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Antimicrobial Activities of Polysaccharides Isolated from Some Plant Seeds

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The present investigation is about the measurement of antimicrobial activity (AMA) of polysaccharides isolated from the seeds of pumpkin (*Cucurbita pepo*), purslane (*Portulaca oleracea*), safflower (*Carthamu stinctorius*),coriander (*Coriandrum sativum*) and rapeseed (*Brassica napus*) 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 (PSs) were extracted, isolated and purified from different seeds separately. Paper chromatographic analysis of the purified PSs, revealed the presence of different percentages of monosaccharides constituents per each seed PS. *In vitro* study was performed to evaluate the obtained PSs activities against 4 microbial strains (*S. aureus, E. coli, B subtilis* and *MRSA*) using agar diffusion method. PSs 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 PSs 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 PSs against *S. aureus, E. coli, B subtilis*

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and *MRSA* at 10% PS within 24 hours. Inhibition zone diameters exhibited different levels of decreases with the PSs concentrations decreases against *S. aureus, E. coli*, *B. subtilis and MRSA*. Inhibition effects of PSs against different microbial strains were found to be depending on PSs concentration. However, the lowest concentration of PSs produced lower inhibitory activity against *S. aureus B*. *subtilis E. coli* and *MRSA*. The results obtained with all PSs showed best AMA against *S. aureus* and *E. coli* than the other two microbial strains used in the present study. Results indicated these PSs have AMA against Gram-positive and Gram-negative bacterium at 10% concentration. The common PSs consider important sources of antimicrobial agents with minimal inhibitory concentrations of 100-500µg/ml for treatment some infectious diseases.

Keywords: Antimicrobial; anticancer; polysaccharides; microorganisms; In vitro.

1.INTRODUCTION

Plant seeds one of the most important economic sources of proteins, lipids and carbohydrates which are essential for human nutrition and treatment of diseases. Plant seeds have a long history as a part of human culture and are associated with people from ancient time as food in many regions of the world and generally consumed for its nutritive values, health benefits, pharmaceutical, biomedical and biological uses [1,2,s3,4] reported plant seeds have medicinal therapeutic properties, used as therapeutic agent for protection against some diseases. Plant seed extracts are showed several active therapeutic compounds represent a source of their chemical constituents, including polysaccharides, tannins, oils, phenolic, flavonoids, saponins, triterpenoids and alkaloids have active antibacterial properties [5, 6,7] they reported these compounds were investigated as antimicrobial *In vitro*. Polysaccharides represent majority of carbohydrates and consider the largest group of natural biopolymers produced in the world used as food, medicine, pharmaceutical, drug agents and have different biological functions [8, 9,10]. Other investigators [7,11,12,] reported the majority of seed polysaccharides have health benefits, pharmaceutical and therapy in medicine. Polysaccharides are non-toxic and water soluble that consequently suitable for different pharmaceutical and biomedical uses and play important roles in several physiological and pathological conditions [13,14]. Polysaccharides are the most abundant natural biopolymers consisting of a large number of monosaccharide have several degree of polymerization to form a variety of branched or linear structures necessary for biological activities [15,16]. Polysaccharide are consider as natural sources play an important role in human growth and development [12,13] reported polysaccharide has interest of some healthy food for patients with cardiovascular diseases as its nutritional and medicinal properties. Recent study has shown that some polysaccharides intake in rats cause improve in some biochemical parameters and reduce the risk of some diseases [7,17]. Different types of natural polysaccharides used as hypoglycemic [16,18], hypolipidemic [19] and anticancer agents [17,20]. Several polysaccharides have antitumor [21,22], therapeutic [7,14,23], antiviral [8,24], antiproliferative [25] and antibacterial activities [26]. Polysaccharides are pharmacologically studied for its antifungal, antiinflammatory, 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 chemically induced colon cancer [27]. Bacteria, *Salmonella* sp., *Pseudomonas* sp., *Escherichia coli* and *Staphylococcus aureus* are responsible for several diseases in humans and animals [28] reported these bacteria are the major agents cause damage in some fields including food industry [29,30]. *Staphylococcus aureus*, *Clostridium perfringens* and *Staphylococcus epidermidis* are producing toxic symptoms in humans and cause diseases [31,32] they reported many gram-negative bacteria are difficult food contaminants and pathogenesis of infections against antibiotics [33,34]. 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 [33,36] due to pathogenic bacterial resistant and side effects of traditional antibiotics [30,31,37].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 [28, 38,39] to overcome the resistance and side effects of the conventional antimicrobial agents [11, 34,40]. 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 [16,40,43] due to human pathogenic microorganisms resistant to antibiotics and failing in treatment of different types of infectious diseases particularly in developing countries [36,44,45]. Several investigators [46, 47] indicates the antibacterial activity is due to different chemical compounds that recognized as active antimicrobial agents. Moreover, polysaccharides component has indirect antimicrobial activity through stimulate phagocytic leukocytes [5,26,48] reported the medicinal importance of plants come from the presence of bioactive polysaccharides in plants seeds. 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]. Antiinfectious and antitumor drugs either under clinical trials or in the market are of natural origin [35,36,49]. Different compoumds were used in medicine as anti-spasmodic and antidiuretic [32,50] 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. Moreover, different plant extracts are widely used as antidiabetic [18], antimicrobial [5], antibacterial [34], antidiuretic [11,50] and anticancer [17]. Polysaccharides are known to exhibit antimicrobial activities against clinical, food-borned pathogens and food spoilage bacteria. Other investigators found specific plant extracted compounds such as dietary fiber, anthraquinones and dihhydroxyanthraquinones have direct antimicrobial activities [35,51]. Pumpkin (*Cucurbita pepo*), purslane (*Portulaca oleracea*), safflower (*Carthamu stinctorius*), coriander (*Coriandrum sativum*) and rapeseed (*Brassica napus*) seeds are commonly used as food or in medicine in many regions of the world. Polysaccharides are biopolymer molecule have complicated structures with a variety of sugar residues, glycosidic linkages (1→3 and1→4), and bonding locations, as a result of this complex intramolecular information leads to have various biological activities against many type of diseases. Moreover, polysaccharides exhibited many conformations and structures that resulted in important biological activities such as antioxidant, antimicrobial, antiglycation, anticancer, hypolipidemic, and prebiotic activities [7,8,11,17].

This study aims to isolate and purify polysaccharides from pumpkin (*Cucurbita pepo*), purslane (*Portulaca oleracea*), safflower (*Carthamu stinctorius*), coriander (*Coriandrum sativum*) and rapeseed (*Brassica napus*) seeds. Antimicrobial activities of the obtained purified polysaccharides (PSs) against four bacterial strains *In vitro* were determined.

2. MATERIALS

2.1 Seed Polysaccharides

Pumpkin (*Cucurbita pepo*), purslane (*Portulaca oleracea*), safflower (*Carthamu stinctorius*), coriander (*Coriandrum sativum*) and rapeseed (*Brassica napus*) seeds 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 48 hours. Seeds 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 (80^{o}C) for 18 hours [52] and homogenized at 100^{o}C using homogenizer (Mechanika precyzyjna warszawa model MPW-309, Poland). Extracts were then collected using cooling centrifuge (Sigma 2K).

Monosaccharides such as, glucose, galactose, fructose, arabinose, mannose, rhamnose, fucose, xylose, maltose, trehalose and raffinose were used as standard obtained from Sigma Chemical Company USA.

2.2 Microorganisms

Four bacterial strains including *Escherichia coli* (*E.coli*), *Staphylococcus aureus* (*S. aureus*), *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°C.

3. METHODS

3.1 Microorganisms Tested

The inhibitory effects of seed polysaccharides 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*. Stock cultures of all microbial strain were grown on nutrient agar plates and maintained in the nutrient agar slants at 4°C. The microbial strains were activated before the antibacterial 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].

3.2 Preparation and Determination of the Purified Polysaccharides (PSs)

Five plant seeds powdered (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 and 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% EtOH then 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 isolated from 5 seed samples were partially purified separately and dried by hot air oven [20,57]. The obtained polysaccharides were weighed and freeze-dried till used. Polysaccharide samples obtained were dissolved individually in deionized water containing 1 % sodium hydroxide, vortex mix and filtered using Whatman filter paper. Solution of polysaccharide was freshly prepared from PS powder to obtain a series of 5-fold dilutions of various concentrations of each polysaccharide in distilled water before added to the agar media used for antimicrobial tests.

3.3 Identification of Monosaccharide (MS)

Monosaccharide content of each polysaccharide sample was identified and measured using paper chromatography [14,58]. Monosaccharides such as glucose, galactose, fructose, arabinose, mannose, rhamnose, fucose, xylose, maltose, trehalose and raffinose were used as standard controls.

3.4 Preparation of PSs 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.

3.4.1 *In vitro* antimicrobial activities (AMA)

Bacterial strains of *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. Antimicrobial activities 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 polysaccharides were dissolved 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 PSs 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. Antimicrobial activities of PSs are the average of triplicate analyses.

3.4.2 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 PSs against the four selected bacterial strains was determined.

4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 Polysaccharides (PS)

The present study was done to evaluate the antimicrobial activity of the purified polysaccharides obtained from different plant seeds against four different bacterial strains. Soluble polysaccharides obtained from five plant seeds revealed the presence of different percentages of polysaccharides as recorded in Table 1. Polysaccharides obtained from pumpkin (*Cucurbita pepo*), purslane (*Portulaca oleracea*), safflower (*Carthamu stinctorius*), coriander (*Coriandrum sativum*) and rapeseed (*Brassica napus*) seeds (3.6, 14.4, 10.8, 8.6 and 12.2 g/100g respectively). Highest PSs were obtained from purslane, rapeseed and safflower seeds than that of pumpkin and coriander seeds (Table 1). Chromatographic analysis of the obtained polysaccharides isolated from different plant seeds, showed the presence of different type and levels of its 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 PSs obtained from different plant seeds. The present results showed small amounts of rhamnose, raffinose and xylose were found in all PSs isolated from the plant seeds used in the present study. Lowest levels of maltose and trehalose were also observed. The present results showed that these differences were not only observed in the levels between PS obtained from plant seed sources, but also in their monosaccharide constituents.

4.1.2 Determination of antimicrobial activity (AMA)

Antimicrobial activity (AMA) of the obtained polysaccharides (PSs) isolated from 5 different plant seeds 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 PSs samples of pumpkin (*Cucurbita pepo*), purslane (*Portulaca oleracea*), safflower (*Carthamu stinctorius*), coriander (*Coriandrum sativum*) and rapeseed (*Brassica napus*) give AMA against *S. aureus, E.coli, B. subtilis* and MRSA. PSs obtained from pumpkin and coriander seeds showed no effect against *B. subtilis* and MRSA (Table 2). However, PSs obtained from 5 different plant seeds were inhibited the growth of *S. aureus*, *E. coli*, *B subtilis* and *MRSA In vitro*.

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). The results obtained with all PS showed best antimicrobial activity against *S. aureus* and *E. coli* than the other two microbial strains used in the present study. The PSs obtained from purslane, safflower and rapeseed were active against both *B.subtilis* and *MRSA* strains. These results indicated that PS has antimicrobial activity against some bacterial strains.

Different values of inhibition zone diameter (12-26mm) were observed at a concentration of 10% for all obtained PSs samples against all bacterial strains. The inhibition zones of pumpkin, purslane, safflower, coriander and rapeseed seeds PSs seeds at 10% concentration were higher (20-26mm) against *S. aureus* as shown in Table (3). Decreasing of inhibition zones were observed with low PSs concentrations (10^{-1}, 10^{-2} and 10^{-3} respectively). Decreases in inhibition zones (16-2**0**mm) against *E.coli* as compared to the PSs effects against *S. aureus* were observed at 10% concentration (Table 3).

Inhibition zone (14-18mm) was observed with the obtained PSs at a concentration of 10% against *B. subtilis* while the inhibition zone (12-16) was observed against *MRSA*. PSs of pumpkin and coriander showed no inhibition zones against *B. subtilis* and *MRSA* (Table 3 and Fig. 1). Moreover, the inhibition zone diameters exhibited different levels of decreases with the all PSs 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 PSs isolated from five plant seeds used in the present study. Inhibition effects of PSs 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 PSs concentrations exhibited increase in the inhibition zone diameter.

Table 1. Polysaccharides (PS) isolated from plant seeds and monosaccharide constituents

Seed samples	PS (g %)	Monosaccharides (g %)										
		Glu.	**Gal.**	**Fr.**	**Arab.**	**Man.**	**Rha.**	**Fuc.**	**Xyl.**	**Mal.**	**Tre.**	**Raff.**
Pumpkin (*Cucurbita pepo*)	3.60	0.60	0.20	0.10	1.00	0.60	0.40	0.20	0.20	tr	Tr	0.2
purslane (*Portulaca oleracea*)	14.40	1.60	1.80	0.40	3.60	4.20	0.1	Tr	0.40	0.8	0.4	0.8
Safflower(*Carthamu stinctorius*)	10.80	1.60	1.20	0.80	2.20	2.80	0.60	0.20	0.80	tr	0.4	0.1
Coriander (*Coriandrum sativum*)	8.60	0.60	0.80	0.40	2.60	3.40	0.20	Tr	0.20	tr	Tr	0.2
Rapeseed (*Brassica napus*)	12.20	1.20	1.60	0.80	4.40	2.40	0.60	0.80	0.20	0.1	Tr	0.1

Mean of three samples.

Table 2. Activity of polysaccharides (PS) on growth of 4 bacterial strains in agar diffusion method

Seed samples	Antimicrobial activity (AMA)			
	S. aureus	***E. coli***	***B. subtilis***	***MRSA***
Pumpkin (*Cucurbita pepo*)	+ve	+ve	-	-
purslane (*Portulaca oleracea*)	+ve	+ve	+ve	+ve
Safflower(*Carthamu stinctorius*)	+ve	+ve	+ve	+ve
Coriander (*Coriandrum sativum*)	+ve	+ve	-	-
Rapeseed (*Brassica napus*)	+ve	+ve	+ve	+ve

Mean of three samples, +ve AMA detect.

Table 3. Minimum inhibitory concentration (MIC) values of ten seed oils

Seed oil samples	**Minimum inhibitory concentration (MIC) for *S. aureus***					
	10	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
Pumpkin (*Cucurbita pepo*)	22 mm	4mm	1 mm	-	-	-
purslane (*Portulaca oleracea*)	26 mm	6 mm	4 mm	-	-	-
Safflower(*Carthamu stinctorius*)	22mm	2mm	2 mm	-	-	-
Coriander (*Coriandrum sativum*)	24mm	4mm	2 mm	-	-	-
Rapeseed (*Brassica napus*)	20mm	2mm	2mm	-	-	-
Seed oil samples	**Minimum inhibitory concentration (MIC) for *E. coli***					
	10	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
Pumpkin (*Cucurbita pepo*)	18mm	4mm	1 mm	-	-	-
purslane (*Portulaca oleracea*)	20 mm	6mm	2mm	-	-	-
Safflower(*Carthamu stinctorius*)	18 mm	4 mm	1 mm	-	-	-
Coriander (*Coriandrum sativum*)	20 mm	2 mm	1 mm	-	-	-
Rapeseed (*Brassica napus*)	16mm	2mm	1mm	-	-	-
Seed oil samples	**Minimum inhibitory concentration (MIC) for *B. subtilis***					
	10	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
Pumpkin (*Cucurbita pepo*)	-	-	-	-	-	-
purslane (*Portulaca oleracea*)	18mm	4 mm	2 mm	-	-	-
Safflower(*Carthamu stinctorius*)	14mm	2mm	1mm	-	-	-
Coriander (*Coriandrum sativum*)	-	-	-	-	-	-
Rapeseed (*Brassica napus*)	16mm	2mm	1mm	-	-	-
Seed oil samples	**Minimum inhibitory concentration (MIC) for *MRSA***					
	10	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
Pumpkin (*Cucurbita pepo*)	-	-	-	-	-	-
purslane (*Portulaca oleracea*)	16mm	2mm	-	-	-	-
Safflower(*Carthamu stinctorius*)	14mm	1mm	-	-	-	-
Coriander (*Coriandrum sativum*)	-	-	-	-	-	-
Rapeseed (*Brassica napus*)	12mm	1mm	-	-	-	-

Mean values of three samples.

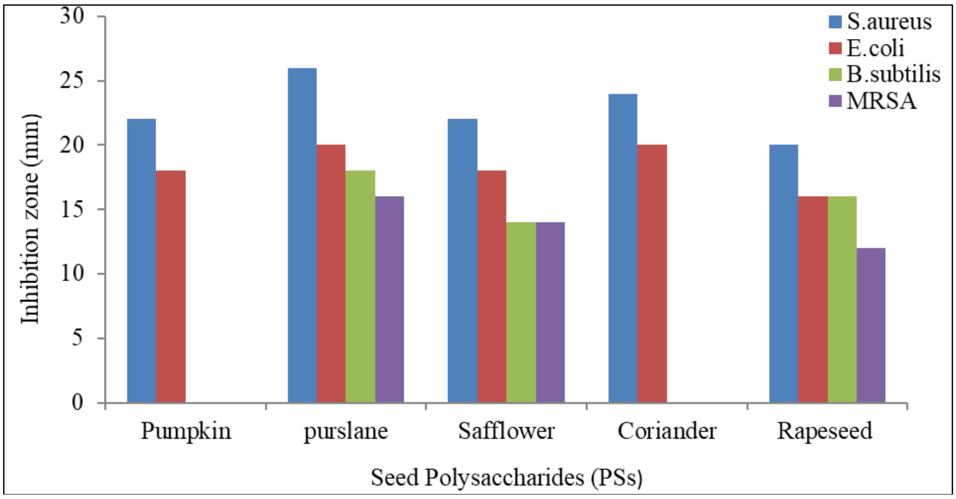

Fig. 1. Antimicrobial activities of seed polysaccharides (PSs) against four bacterial strains

4.1.3 Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined for the all obtained PSs and the results are given in Table 3. PSs of pumpkin, purslane, safflower, coriander and rapeseed seeds PSs at 10% concentration exhibited the best antibacterial activity against *S. aureus* and *E. coli*. PSs of purslane, safflower and rapeseed exhibited high activity against *B. subtilis* and *MRSA* than the other obtained PSs. The PSs were more effective against *S. aureus* with a zone of inhibition (20-26) and were least effective against the other tested strains. Among the other bacterial strains studied, *E. coli* showed a zone of inhibition (16-20mm), *B. subtilis* (14-18mm) and *MRSA* showed inhibition zone (12-16mm) at conc. 100µg. The MIC value of PSs were found to have Low MIC value of 0.5mg/ml for *S. aureus* and *E. coli*. PSs showed a higher MIC value of 2mg/ml with *B. subtilis* and *MRSA*. These results were indicated higher activity of PSs with *S. aureus,* and *E. coli* and less activity of the PS with *B. subtilis* and *MRSA*. PSs obtained from different plant seeds were inhibited the growth of *S. aureus, E. coli, B. subtilis* and *MRSA In vitro* This common plant seeds consider an important sources of antimicrobial substances with minimal inhibitory concentration (MIC).

4.2 Discussion

The present work was done to investigate the antimicrobial activity of polysaccharides (PSs) on growth inhibition of four different bacterial strains. Polysaccharides obtained from pumpkin (*Cucurbita pepo*), purslane (*Portulaca oleracea*), safflower (*Carthamu stinctorius*), coriander (*Coriandrum sativum*) and rapeseed (*Brassica napus*) seeds (3.6, 14.4, 10.8, 8.6 and 12.2 g/100g respectively). Highest PSs were obtained from purslane, rapeseed and safflower seeds than that of pumpkin and coriander seeds (Fig. 2). Similar results were recorded by several investigators [14,10, 61].

Chromatographic analysis of the obtained polysaccharides revealed different type and levels of monosaccharide constituents such as glucose, galactose, fructose, arabinose, mannose, rhamnose, xylose, maltose, trehalose and raffinose (Table 1). Similar results were obtained by other investigators using cabbage, sugar beet, Jerusalem artichoke, rhubarb and *Raphanus sativus* [14,20,27]. The differences were not only observed in the levels between PSs obtained from plant seed sources, but also in their monosaccharide constituents [10,20]. Similar results were obtained by other investigators [14,27,62]. Different PSs of plant seeds contained highest amounts of monosaccharide comprising mostly glucose, galactose, arabinose and mannose usually arising from glucane, galactan, galactan-mannan and arabinan-galactan. Other studies [7,20,63] showed that a large proportion of the polysaccharide chains is conjugated with the polypeptide and obtained L-arabino-D-galactan isolated from radish both contained arabinose, galactose and fucose [10,27]. Predominant monosaccharides in all PSs obtained from different plant seeds were glucose, mannose and arabinose. Results showed small amounts of rhamnose,fucose and xylose in all seeds PSs. Many investigators [8,16,43], reported the monosaccharides, galactose and mannose are the main polymer of seeds polysaccharide were identified by paper chromatography. These PSs are very viscous when dissolved in water, have biological and physiological importance [12] and has different effects against different diseases [14,18]. The obtained PSs 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 studies [14,20]. The present work was done to investigate the antimicrobial activity of polysaccharides (PSs) on growth inhibition of four different bacterial strains. Different studies on polysaccharides obtained from plant origin showing good antibacterial effects against some common pathogens such as *B. subtilis, E. coli* and *S. aureus* [7,64,65] and / or able to rescue cell viability from rotavirus infection [66,67] reported new antimicrobial substances were isolated from radish seeds (*Raphanus sativus*). However, several investigators suggests that b-glucans and other polysaccharides are effective in treating diseases, microbial infections, cancer and diabetes [27, 34,64].

4.2.1 Determination of antimicrobial activity (AMA)

Antimicrobial activity (AMA) of the obtained polysaccharides (PSs) isolated from 5 different plant seeds 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 PSs samples of pumpkin (*Cucurbita pepo*), purslane (*Portulaca oleracea*), safflower (*Carthamu stinctorius*), coriander (*Coriandrum sativum*) and rapeseed (*Brassica napus*) give AMA against *S. aureus, E.coli, B. subtilis* and MRSA. The present results showed inhibited growth of *S. aureus* by the all PSs isolated from different plant seeds used in the present study (Table 2). The results obtained were found to be similar the results reported by other investigators [7,63,64]. PSs obtained from pumpkin and coriander seeds showed no effect against *B. subtilis* and MRSA (Table 2). However, PSs obtained from 5 different plant seeds were inhibited the growth of *S. aureus*, *E. coli*, *B subtilis* and *MRSA In vitro*. The antimicrobial activity of PSs was done at different concentrations using the diffusion method test and inhibition zones were measured in mm diameter (Table 3). The results obtained with all PS showed best antimicrobial activity against *S. aureus* and *E.coli* than the other two microbial strains used in the present study. The PSs obtained from purslane, safflower and rapeseed were active against both *B.subtilis* and *MRSA* strains. These results indicated that PS has antimicrobial activity against some bacterial strains. Several investigators reported some plant seed polysaccharides are effective in treating diseases of microbial infections [64,68]. Other investigators [18,27] used polysaccharides in treating different diseases (diabetes, hyperlipidemia and cancer). However, different effects of polysaccharides were dependent on their structure, type and dose [7,10,20]. Different values of inhibition zone diameter (12-26mm) were observed at a concentration of 10% for all obtained PSs samples against all bacterial strains. The inhibition zones of pumpkin, purslane, safflower, coriander and rapeseed seeds PSs seeds at 10% concentration were higher (20-26mm) against *S. aureus* as shown in Table 3. Decreases in inhibition zones (16-20mm) against *E.coli* as compared to the PSs effects against *S. aureus* were observed at 10% concentration (Table 3). Decreasing of inhibition zones were observed with low PSs 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 (14-18mm) was observed with the obtained PSs at a concentration of 10% against *B. subtilis* while the inhibition zone (12-16) was observed against *MRSA*. PSs of pumpkin and coriander showed no inhibition zones against *B. subtilis* and *MRSA* (Table 3 and Fig. 1). Moreover, the inhibition zone diameters exhibited different levels of decreases with the all PSs 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 PSs isolated from five plant seeds used in the present study. Inhibition effects of PSs 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 seed polysaccharides [7,20,27,64]. However, the present results indicated that the increase of PSs concentrations exhibited increase in the inhibition zone diameter.

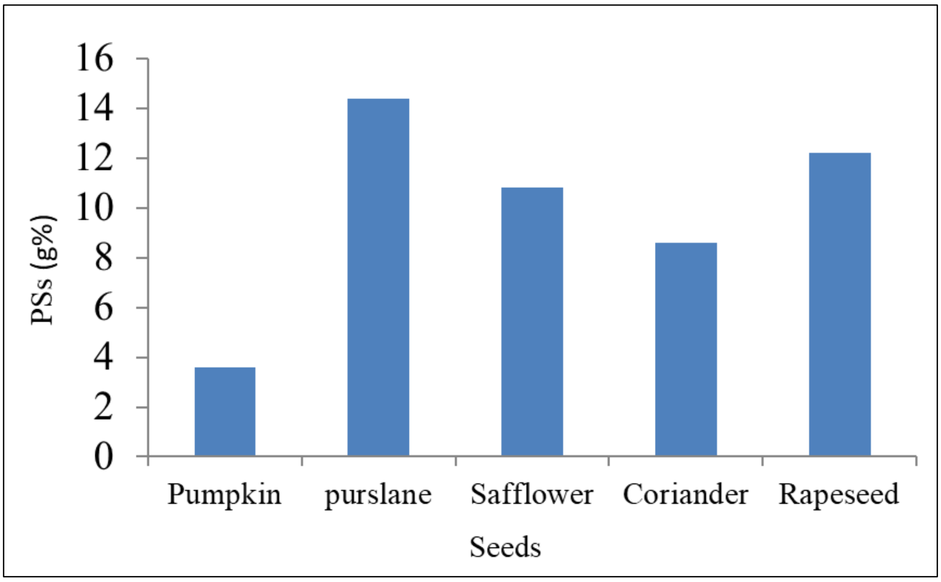

Fig. 2. Polysachharides isolated from plant seeds

4.2.2 Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined for the all obtained PSs and the results are given in Table 3. PSs of pumpkin, purslane, safflower, coriander and rapeseed seeds PSs at 10% concentration exhibited the best antibacterial activity against *S. aureus* and *E. coli*. PSs of purslane, safflower and rapeseed exhibited high activity against *B. subtilis* and *MRSA* than the other obtained PSs. The PSs were more effective against *S.aureus* with a zone of inhibition (20-26) and were least effective against the other tested strains. These results are close related to those obtained by other studies [69,70]. Among the other bacterial strains studied, *E. coli* showed inhibition zone (16-20mm), *B. subtilis* (14-18mm) and *MRSA* (12-16mm) at conc. 100µg. The MIC values of PSs were found to have Low MIC value of 1mg/ml for *S. aureus* and *E. coli*. PSs showed a higher MIC value of 5mg/ml with *B. subtilis* and *MRSA*. These results were indicated higher activity of PSs with *S. aureus,* and *E. coli* and less activity of the PS with *B. subtilis* and *MRSA*. PSs obtained from different plant seeds were inhibited the growth of *S. aureus, E. coli, B. subtilis* and *MRSA In vitro*. Several investigators suggests that some plant seed polysaccharides are effective in treating diseases of microbial infections [7,64,68,71]. Other investigators [13,17,18,72] used plant polysaccharides in treating different diseases (diabetes, hyperlipidemia and cancer). However, different effects of polysaccharides were dependent on their structure, type and dose [7,20,73]. This common plant seeds consider an important sources of antimicrobial substances with minimal inhibitory concentration (MIC) of 60–100 µg/ml.

5. CONCLUSION

The results demonstrate that bacterium S. aureus, E. coli and MRSA were being inhibited by PSs isolated from some plant seeds used in the present study. Inhibition zone of S. aureus was found at 10% PS, whereas no inhibition zone was observed on lower concentrations of PS. Polysaccharide from plant seeds used in the present study produce inhibitory activity against S. aureus, E. coli, B. subtilis and MRSA.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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