin-Resistant Staphylococcus aureus (MRSA) as standard strain.

Materials and Methods

Plant leaves

Parsley (Petroselinum sativum), Spinach (Spinocia oleracea), Watercress (Eruca sativa), Radish (Raphanus sativus) and Lettuce (Lactuca sativa) leaves were obtained locally from markets in Cairo, Egypt, washed with tap-water followed by distilled water and drying in an oven at 50 C for 24 hours. Leaves were then ground using food grinder (mincer) to a very fine powder, sifted through a 16mesh sieve, packed in bags, and stored at room temperature till used. Polysaccharides were extracted with water using hot water bath (80oC) for 18 hours and homogenized at 100ºC using homogenizer (Mechanika precyzyjna warszawa model MPW-309, Poland) [52]. Extracts were then collected using cooling centrifuge (Sigma 2K). Qualitative and quantitative determinations of monosacharides of each soluble polysaccharides (PS) were measured [53]. Monosaccharides such as, glucose, galactose, fructose, arabinose, mannose, rhamnose, fucose, xylose, maltose, trehalose and raffinose were used as standard were obtained from Sigma Chemical Company USA.

Microorganisms

Four bacterial strains including Staphylococcus aureus (S. aureus), Escherichia coli (E.coli), Bacillus subtilis (B. subtilis) and Methicillin-Resistant Staphylococcus aureus (MRSA) as standard strain. All bacteria were obtained from Mercin faculty of agricultural, Ain shams University Cairo, Egypt. Stock cultures of all microbial strain were grown on nutrient agar plates and maintained in the nutrient agar slants at 4 ºC.

Microorganisms Tested

The inhibitory effects of each polysaccharides (PS) obtained separately were carried out on four strains of bacteria. The bacterial strains used in the present study were S.aureus, E.coli, B.subtilis and MRSA. The microbial strains were activated before the antimicrobial test. After removal from the refrigerator, strains were incubated overnight in nutrient broth and then streaked on nutrient agar plate and kept for 24 hours at 37 ºC [53,54].

Preparation and Determination of the Purified Polysaccharides (PS)

Five plant leaves (100gm/each) were soaked separately in 500 ml water, stirred for 4hrs using mechanical magnetic stirrer and extraction technique with boiling water for 18 hours was done, then cooled at room temperature [52,55]. Solutions after cooling were centrifuged after filtered to remove insoluble matters and five volumes of ethanol (98% v/v) were added to precipitate crude polysaccharides. The precipitates were collected by centrifugation and washed successively with ethanol, followed by drying at 60°C, yielding crude polysaccharide. The crude polysaccharides were dissolved in water and using trichloroacetic acid (TCA) method to remove proteins [56]. Three volumes of 98% ethanol (EtOH) were added to the filtrate and the precipitate was recovered after centrifugation, dissolved in water, dialyzed against water for 72h at 4 ºC [27]. The polysaccharides (PS) isolated separately from 5 plant leave samples were partially purified separately and dried by hot air oven [20,57]. The obtained PS were weighed and freeze-dried till used. PS samples obtained were dissolved individually in deionized water containing 1 % sodium hydroxide, vortex mix. Solution of each PS was freshly prepared from PS powder to obtain a series of 5-fold dilutions of various concentrations of each PS in distilled water before added to the agar media used for antimicrobial tests.

Identification of Monosaccharide (MS)
Monosaccharide content of each PS sample was identified and measured using paper chromatography [14,58]. Monosaccharides such as glucose (Glu.), galactose (Gal.), fructose (Fr.), arabinose (Arab.), mannose (Man.), rhamnose (Rha.), fucose (Fuc.), xylose (Xyl.), maltose (Mal.), trehalose (Tre.) and raffinose (Raff.) were used as standard controls.

Preparation of PS Stock Solutions
Each PS sample was weighted and diluted with DEMSO according to the solubility of polysaccharides powder. 100µ from each stock solution was diluted serially via 5-fold dilution (from 10-1 to 10 -5) in ependorf, 50µ was taken from each dilution of samples[58].

Antimicrobial Activities (AMA) in Vitro
Bacterial strains, E.coli, S.aureus, B subtilis and MRSA cultures were incubated at 37 ˚C for 24-48h, Each bacterial strain sub-cultured and strecked on agar medium and the AMA of each strain was detected against each PS sample. AMA were measured using agar-well diffusion method [54,59]. 0.1 ml of each culture of each bacteria strain was introduced into a sterile Petri dish containing nutrient agar. Sterile nutrient agar has cooled and allowed to set. Three wells were made on the set medium at suitable space. The dried purified PS were dissolved separateley in 1% DEMSO and prepared at concentration of 200µg/ml. The wells were respectively filled with different concentrations (100, 50, 25 and 12.5 mg/ml) of each PS separately and they were incubated in an incubator at 37°C for 24 h. The all PS solutions were diffused around the wells in Petri dishes and they were surrounded by circular clear zones of inhibition that could be analyzed. The results were recorded by measuring the diameters of growth inhibition zone around each bacterial strain in millimeter (mm). These clear inhibition zones around the wells indicate the presence of antimicrobial activity. AMA of PS measured by the average of triplicate samples analysis.

Determination of Minimum Inhibitory Concentration (MIC)
Agar diffusion test was used for determination of MIC [54,59]. Muller hinton agar medium was used and a clear circular zone of growth inhibition (mm) was measured [60]. MIC of different PS against the four selected bacterial strains was determined.

Results Polysaccharides (PS)
Polysaccharides (PS) were isolated and purified from five different plants leaves and were used to estimate the antimicrobial activity (AMA) of each PS separately against four different bacterial strains (S.aureus, E.coli, B.subtilis and MRSA). Polysaccharides (PS) obtained from five different plants leaves revealed the presence of various percentages of PS as shown in Table (1). Polysaccharides obtained from Parsley (Petroselinum sativum), Spinach (Spinocia oleracea), Watercress (Eruca sativa), Radish (Raphanus sativus) and Lettuce (Lactuca sativa) leaves (18.20, 16.80, 14.40, 12.40 and 10.60 g/100g respectively). Highest PS were obtained from Parsley, Spinach and Watercress leaves than that of Radish and Lettuce leaves (Table 1). Chromatographic analysis of the obtained PS isolated from different plant leaves separately, showed the presence of various types and levels of monosaccharide constituents such as glucose, galactose, fructose, arabinose, mannose, rhamnose, xylose, maltose, trehalose and raffinose (Table 1). Glucose, galactose, mannose and arabinose were found to be the predominant monosaccharide in all PS obtained from different plant leaves. Results showed small amounts of rhamnose, raffinose and xylose were found in all PS isolated from the plant leaves used in the present study. Lowest levels of maltose and trehalose were also observed. The present results finding that these differences were not only observed in the levels between all PS obtained from five different plant leave sources, but also in their monosaccharide constituents.

Table 1: Polysaccharides (PS) Produced from Plant Leaves and Monosaccharide Constituents

Leaves samples	PS (g %)	Monosaccharides (g %)										
		Glu.	Gal.	Fr.	Ara b.	Ma n.	Rha.	Fuc.	Xyl.	Mal.	Tre.	Raff.
Pparsley (Petroselinum sativum)	18.20	4.12	1.52	1.60	2.00	2.20	1.56	1.62	1.14	0.52	0.60	1.32
Spinach (Spinocia oleracea)	16.80	3.02	1.98	2.08	1.78	1.84	1.08	1.02	1.04	0.68	0.88	1.40
Watercress (Eruca sativa)	14.40	2.18	1.90	2.10	1.32	1.56	0.82	0.98	0.80	0.78	0.66	1.30
Radish (Raphanus sativus)	12.40	2.60	1.40	1.60	1.40	1.44	0.90	0.64	0.38	0.46	0.56	1.02
Lettuce (Lactuca sativa)	10.60	2.56	1.20	0.96	1.30	1.36	0.56	0.44	0.52	0.48	0.58	0.64

Mean of three samples.

Determination of Antimicrobial Activity (AMA)
Antimicrobial activity (AMA) of the obtained polysaccharides (PS) isolated from 5 different plant leaves were determined against four strains of bacteria (S. aureus, E.coli, B. subtilis and MRSA) as shown in Table (2). The present results showed the PS samples obtained from Parsley (Petroselinum sativum), Spinach (Spinocia oleracea), Watercress (Eruca sativa), Radish (Raphanus sativus) and Lettuce (Lactuca sativa) leaves have AMA against S. aureus, E.coli, B. subtilis and MRSA (Table 2). However, PS obtained from 5 different plant leaves were found to be inhibited the growth of S. aureus, E. coli, B subtilis and MRSA in vitro.

Table 2: Activity of Polysaccharides (PS) on Growth of 4 Bacterial Strains in Agar Diffusion Method

Leave samples	Antimicrobial activity (AMA)			
	S. aureus	E.coli	B subtilis	MRSA
Pparsley (Petroselinum sativum)	+ve	+ve	+ve	+ve
Spinach (Spinocia oleracea)	+ve	+ve	+ve	+ve
Watercress (Eruca sativa)	+ve	+ve	+ve	+ve
Radish (Raphanus sativus)	+ve	+ve	+ve	+ve
Lettuce (Lactuca sativa)	+ve	+ve	+ve	+ve

Mean of three samples, +ve AMA detect.

The antimicrobial activity of PS was done at different concentrations using the diffusion method test and inhibition zones were measured in mm diameter (Table 3). Results obtained with the all PS showed higher antimicrobial activity against S. aureus and E.coli than that of B subtilis and MRSA. The PS obtained from Parsley (Petroselinum sativum), Spinach (Spinocia oleracea) and Watercress (Eruca sativa) were found to be active against both B.subtilis and MRSA strains. The PS obtained from Radish (Raphanus sativus) and Lettuce (Lactuca sativa) leaves have lower active against B subtilis and MRSA These results indicated that PS has different levels of AMA against bacterial strains.

Different values of inhibition zone diameter (10-28mm) were observed at a concentration of 10% for all obtained PSs samples against all bacterial strains. The inhibition zones of Parsley (Petroselinum sativum), Spinach (Spinocia oleracea), Watercress (Eruca sativa), Radish (Raphanus sativus) and Lettuce (Lactuca sativa) leaves PS at 10% concentration were higher (20-28 mm) against S. aureus as shown in table (3). Decreasing of inhibition zones were observed with low PS concentrations (10-1, 10-2 and 10-3 respectively). Decreases in inhibition zones (14-22mm) against E.coli as compared to the PS effects against S. aureus were observed at 10% concentration (Table 3).

Inhibition zone (12-18mm) was observed with the obtained PS at a concentration of 10% against B. subtilis while the inhibition zone (10-14 mm) was observed against MRSA (Table 3 and Figure 1).Moreover, the inhibition zone diameters exhibited different levels of decreases with the all PS concentrations decrease against S. aureus, E.coli ,B. subtilis and MRSA (Table 3). The results suggest that S. aureus and E.coli were being inhibited in the presence of PS isolated from five plant leaves. Inhibition effects of PS obtained against different bacterial strains were found to be depending on the concentrations used. No inhibition zone against four bacterial strains was obtained at low all PS concentrations (10-3, 10-4 and 10-5). However, the present results indicated that the increase of PS concentrations exhibited increase in the inhibition zone diameter.

Table 3: Minimum Inhibitory Concentration (MIC) Values of Five Leave PS Samples

Leave PS samples	Minimum inhibitory concentration (MIC) for S.aureus					
	10	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Parsley (Petroselinum sativum)	26 mm	6mm	2 mm	-	-	-
Spinach (Spinocia oleracea)	24 mm	4 mm	2 mm	-	-	-
Watercress (Eruca sativa)	26mm	4mm	2 mm	-	-	-
Radish (Raphanus sativus)	28mm	6mm	2 mm	-	-	-
Lettuce (Lactuca sativa)	20mm	2mm	2mm	-	-	-
Leave PS samples	**Minimum inhibitory concentration (MIC) for E.coli**					
	10	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Parsley (Petroselinum sativum)	22mm	2mm	1 mm	-	-	-
Spinach (Spinocia oleracea)	18 mm	4mm	1mm	-	-	-
Watercress (Eruca sativa)	16 mm	2 mm	1 mm	-	-	-
Radish (Raphanus sativus)	16 mm	4 mm	2 mm	-	-	-
Lettuce (Lactuca sativa)	14mm	2mm	1mm	-	-	-
Leave PS samples	**Minimum inhibitory concentration (MIC) for B.subtilis**					
	10	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Parsley (Petroselinum sativum)	18mm	4mm	1mm	-	-	-
Spinach (Spinocia oleracea)	14mm	6 mm	2 mm	-	-	-
Watercress (Eruca sativa)	12mm	4mm	2mm	-	-	-
Radish (Raphanus sativus)	14mm	2mm	2mm	-	-	-
Lettuce (Lactuca sativa)	12mm	2mm	1mm	-	-	-

Leave PS samples	Minimum inhibitory concentration (MIC) for MRSA					
	10	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Parsley (Petroselinum sativum)	14mm	4mm	2mm	-	-	-
Spinach (Spinocia oleracea)	12mm	2mm	4mm	-	-	-
Watercress (Eruca sativa)	10mm	2mm	2mm	-	-	-
Radish (Raphanus sativus)	12mm	1mm	1mm	-	-	-
Lettuce (Lactuca sativa)	10mm	2mm	1mm	-	-	-

Mean values of three samples.

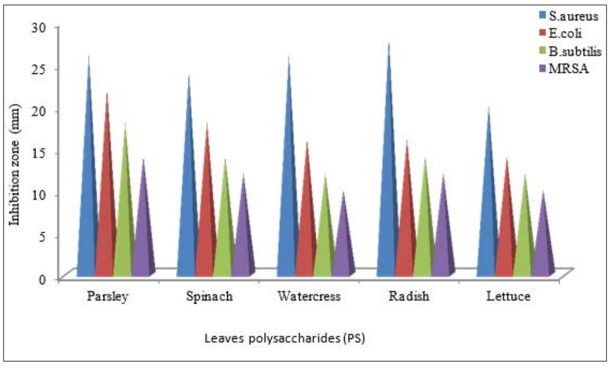

Figure (1) : Antimicrobial activity of PS against 4 bacterial strains.

Determination of Minimum Inhibitory Concentration (MIC)
Minimum inhibitory concentration (MIC) of 5 obtained PS was determined and the results are given in Table (3) and Figure (1). PS of Parsley, Spinach, Watercress, Radish and Lettuce leaves polysaccharides (PS) at 10% concentration exhibited the best antibacterial activity against S. aureus, E.coli amd B. subtilis. Higher activity of PS obtained from Parsley, Spinach, Watercress were observed against S. aureus, E.coli and MRSA than that of those PS obtained from Radish and Lettuce leaves. Results showed the PS were more effective against S.aureus with a higher zone of inhibition (20-28) while lower effective against E.coli, B. subtilis and MRSA. Other bacterial strains in the present study, E.coli showed a zone of inhibition (14- 22mm), B. subtilis (12-18mm) and MRSA showed inhibition zone (10-14mm) at conc. 100µg. The MIC values of PS were found to have Low MIC value of 0.50 mg/ml for S. aureus and E.coli. PS showed a higher MIC value of 2mg/ml with B. subtilis and MRSA. These results were indicated higher activity of PS with S. aureus, and E.coli and less activity with B. subtilis and MRSA (Table 3 and Figure 1). PS obtained from different plant leaves were inhibited the growth of S. aureus, E. coli, B. subtilis and MRSA in vitro This common plant leaves consider an important sources of antimicrobial substances with MIC.

Discussion

Polysaccharides (PS)
The present work was done to investigate the antimicrobial activity (AMA) of polysaccharides (PS), isolated feom different plant leaves, on growth inhibition of four different bacterial strains (S. aureus, E. coli, B. subtilis and MRSA). Minimal inhibitory concentration (MIC) was also investigated (60) Polysaccharides (PS) obtained from Parsley, Spinach, Watercress, Radish and Lettuce leaves (18.20, 16.80, 14.40, 12.40 and 10.60 g/100g respectively) [60]. Higher PS was obtained from Parsley, Spinach, Watercress than that of PS obtained from Radish and Lettuce leaves (Table 1). Similar results were recorded by several investigators [14,10, 61]. Chromatographic analysis of the obtained PS revealed different type and levels of monosaccharide constituents such as glucose, galactose, fructose, arabinose, mannose, rhamnose, xylose, maltose, trehalose and raffinose (Table 1). These results are similar to those obtained by other investigators using cabbage, sugar beet and Radish [14,20,27]. The differences were not only observed in the levels between PS obtained from each plant leave sources, but also in their monosaccharides constituents [10,20]. Other investigators stated the same differences in the levels and types of PS and their monosaccharides constituents[14,27,62]. Different PS of plant leaves contained highest amounts of monosaccharides comprising mostly glucose, galactose, arabinose and mannose usually arising from glucane, galactan, galactan-mannan and arabinan-galactan. Other studies reported a large proportion of polysaccharide chains is conjugated with the polypeptide and resulted L-arabino-D-galactan isolated from radish both contained arabinose, galactose and fucose [7,10,20,27,63]. Predominant monosaccharides in all PS obtained from different plant leaves were glucose, mannose and arabinose. Results showed less levels of rhamnose,fucose and xylose in all PS obtained. Many investigators, reported the monosaccharides, galactose and mannose are the main polymer of polysaccharides were identified by paper chromatography [8,16,43]. These PS are very viscous when dissolved in water, have biological and physiological importance and has different effects against different diseases [12,14,18]. Thus, the obtained PS have effective in the treatment of infectious diseases, due to their structure containing mainly galacto-mannan and/or arabino-galactan [17,64]. These finding are in accordance with other investigators they indicated galacto-mannan and arabino-galactan in polysaccharides has effects against different diseases particularly infectious diseases[14,20]. The present study was done to investigate the antimicrobial activity (AMA) of polysaccharides (PS) on growth inhibition of four different bacterial strains. Recent studies using polysaccharides produced from plant origin showing antibacterial activity against some common pathogens such as B. subtilis, E. coli and S. aureus and able to rescue cell viability from rotavirus infection, reported new antimicrobial substances were isolated from different plant sources[7,64-67]. However, many investigators suggests that b-glucans and other polysaccharides are effective in treating diseases, microbial infections, cancer and diabetes [27, 34,58,64].

Determination of Antimicrobial Activity (AMA)
Antimicrobial activity (AMA) of polysaccharides (PSs) isolated from 5 different plant leaves were determined against four strains of bacteria (S. aureus, E.coli, B. subtilis and MRSA) as shown in Table (2). S.aureus represented gram-positive bacteria that can cause skin infection and E. coli represented gram- negative bacteria which can be found in gastrointestinal tract. Moreover, S. aureus, responsible for several diseases in humans and animals. The present results showed the PS samples of Parsley, Spinach, Watercress, Radish and Lettuce leaves have AMA against S. aureus, E.coli, B. subtilis and MRSA (Table 2). The present results showed inhibited growth of S. aureus by the all PS isolated from

different plant leaves (Table 2). These results were found to be similar to the results obyained by other investigators [7,63,64]. PS obtained from Parsley, Spinach, Watercress Radish and Lettuce leaves showed higher effect against S. aureus, E.coli and B. subtilis more than that of MRSA (Table 2). However, PS obtained from 5 different plant leaves were inhibited the growth of S. aureus, E. coli, B subtilis and MRSA in vitro. AMA of PS obtained was done at different concentrations using the diffusion method test and inhibition zones were recorded in mm diameter (Table 3). The results obtained with all PS showed best AMA against S. aureus and E.coli than the other two microbial strains used in the present study. The PS obtained from Parsley, Spinach, Watercress were found to be more active against S. aureus, B.subtilis and MRSA strains. These results indicated that PS has AMA against some bacterial strains. Other investigators reported some plant leaves polysaccharides are effective and used in treating diseases of microbial infections [64,68]. Other studies used polysaccharides in treating of different diseases (diabetes, hyperlipidemia and cancer)[18,27]. However, different effects of polysaccharides were dependent on their structure, type and dose [7,10,20]. Different values of inhibition zone diameter (10-28mm) were observed at a concentration of 10% for all obtained PS samples against all bacterial strains. The inhibition zones of Parsley, Spinach, Watercress Radish and Lettuce leaves PS at 10% concentration were higher (20-28mm) against S. aureus as shown in table (3). Decreases in inhibition zones (14-22mm) against E.coli as compared to the PS effects against S. aureus were observed at 10% concentration (Table3). Decreasing of inhibition zones were observed with low PS concentrations (10-1, 10-2 and 10-3 respectively). These results are in accordance with those reported by other investigators [7,41,64]. Inhibition zone (12-18mm) was observed with the obtained PS at a concentration of 10% against B. subtilis while the inhibition zone (10-14mm) was observed against MRSA. PS of Radish and Lettuce leaves showed small inhibition zones against B. subtilis and MRSA (Table 3 and Figure 1). Moreover, the inhibition zone diameters exhibited different levels of decreases with the all PS concentrations decrease against S. aureus, E.coli ,B. subtilis and MRSA (table 3). The results suggest that S. aureus and E.coli were being inhibited in the presence of PS isolated from five plant leaves used in the present study. Inhibition effects of PS obtained against different bacterial strains were found to be depending on the concentrations used. No inhibition zone against four bacterial strains was obtained at low all PS concentrations (10-3, 10-4 and 10-5). Similar results were reported by other investigators used different plant sources for production of polysaccharides [7,20,27,64]. Moreover, the present results indicated that the increase of PS concentrations exhibited increase in the inhibition zone diameter.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of the all PS obtained was determined and the results are given in table (3). PS of Parsley, Spinach, Watercress Radish and Lettuce leaves at 10% concentration exhibited the higher AMA against S. aureus, E.coli. and B. subtilis than that of MRSA. PS of Parsley, Spinach, Watercress exhibited high activity against B. subtilis and MRSA than the other obtained PS used in the present study. The PS showed more effective against S.aureus with a zone of inhibition (20-28mm) and were least effective against the other tested strains. These results are close related to those obtained by other studies [58,69-71]. Among the other bacterial strains studied, E.coli showed inhibition zone (14-22mm). B. subtilis (12-18mm) and MRSA (10-14mm) at conc. 100µg. The MIC values of PS were found to have Low MIC value of 1mg/ml for S. aureus and E.coli. PS showed a higher MIC value of 5mg/ml with B. subtilis and MRSA. These results indicated a higher activity of PS with S. aureus, and E.coli and less activity with B. subtilis and MRSA. PS obtained were inhibited the growth of S. aureus, E. coli, B. subtilis and MRSA in vitro. Several investigators suggests that some plant leaves contain different type of polysaccharides have effective in treating diseases of microbial infections [7,64,68]. Other investigators, they used plant polysaccharides in treating different diseases (diabetes, hyperlipidemia and cancer) [13,17,18,72]. However, different effects of polysaccharides were dependent on their structure, type and dose [7,20,73]. This common plant leaves consider an important sources of antimicrobial substances with minimal inhibitory concentration (MIC) of 60–100 µg/ml.

Conclusion

The present results demonstrate that bacterium S. aureus, E. coli, B. subtilis and MRSA were being inhibited by PS isolated from five plant leaves used in the present study. Inhibition zones of S. aureus E. coli, B. subtilis and MRSA were found to be higher at 10% PS, whereas no inhibition zone was observed on lower concentrations of PS (10-3-10-5). Polysaccharide (PS) isolated from plant leaves used in the present study produce inhibitory activity against S. aureus, E. coli, B. subtilis and MRSA in vitro.

Acknowledgments

The author acknowledges the National Research Centre, Dokki, Cairo, Egypt. They funded this work. This work was supported by grants from NRC, Egypt.

References

1. Yuhui W, Haodi L, Baoying W, Gülzire G, Jingliang Q, et al. (2025) Recent advances in the biosynthesis of polysaccharide-based antimicrobial glycoconjugate vaccines. Front Microbiol 15: 1457908.
2. Jukanti AK, Gaur PM, Gowda CLL, Chibbar RN (2012) Nutritional quality and health benefits of chickpea (Cicer arietinum L.) British Journal of Nutrition 108: 1: S11-26.
3. Hang H, Chen R, Wang C, Sun Y, Du D (2025) A review of the extraction processes and biological characteristics of Chrysanthemum polysaccharides. Int J Biol Macromo l285: 138224.
4. Kim MJ, Moon Y, Tou JC, Mou B, Waterland NL (2016) Nutritional value, bioactive compounds and health benefits of lettuce (Lactuca sativa L.) J Food Compos. Anal 49: 19-34.
5. Rawani A, Pal S, Chandra G (2011) Evaluation of antimicrobial properties of four plants extacts against human pathogens. Asian Pac J Trop Biomed 1: S71-S75.
6. Bhat BA, Ahmad M, Amin T, Ahmad A, Shah WA (2015) Evaluation of phytochemical screening, anticancer and antimicrobial activities of Robinia pseudoacacia. Am J Pharm Health Res 3: 62-69.
7. Yucui L, Linyin Q, Yuanhui M, Xianmei L, Qianni W, et al. (2024) Antibacterial activity of a polysaccharide isolated from litchi (Litchi chinensis Sonn.) pericarp against Staphylococcus aureus and the mechanism investigation. International Journal of Biological Macromolecules 279: 134788.
8. Mazumder S, Ghosal PK, Pujol CA, Carlucci M, Damonte EB, et al. (2002) Isolation, chemical investigation and antiviral activity of polysaccharides from Gracilaria corticata (Gracilariaceae, Rhodophyta). Inter J Biol Macromol 31: 87-95.

9. Maciel JS, Chaves LS, Souza BWS, Teixeira DIA, Freitas ALP, et al. (2008) Structural characterization of cold extracted fraction of soluble sulfated polysaccharides from red sea weed Gracilaria birdiae. Carbohydr Res 71: 559565.
10. Wang Libo, Cheng Long, Li Fangcheng, Li Tengfei, Yu Zeyuan, et al. (2018) Optimization of Ultrasound-Assisted Extraction and Structural Characterization of the Polysaccharide from Pumpkin (Cucurbita moschata) Seeds. Molecules 23: 1207.
11. Sato Y, Suzaki S, Nishikawa T, Kihara M, Shibata H, et al. (2000). Phytochemical flavones isolated from Scutellaria barbata and antibacterial activity against methicillin-resistant Staphylococcus aureus. J Ethnopharmacol 72: 483-488.
12. Jahangir M, Kim HK, Choi YH (2009) Health-Affecting compounds in Brassicaceae. Comprehensive reviews in food science and safety 8: 31-39.
13. Huan Shen, Guo Tang, Guang Zeng, Yongjin Yang, Xingwei Cai, et al. (2013) Purification and characterization of an antitumor polysaccharide from Portulaca oleracea L 93: 395-400.
14. Wu X, Mao G, Fan Q, Zhao T, Zhao J, et al. (2012) Isolation, purification, immunological and anti-tumor activities of polysaccharides from Gymnema sylvestre. Food Research International 48: 935-939.
15. Persin Z, Stana-Kleinschek K, Foster TJ, Van Dam JEG, Boeriu CG, et al. (2011) Challenges and opportunities in polysaccharides research and technology: The EPNOE views for the next decade in the areas of materials, food and health care, Carbohydrate Polymers 84: 22-32,
16. Pranjal S, Bhanu PS, Dash SK (2013) Isolation and Characterisation of Some Natural Polysaccharides as Pharmaceutical Excipients International Journal of Pharm. Tech Research 5: 1196-1206.
17. Mengyi Zhou, Lei Yang, Shilong Yang, Fengyi Zhao, Li Xu, et al. (2018) Isolation, characterization and in vitro anticancer activity of an aqueous galactomannan from the seed of Sesbania cannabina Internal J. of Biol. Macromolecules 113: 1241-124.
18. Zichao W, Yi Z, Yiwei H, Libo Y, Xirui L, et al. (2024) Improvement of antibacterial activity of polysaccharides via chemical modification: A review Int J Biol Macromol 269: 132163.
19. Mao WJ, Li BF, Gu QQ, Fang YC, Xing HT (2004) Preliminary studies on the chemical characterization and antihyperlipidemic activity of polysaccharide from the brown alga Sargassum fusiforme. Hydrobiologia 512: 263-266.
20. Jwanny EW, Moharib SA, Rasmy GE (2009) Effect of two polysaccharides on chemically- induced colorectal cancer in rats. Advav in Food Sci 31: 202-209.
21. Ji YB, Gao SY (2005) Effects and mechanism of Sargassum fusiforme polysaccharides on antitumor in vitro. Chin J Clin Rehabil 34: 190-192.
22. Chena G, Jie X, Xia M, Yi H, Xiaogang L, et al. (2012) Characterization and antitumor activities of the water-soluble polysaccharide from Rhizoma Arisaematis. Carbohydrate Polymers. 90: 67-71.
23. Raja RDA, Jeeva S, Prakash JW, Antonisamy JM, Irudayaraj V (2011) Antibacterial activity of selected ethnomedicinal plants from South India. Asian Pac J Trop Med 4: 375-378.
24. Sinha S, Astani A, Ghosh T, Schnitzler P, Ray B (2010) Polysaccharides from Sargassum tenerrimum: Structural features, chemical modification and antiviral activity. Phytochem 71: 235-242.
25. Chen YY, Chang HM (2004) Antiproliferative and differentiating effects of polysaccharide fraction from fuling (Poriacocos) on human leukemic U937 and HL-60 cells. Food and Chemical Toxicology 42:759-769.
26. Turkoglu A, Kivrak I, Mercan N, Duru ME, Gezer K, et al. (2006) Antioxidant and antimicrobial activities of Morchella conica Pers. Afr. J. Biotechnol 5: 1146-1150.
27. Abd-Elmoneim MA, Bakar AA, Awad IM, Mohamed EM, Moharib SA (2013) Anticarcinogenic effect of Raphanus Sativus on 1, 2 dimethylhydrazine (DMH) induced colon cancer in rats. The Egyptian J of Hospital Med 51: 473-486.
28. Aguilar B, Amorena B, Iturralde M (2001) Effect of slime on adherence of Staphylococcus aureus isolated from bovine and ovine mastitis. Veterinary Microbiology 78: 183-191.
29. Ailbhe McG, Rahel S Das, Arturo BS, Julie M, Noelia F, et al. (2025) Antimicrobial Activities of Polysaccharide-Rich Extracts from the Irish Seaweed Alaria esculenta, Generated Using Green and Conventional Extraction Technologies. Against Foodborne Pathogens Mar Drugs 18: 23-46.
30. Huang YC, Chou YH, Su LH, Lien RI, Lin TY (2006) Methicillin- Resistant Staphylococcus aureus colonization and Its association with infection among infants hospitalized in neonatal intensive care units. Pediatrics 118: 469-474.
31. Masako K, Hideyuki I, Shigeyuki O, Zenro I (2005) A novel method to control the balance of skin microflora. I. Attack on biofilm of Staphylococcus aureus without antibiotics. J Dermatol Sci 38: 197-205.
32. Akrayi HFS (2012) Antibacterial effect of seed extracts of cardamom (Elettaria cardamomum) against Staphylococcus aureus and Proteus mirabilis). J of pure sci 17: 14-18.
33. Sheikh M, Islam I, Rahman MS, Rahman MM (2010) Control of some human pathogenic bacteria by seed extracts of cumin (CuminumCyminum) Agricconcepec Sci 5: 39-44.
34. Geetha RV, Anitha R, Lakshmi T (2011) Evaluation of anti-bacterial avtivity of fruit rend extract of Gsrcina mangostana LINN on enteric pathogens - an in vitro study. Asian J Pharm Clin Res 4: 115-118.
35. Foo LY, Lu Y, Howell AB, Vorsa N (2000) A-type proanthocyanidin trimmers from cranberry that inhibit adherence of uropathogenic P-fimbriated Escherichia coli. J Nat Prod 63: 1225-1228.
36. Gould IM (2009) Antibiotic resistance: the perfect storm. Internal J of Antimicrob Agents 34: S2-S5.
37. Levy SB, Marshall B (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nature Medicine 10: S122-S129.
38. Gao S, Wang W, Li J, Wang Y, Shan Y, et al. (2025) Unveiling polysaccharides of Houttuynia cordata Thunb.: Extraction, purification, structure, bioactivities, and structure-activity relationships. Phytomedicine 138: 156436.
39. Cragg GM, Newman DJ (2013) Natural products: A continuing source of novel drug leads. Biochim Biophys Acta Gen Subj 1830: 3670-3695.
40. Saleem M, M Nazir, MS Ali, H Hussain, YS Lee, et al. (2010) Antimicrobial natural products: an update on future antibiotic drug candidates. Natural Product Reports 27: 238-254.
41. Mukherjee PK, Saritha GS, Suresh B (2002) Antimicrobial potential of two different Hypericum species available in India. Phytother Res 16: 692-695.
42. Brock TD, Madigan MT, Martinko JM, Parker J (1994) Biology of Microorganisms, 7th ed., Prentice Hall. Englewood Cloffs NJ 118-124.
43. Chandramouli Y, Firoz S, Vikram A, Padmaja C, Chakravarthi RN (2012) Design and evaluation of controlled relelese

matrix tablets of acyclovir sodium using tamarind seed Polysaccharides, Journal Of Pharmaceutical Biology 2: 55-62.
44. Karaman I, Sahin F, Güllüce M, Ögütçü H, Sengül M, et al. (2003) Antimicrobial activity of aqueous and methanol extracts of Juniperus oxycedrus L. J Ethnopharma 85: 213-235.
45. Paterson DL (2006) Resistance in Gram-negative bacteria Enterobacteriaceae. Am J Infect Control 119: 20-28.
46. Garcia-Sosa K, Villarreal-Alvarez N, Lubben P, Peña-Rodriguez LM (2006) Chrysophanol, an antimicrobial anthraquinone from the root extract of Colubrina greggii. J Mex Chem Soc 50: 76-78.
47. Lee JH, Shim JS, Chung MS, Lim ST, Kim KH (2009) Inhibition of pathogen adhesion to host cells by polysaccharides from Panax ginseng. Biosci Biotechnol Biochem 73: 209-212.
48. Pugh N, Ross SA, Elsohly MA, Pasco DS (2001) Characterisation of Aloeride, a new high molecular weight polysaccharide from Aloe vera with potent immunostimulatory activity. J Agric Food Chem 49: 1030-1034.
49. Butler MS (2008) Natural products to drugs: natural product derived compounds in clinical trials. Natural Product Reports 25: 475-516.
50. Jebur MH, Bnuyan Dr Il A, Yasri Dr AA, Hindi Dr NKK (2014) Antimicrobial effect of seed extracts leaves and crude oil of Cardamom (Elettaria cardamomum) against different types of bacteria in HILLA CITY, IRAQ. World Journal of Pharmaceutical Research 3: 4934-4944.
51. Wu YW, Ouyang J, Xiao XH, Gao WY, Liu Y (2006) Antimicrobial properties and toxicity of anthraquinones by microcalorimetric bioassay. Chinese J Chem 24: 45-50.
52. Chihara G, Hamuro J, Maeda Y, Arai Y, Fukuoka F (1970) Fractionation and purification of the polysaccharides with marked antitumor activity especially lentinan from Lentinus edodes (Berk.) Sing. (an edible mushroom). Cancer Res 30: 2776-2781.
53. Wilson CM (1959) Quantitative determination of sugars on paper chromatograms. Anal. Chem 31: 1199-1201.
54. Bauer AW, Kirby MD, Sherris SC, Turck M (1966) Antibiotic susceptibility testing by standard single disc diffusion method. J Ethnopharmacol 45: 493-496.
55. Staub AM (1965) Methods in Carbohydrate Chemistry 5: 5-7.
56. Cerning J, Renard C, Thibault J, Bouillanne C, Lan-don M, et al. (1994) Carbon source requirements for exo-polysaccharide production by Lactobacillus casei CG11 and partial structure analysis of the polymer. Appl Environ Microbiol 60: 3914-3919.
57. Pongsamart S, Panmaung T (1998) Isolation of polysaccharides from fruit-hulls of durian (Durio zibethinus L.). Songklanakarin J Sci Technol 20: 323-332.
58. Yucui L, Linyin Q, Yuanhui M, Xianmei L, Qianni W, et al. (2024) Antibacterial activity of a polysaccharide isolated from litchi (Litchi chinensis Sonn.) pericarp against Staphylococcus aureus and the mechanism investigation. International Journal of Biological Macromolecules 279: 134788
59. Overlay P, Guoliang W, Xi Y, Xiangru C, Jiaoyan H, et al. (2024) Construction and antibacterial activities of walnut green husk polysaccharide based silver nanoparticles (AgNPs). International Journal of Biological Macromolecules 276: 133798.
60. Eloff JN (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64: 711-713.
61. Singh RB (2013) Nature of seeds polysaccharide obtained from medicinal plant of Wrightia tinctoria R. BR. (Roxb). Adv in appl Sci Res 4: 115-118.
62. Rashed AN, Afifi FU, Disi AM (2003) Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L. (growing in Jordan) in Mus musculus JVI-1. J Ethnopharmacol 88: 131-136.
63. Gutiérrez RMP, Perez RL (2004) Raphanus sativus (Radish): Their Chemistry and Biology. The Sci World J 4: 811-837.
64. Al-Snafi AE (2015) The chemical constituents and pharmacological importance of Carthamus Tinctorius- an overview. Journal of Pharmaceutical Biology 5: 143-166.
65. Wang F, Li J, Jiang Y (2010) Polysaccharides from mulberry leaf in relation to their antioxidant activity and antibacterial ability. Journal of Food Process Engineering 33: 39-50.
66. Baek SH, Lee JG, Park SY, Bae ON, Kim DH, et al. (2010) Pectic polysaccharides from Panax ginseng as the antirotavirus principals in ginseng. Biomacromolecules 11: 2044-2052.
67. Jong-Heum P, Heuyn-Kil S, Cher-Won H (2001) New antimicrobial activity from Korean radish seeds (Raphanus sativus L.). J Microbiol Biotechnol 11: 337-341.
68. Bao X, Wang Z, Fang J, Li X (2002) Structure features of an immunostimulating and antioxidant acidic polysaccharide from the seeds of Cuscuta chinensis. Planta Medica 68: 237-243.
69. Kubo I, Fujita K, Kubo A, Nihei K, Ogura T (2004) Antibacterial activity of coriander volatile compounds against salmonella. J Agric Food Chem 52: 3329-3332.
70. Keskin D, Toroglu S (2011) Studies on antimicrobial activities of solvent extracts of different spices. J Environ Biol 32: 251-256.
71. Giulia AR, Rosana BRF (2022) Antimicrobial Polysaccharides Obtained From Natural Sources. Future Mycrobiology 17: 701-716.
72. Sanchita M, Nagapriya B, Soumyava B, Tirupathi R (2024) Ultrastructural polymicrobial Staphylococcus aureus–Pseudomonas aeruginosa interactions and antimicrobial resistance in ex vivo cornea model. Future Mycrobiology 20: 117-135.
73. Yin Z, Xinxin C, Tingting C, Xiaoqiang C (2022) A review of the antibacterial activity and mechanisms of plant polysaccharides. Trends in Food Science and Technology 123: 264-280.