PALGO JOURNAL OF AGRICULTURE

ISSN 2476-8359
Volume 5 Issue 1, August 2018.Page 1-8
http://www.palgojournals.org/PJA/Index.htm
Authors Email:smoharib@yahoo.com

ANTIMICROBIAL ACTIVITY OF POLYSACCHARIDES ISOLATED FROM SOME PLANT SEEDS IN VITRO

Sorial A. Moharib

Biochemistry Department, National Research Centre, Cairo. Egypt.

Accepted 17 July, 2018

The present investigation is about the determination of the antimicrobial activity of polysaccharide samples isolated from ten seeds of some plants. Polysaccharides (PS) were isolated from seeds of some plants with water and precipitation with ethanol to obtain crude PS and then purified PS. The obtained polysaccharide have different constituents of monosaccharides obtained. Paper chromatographic analysis revealed the presence of glucose, galactose, arabinose, mannose, rhamnose, and xylose as compared to standard sugars. In vitro study was performed to evaluate the obtained PS activities against 4 microbial strains (*S. aureus, E. coli, B subtilis* and *MRSA*) using agar diffusion method. All PS samples obtained were exhibited antimicrobial activity against *S. aureus* but *Raphanus sativus*, *Anethum graveolens* and *Lactuca sativa* showed antibacterial activity against *E. coli, B subtilis* and *MRSA*. PS at 10% concentration showed different inhibition zones against *S. aureus*, *E.coli*; and *MRSA*. Minimal inhibitory concentrations (MIC) of Polysaccharide against *S. aureus, E.coli, B subtilis* and *MRSA* were found in the range of 0.5-2 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.

Keywords: Antimicrobial, Polysaccarides, Seeds, Microorganisms.

1.INTRODUCTION

Infectious diseases constitute a major health problem, particularly in developing countries, where the rate of mortality and morbidity is very high. Conventional chemotherapy drugs for treatment and medicines are high cost and ineffective against MRSA(Mack et al. 1992 and Aguilar et al. 2001). Carbohydrates are biosynthesized by plants and photosynthesizing microorganisms. The majority of these carbohydrates are polysaccharides that represent largest group of natural biopolymers produced in the world used as food, medicine, pharmaceutical, drug agents and have different biological functions (Mazumder et al. 2002, Maciel et al. 2008, Persin et al., 2011 and Moharib 2016). These 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 (Wang et al.2010 and Wu et al. 2012). Polysaccharides are biological macromolecules group consisting of a large number of monosaccharide joined by glycosidic linkages and have several degree of polymerization to form a variety of branched or linear structures necessary for biological activities and various mechanisms (Pranjalet al.2013). Different types of natural polysaccharides used as hypoglycemic (Moharib and El-Batran 2008 and Pranjal et al. 2013), hypolipidemic (Moharib, 2006). andanti cancer agents (Jwanny et al. 2009 and Moharib et al. 2014). Several polysaccharides have antitumor, therapeutic, antiviral, and antibacterial activities (Chen et al. 2004, Raja et al., 2011,Chena et al. 2012 and Wu et al. 2012). Furthermore, activity against a variety of infectious agents has been attributed to isolated bioactive polysaccharides; for instance, antibacterial (Premanath et al., 2011), antiviral (Mazumder et al. 2002 and Sinha et al. 2010). Antimicrobial drugs growing rapidly and strong demand to find new antimicrobial agents from natural inexpensive sources used in treatment of infectious diseases without side effects (Bern et al., 1992, Mukherjee et al., 2002, Turkoglu et al., 2006 and Chandramouliet al. 2012). Several studies was made for production of new antimicrobial agents from natural sources (Garcia-Sosa et al., 2006, Dabai et al., 2007, Chandramouliet al. 2012 and Pranjal et al.2013) due to human pathogenic microorganisms resistant to antibiotics and failing in treatment of some infectious diseases, particularly in developing countries (Mack et al. 1992 and Paterson, 2006). Several investigators (Lee et al. 2009 and Premanath et al. 2011), 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 (Pugh et al.2001, Turkoglu et al. 2006 and Rawani et al. 2011),reported the medicinal importance of plants come from the

presence of bioactive polysaccharides in plants seeds.
The aim of the present study was done to isolation and characterization of polysaccharides from some plant seeds. Antimicrobial activity of polysaccharides was measurement in vitro against 4 microbial strains.

2. MATERIALS AND METHODS

2.1. Materials

The plant material used in this study was collected locally from markets in Cairo, Egypt.

1-Ten samples from plant seeds were obtained and washed with tap-water followed by distilled water then they were ground in a food grinder (mincer) to a very fine powder, sifted through a 16mesh sieve, packed in bags, and stored at room temperature till used.

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

3- Four bacterial strains including Escherichia coli (E.coli), Staphylococcus aureus (S. aureus), Bacillus subtilis (B subtilis) and MRSA. 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.

2.2. Microorganisms tested

The inhibitory effects of seed polysaccharides were carried out on four species bacteria. The bacterial strains used were *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *MRSA* (standard strain of *MRSA*). Stock cultures of all microbial strain were grown in the nutrient agar slants at 4°C and subjected to antimicrobial testing using overnight culture kept for 24 hours at 37 ºC (Bauer et al., 1966, Brock et al. 1994 and Premanath et al. 2011).

2.3. Preparation and determination of purified PS

10 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 (Staub, 1965 and Chihara et al., 1970). Solutions after cooling were centrifuged and filtered to remove insoluble matters and five volumes of ethanol (98%,v/v) was 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 (Cerning et al 1994). 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 (Abd el Monem et al., 2013). The polysaccharides isolated from 10 seed samples were partially purified separately and dried by hot air oven (Pongsamart and Panmaung, 1998 and Jwanny et al. 2009). 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 obtained 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.

2.4. Identification of Monosaccharide

Monosaccharide contents of each polysaccaride sample was identified and measured using paper chromatography (Wilson, 1959 and Wu et al. 2012). Monosaccharides such as glucose, fucose, maltose, mannose, galactose, arabinose, trehalose, raffinose, xylose and rhamnose were used as standard controls.

2.5. Preparation of stock solution

Each PS sample was weighted and diluted with DEMSO according to the solubility of the polysaccharide 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.

2.6. Determination of antimicrobial activity (AMA)

The 4 bacterial strains of *E.coli, S.aureus, B subtilis* and *MRSA* cultured 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 the 10 samples. Antimicrobial activity was measured using agar-well diffusion method (Lorian, 1991 and Brock et al., 1994). 0.1 ml of each culture of bacteria was introduced into a sterile Petri dish containing nutrient agar. Wells were made on the set medium at suitable space. The dried purified polysaccharides were dissolved in 1% dimethyl sulfoxide and prepared at concentration of 200 µg/ ml. The wells were respectively filled with different concentrations (50, 25 and 12.5 mg/ml) of the PS and they were incubated in an incubator at 37°C for 24 h. The 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 bacterial strains in millimeter (mm). The clear inhibition zones around the wells indicate the presence of antimicrobial activity. All data of antimicrobial activity are the average of triplicate analyses.

2.7. Determination of minimum inhibitory concentration (MIC)

Agar diffusion test was used for determination of MIC (Lorian, 1991 and Brock et al., 1994). Muller hinton agar medium was used and a clear circular zone of growth inhibition (mm) was measured ((Eloff, 1998,).MIC of different isolated PS against the four selected bacterial strains were determined.

3. RESULTS AND DISCUSSION

3.1. Polysaccharides (PS)

The main objective of this study was to evaluate the antimicrobial activity of the purified polysaccharides obtained from different plant seeds against four different bacterial strains. Screening of soluble polysaccharides isolated from different plant seeds revealed the presence of different percentages of soluble polysaccharides (Table 1).Polysaccharides obtained from *Raphanus sativus*, *Lupins*, *Eruca sativa* and *Lactuca sativa* (6.8,4.6, 4.2 and 4.0 g/100g respectively) were found to be more than that of *Hordeum vulgare*, *Cicer arietinum*, *Pisum sativum*, *Brassica napus*, *Triticum aestivum* and *Viqna unguiculata* (3.8, 3.2, 2.6, 2.4, 2.2 and 1.2 g/100g respectively). The present results also showed the presence of higher amount of polysaccharides in seeds of *Raphanus sativus*, *Lupins*, *Eruca sativa* (6.8,4.6 and 4.2 g/100g respectively) than that of the other seeds (Table 1). Similar results were recorded by several investigators (Wu et al. 2012 and Singh, 2013). Chromatographic analysis of the obtained polysaccharides revealed the presence of different type and levels of individual monosaccharide of all plant seeds such as mannose, galactose, glucose rhamnose, arabinose, xylose. Similar results were obtained by other investigators using cabbage, sugar beet, Jerusalem artichoke, rhubarb and Raphanussativus (Jwanny et al., 2009; Wu et al. 2012 and Abd-Elmoneim et al. 2013). Results also indicated that Glucose, Galactose, Mannose and Arabinose were the predominant monosaccharide in all PS obtained. However, differences were not only observed in the levels between PS obtained from plant seed sources, but also in their monosaccharide constituents. Similar results were obtained by other investigators (Rashad et al. 2000, Sun 2011 and Wu et al. 2012). Different PS 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. Yoichi et al. (1987) and Gutiérrez, and Perez (2004) and Moharib and and Awad (2012) 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. The present results showed small amounts of rhamnose,fucose and xylose in all seeds. Mazumder et al. (2002), Chandramouli et al. (2012) and Pranjal et al. (2013) , reported the monosaccharides galactose and mannose are the main polymer of seeds polysaccharide were identified by paper chromatography. These PS are very viscous when dissolved in water, have biological and physiological importance (Borchani et al. 2011 and Moharib et al. 2014) and has different effects against different diseases (Moharib 2006, Sun 2011 and Wu et al. 2012). The obtained PS have effective in the treatment of infectious diseases, due to their structure containing mainly galacto-mannan and/or arabino-galactan (Yoichi et al. 1987 and Abd-Elmoneim et al. 2013). These finding are in accordance with other studies (Jwanny et al., 2009; Wu et al. 2012). The present work was done to investigate the antimicrobial activity of polysaccharides (PS) on growth inhibition of four different bacterial strains. Different studies on polysaccharides obtained from plant origin showing good anti-bacterial effects against some common pathogens such as *B. subtilis, E. coli* and *S. aureus* (Wang and Jiang, 2010) and / or able to rescue cell viability from rotavirus infection (Baek et al., 2010).Jong-Heum et al. (2001) reported new antimicrobial substances were isolateed 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 (Moharib and El-Batran 2008, Jwanny et al. 2009, Geetha et al. 2011 and Moharib et al. 2014).

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

Plant seed samples	Soluble PS (g%)	Monosaccharides (g %)						
		Glu.	Gal.	Arab.	Mann.	Rha.	Fuc.	Xyl.
1-Cowpea (*Viqna unguiculata*)	1.2	0.1	0.4	0.1	0.05	0.1	trace	Trace
2-Pea (*Pisum sativum*)	2.6	0.6	1.4	0.1	0.2	0.2	trace	Trace
3-Lupine (*Lupinus* species)	4.2	0.2	2.5	trace	0.1	0.2	0.1	trace
4-Chick peas (*Cicer arietinum*)	3.2	0.5	1.8	0.3	0.2	0.2	trace	trace
5-Wheat (*Triticum aestivum*)	2.2	0.6	0.8	0.3	0.2	trace	trace	0.2
6-Cabbage (*Brassica napus*)	2.4	0.4	1.4	0.2	0.1	0.1	trace	0.1
7-Watercress (*Eruca sativa*)	4.2	0.8	2.6	0.3	0.2	0.1	0.1	trace
8-Radish (*Raphanus sativus*)	6.8	1.4	3.8	0.6	0.4	0.3	0.1	0.1
9-Dill(*Anethum graveolens*)	3.8	0.8	0.1	2.4	0.1	0.1	0.1	0.2
10-Lettuce (*Lactuca sativa*)	4.0	1.0	1.4	0.4	0.6	0.2	trace	0.1

Mean of three samples

3.2. Determination of antimicrobial activity (AMA)

Measurements of antimicrobial activity (AMA) of the obtained polysaccharides (PS) isolated from different plant seeds were determined against four strains of bacteria (*S. aureus, E.coli, B. subtilis* and *MRSA*)as shown in Table (2). The one bacterial strains, S. aureus represented a gram-positive bacteria that can cause skin infection and E. coli represented a 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 inhibited growth of S. aureus by the all PS isolated from different plant seeds used in the present study. On contrast some PS isolated from different plant seeds showed no effect against MRSA except *Raphanus sativus* and *Lactuca sativa* (Table 2). The present results showed ten PS samples cowpea (*Viqna unguiculata*, Pea (*Pisum sativum*), Wheat (*Triticum aestivum*), Chick peas (*Cicer arietinum*), Lupine (*Lupinus species*) ,Cabbage (*Brassica napus*), Radish(*Raphanus sativus*), Watercress (*Eruca sativa*), Lettuce (*Lactuca sativa*) and Dill(Anethum graveolens)give AMA against *S. aureus*. Two samples of isolated PS (Radish and Lettuce) showed AMA against *E.coli, B subtilis* and *MRSA* (Table 2). However, the PS isolated from Cowpea, Pea, Wheat, Chick peas, Lupine, Cabbage, Watercress and Dill showed no effect against *E.coli* and *MRSA* (Table 2). However, PS obtained from different plant seeds were inhibited the growth of *S. aureus*, *E. coli*, *B subtilis* and *MRSA* in vitro. The results obtained were found to be similar the results reported by other investigators (Abdou et al. 1972, Cowan 1999 and Gutiérrez, and Perez 2004).

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

Plant seed samples	Antimicrobial activity (AMA)			
	S. aureus	*E.coli*	*B subtilis*	MRSA
1-Cowpea (*Viqna unguiculata*)	+ve	-	-	-
2-Pea (*Pisum sativum*)	+ve	-	-	-
3-Lupine (*Lupinus* species)	+ve	-	-	-
4-Chick peas (*Cicer arietinum*)	+ve	-	-	-
5-Wheat (*Triticum aestivum*)	+ve	-	-	-
6-Cabbage (*Brassica napus*)	+ve	-	-	-
7-Watercress (*Eruca sativa*)	+ve	-	-	-
8-Radish (*Raphanus sativus*)	+ve	+ve	+ve	+ve
9-Dill(*Anethum graveolens*)	+ve	-	-	-
10-Lettuce (*Lactuca sativa*)	+ve	+ve	-+ve	+ve

Mean of three samples

The antimicrobial activity of PS at different concentrations was done using diffusion method test and inhibition zones were measured in cm diameter (Fig 1) and recorded (table 3). The results obtained with all PS showed best antimicrobial activity against *S. aureus* than the other microbial strains used in the present study. The PS obtained from Radish (*Raphanus sativus*) and Lettuce (*Lactuca sativa*)were active against both *S. aureus,E.coli* and *MRSA* strains. The Watercress (*Eruca sativa*) PS also active against *E.coli*. These results indicated that PS has antimicrobial activity against some bacterial strains. Several investigators suggests that some plant seed polysaccharides are effective in treating diseases of microbial infections (Bao et al. 2002). Other investigators (Moharib and El-Batran 2008, Abd-Elmoneim et al. 2013 and Moharib et al. 2014) used plant polysaccharides in treating different diseases (diabetes, hyperlipidemia and cancer). However, different effects of polysaccharides were dependant on their structure, type and dose (Jwanny et al. 2009, Suresh et al. 2012).

Generally the inhibition zone was observed on agar media with all PS against *S. aureus* (Figure 1). Different values of

Table 3. Minimum inhibitory concentration (MIC) values of polysaccharide (PS) isolated from ten plant seed

Seed polysaccharide (PS) samples	Minimum inhibitory concentration (MIC) for *S.aureus*					
	10	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
1-Cowpea (*Viqna unguiculata*)	0.6 cm	-	-	-	-	-
2-Pea (*Pisum sativum*)	1.0 cm	0.4cm	0.2cm	0.1cm	-	-
3-Lupine (*Lupinus* species)	0.8cm	-	-	-	-	-
4-Chick peas (*Cicer arietinum*)	0.6 cm	0.2cm	0.1cm	-	-	-
5-Wheat (*Triticum aestivum*)	0.4 cm	-	-	-	-	-
6-Cabbage (*Brassica napus*)	0.6 cm	0.3cm	0.2 cm	0.1cm	-	-
7-Watercress (*Eruca sativa*)	0.6 cm	-	-	-	-	-
8-Radish (*Raphanus sativus*)	1.4 cm	-	-	-	-	-
9-Dill(*Anethum graveolens*)	0.4 cm	-	-	-	-	-
10-Lettuce (*Lactuca sativa*)	0.8 cm	-	-	-	-	-
Seed PS samples	Minimum inhibitory concentration (MIC) for *E.coli*					
	10	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
1-Cowpea (*Viqna unguiculata*)	-	-	-	-	-	-
2-Pea (*Pisum sativum*)	0.4 cm	0.2cm	0.1 cm	-	-	-
3-Lupine (*Lupinus* species)	-	-	-	-	-	-
4-Chick peas (*Cicer arietinum*)	0.6 cm	0.3cm	0.1 cm	-	-	-
5-Wheat (*Triticum aestivum*)	-	-	-	-	-	-
6-Cabbage (*Brassica napus*)	-	-	-	-	-	-
7-Watercress (*Eruca sativa*)	0.4 cm	-	-	-	-	-
8-Radish (*Raphanus sativus*)	1.2 cm	0.6 cm	-	-	-	-
9-Dill(*Anethum graveolens*)	-	-	-	-	-	-
10-Lettuce (*Lactuca sativa*)	0.8 cm	0.4 cm	-	-	-	-
Seed PS samples	Minimum inhibitory concentration (MIC) for *B. subtilis*					
	10	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
1-Cowpea (*Viqna unguiculata*)		-	-	-	-	-
2-Pea (*Pisum sativum*)	0.4 cm	0.2 cm	-	-	-	-
3-Lupine (*Lupinus* species)	-	-	-	-	-	-
4-Chick peas (*Cicer arietinum*)	0.6 cm	0.2 cm	-	-	-	-
5-Wheat (*Triticum aestivum*)	-	-	-	-	-	-
6-Cabbage (*Brassica napus*)	-	-	-	-	-	-
7-Watercress (*Eruca sativa*)	-	-	-	-	-	-
8-Radish (*Raphanus sativus*)	1.0 cm	-	-	-	-	-
9-Dill(*Anethum graveolens*)	-	-	-	-	-	-
10-Lettuce (*Lactuca sativa*)	0.6 cm	0.4 cm	-	-	-	-
Seed PS samples	Minimum inhibitory concentration (MIC) for *MRSA*					
	10	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
1-Cowpea (*Viqna unguiculata*)	-	-	-	-	-	-
2-Pea (*Pisum sativum*)	0.4 cm	0.2 cm	-	-	-	-
3-Lupine (*Lupinus* species)	-	-	-	-	-	-
4-Chick peas (*Cicer arietinum*)	0.6 cm	0.2 cm	-	-	-	-
5-Wheat (*Triticum aestivum*)	-	-	-	-	-	-
6-Cabbage (*Brassica napus*)	-	-	-	-	-	-
7-Watercress (*Eruca sativa*)			-	-	-	-
8-Radish (*Raphanus sativus*)	1.0 cm	0.4	0.1 cm	-	-	-
9-Dill(*Anethum graveolens*)	-	-	-	-	-	-
10-Lettuce (*Lactuca sativa*)	0.6 cm	0.4 cm	0.1 cm	-	-	-

Mean of three samples

inhibition zone diameter (0.2-1.4 cm) were observed at a concentrations of 10% for all obtained PS samples. The inhibition zones of Pea (*Pisum sativum*), Chick peas (*Cicer arietinum*) and Cabbage (*Brassica napus*) seeds PS against *S. aureus* was decreasing at a low PS concentrations (10-1, 10-2 and 10-3 respectively) as shown in table (3). Results (table 3) showed the PS obtained from Radish (*Raphanus sativus*), Lupine (*Lupinus species*) and Lettuce (*Lactuca sativa*) seeds at 10% concentration, exhibited higher inhibition zones (0.8-1.4 cm) against *S. aureus*. Decreases in inhibition zones (0.4-1.2 cm) against *E.coli* and(0.4-1.0 cm) against *B. subtilis* and *MRSA* as compared to the PS effect against *S. aureus* (table3) were observed at 10% concentration .Inhibition zone ranged from 1.2 to 1.4 cm

in diameter was observed with PS isolated from Radish seeds at a concentration of 10% against *S.aureus* and *E.coli* respectively while the inhibition zone of 1.0 cm was observed against *B. subtilis* and *MRSA*. PS of Radish showed diameter of 0.6 0.and 0.4 at a concentration of 10^{-1} against *E.coli* and *MRSA* (Figure 1).Moreover, the inhibition zone diameters exhibited different levels of decreases with the Radish, Lettuce, Pea, Chick peas and Cabbage seeds PS concentrations decrease against *S. aureus, E.coli ,B. subtilis* and *MRSA* (table 3). The concentration of Lettuce (*Lactuca sativa*) PS sample at 10% concentration showed inhibition zone of 0.8 and 0.6 cm against *S.aureus, E.coli* and *B. subtilis* and *MRSA* (table 3). Moreover *E.coli* was not inhibited with the other PS samples used in these study. The results suggest that *S. aureus* was being inhibited in the presence of PS isolated from some plant seeds 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 bacterial strains was obtained at low PS concentrations (10^{-4} and 10^{-5}). However, the present results indicated that the increase of PS concentrations exhibited increase in the inhibition zone diameter.

3.3. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined for the all obtained PS and the results are given in table 3. PS at 10% concentration exhibited best antibacterial activity against S. aureus. PS of Radish (*Raphanus sativus*) and Watercress (*Eruca sativa*) exhibited high activity against *E.coli* and *MRSA* than the other obtained PS. The PS was more effective against *S.aureus* with a zone of inhibition of 1.0cm and was least effective against the other tested strains. Among the other bacterial strains studied, *E.coli* showed a zone of inhibition of 0.6cm diameter and *MRSA* showed inhibition zone of 0.4cm diameter (at conc. 100µg.). The MIC value of PS was found to have Low MIC value of 0.5mg/ml for *S. aureus* and *E.coli* . With *MRSA*, PS showed a higher MIC value of 2mg/ml. These results were indicated higher activity of PS with *S. aureus, B. subtilis* and E.coli and less activity of the PS with *MRSA*. PS 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) of 30–60 µg/ml

CONCLUSION

The results demonstrate that bacterium S. aureus, E. coli and MRSA were being inhibited by PS 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

ACKNOWLEDGMENTS

The author acknowledge the National Research Centre, Dokki, Cairo, Egypt. They funded this project with no (9100108).

REFERENCES

Abd-Elmoneim, M.A., Bakar, AA. , Awad, I. M , Mohamed, E.M. and Moharib, S.A. (2013). Anticarcinogenic effect of *Raphanus Sativus* on 1, 2 dimethylhydrazine (DMH) induced colon cancer in rats. The Egyptian J. of Hospital Med..

Abdou, I.A., Abou-Zeid, A.A., El-Sherbeeny, M.R., and Abou-El-Gheat, Z.H. (1972) Antimicrobial activities of *Allium sativum*, *Allium cepa*, *Raphanussativus*, *Capsicum frutescens, Eruca sativa, Allium kurrat* on bacteria. Qual. Plant. Mater. Veg. 22, 29–35.

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.

Baek, S.H., Lee, J.G., Park, S.Y., Bae, O.N., Kim, D.H. and Park, J.H. (2010).Pectic polysaccharides from *Panax ginseng* as the antirotavirus principals in ginseng, Biomacromolecules, 11, 2044–2052.

Bao X, Wang Z, Fang J, Li X. (2002).Structure features of an immunostimulating and antioxidant acidic polysaccharide from the seeds of *Cuscutachinensis*.PlantaMedica, 2002; 68:237-243

Bauer, A.W., Kirby, M. D., Sherris, S.C. and Turck, M. (1966).Antibiotic susceptibility testing by standard single disc diffusion method. J. Ethnopharmacol. 45: 493-496.

Bern, C.; Martines, J.; de Zoysa, I.; Glass, R.I. (1992). The magnitude of the global problem of diarrhoeal disease: a ten-year update. Bull WHO Vol.70, pp 705-714, ISSN 0042-9686

Borchani, C.,Besbes, S.,Masmoudi, M.,Blecker, C., Paquot,M. and Attia,H. (2011) Effect of drying methods on physico-chemical and antioxidant properties of date fibre concentrates Food Chem. 125, 1194-1201.

Brock, T.D., Madigan, M.T., Martinko, J.M. and Parker, J. (1994). Biology of Microorganisms, 7th ed., Prentice Hall, Englewood Cloffs, N.J., pp. 118-124.

Cerning J., Renard C., Thibault J., Bouillanne C., Lan-don M., Desmazeaud M. and Topisirovic L. (1994).Carbon source requirements for exopolysaccharideproduction by *Lactobacillus casei* CG11 and partial structure analysis of the polymer. Appl. Environ.Microbiol.60, 3914Ð3919.

Chandramouli Y., Firoz S., Vikram A., Padmaja C., Chakravarthi R.N., (2012) Design and evaluation of controlled relelese matrix tablets of acyclovir sodium using tamarind seed Polysaccharides, Journal Of Pharmaceutical Biology 2, 55-62.

Chen YY, Chang H.M. Antiproliferative and differentiating effects of polysaccharide fraction from fuling (*Poriacocos*) on human leukemic U937 and HL-60 cells. Food and Chemical Toxicology, 2004; 42:759-769.

Chena, G., Jie, X., Xia, M., Yi, H., Xiaogang, L., Ying, J.andXinsheng, H. (2012).Characterization and antitumor activities of the water-soluble polysaccharide from *Rhizoma Arisaematis* Carbohydrate Polymers, 90, 67-71.

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.

Cowan MM (1999). Plant products as antimicrobial agents.Clin.Microbiol. Rev., 12, 564-582.

Dabai YU, Muhammad S, Aliyu BS. 2007. Antibacterial activity of anthraquinone fraction of *Vitex doniana*. Pakistan J BiolSci, 1-3

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.

Garcia-Sosa K, Villarreal-Alvarez N, Lubben, P, Peña-Rodriguez L.M. (2006).Chrysophanol, an antimicrobial anthraquinone from the root extract of *Colubrina greggii*. J Mex Chem. Soc, 50: 2, 76-78.

Geetha, R.V., Anitha, R. and 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, Vol 4, 115-118.

Gutiérrez,R.M.P. and Perez,R.L. (2004). *Raphanus sativus* (Radish): Their Chemistry and Biology. The Sci. World J. 4, 811–837.

Jong-Heum, P., Heuyn-Kil, S., and Cher-Won, H. (2001) New antimicrobial activity from Korean radish seeds (*Raphanus sativus* L.) J. Microbiol. Biotechnol. 11, 337–341.

Jwanny, E.W., Moharib, S.A. and Rasmy, G.E. (2009).Effect of two polysaccharides on chemically-induced colorectal cancer in rats. Advav.in Food Sci, 31, 202-209.

Lee, J.H., Shim, J.S., Chung, M.S., Lim, S.T., and Kim, K.H. (2009) Inhibition of pathogen adhesion to host cells by polysaccharides from *Panax ginseng*. Biosci. Biotechnol. Biochem. 73: 209–212.

Lorian, V. (1991). Antibiotics in Laboratory Medicine, Williams Wilkins, Baltimore, pp. 739-786.

Maciel JS, Chaves LS, Souza BWS, Teixeira DIA, Freitas ALP, Feitosa JAP, et al. (2008).Structural characterization of cold extracted fraction of soluble sulfated polysaccharides from red sea weed *Gracilaria birdiae*.Carbohydr Res. 71(4): 559565.

Mack, D.; Siemssen, N.; Laufs, R. (1992). Parallel Induction by glucose of adherence and a polysaccharide antigen specific for plastic-adherent *Staphylococcus epidermidis*: evidence for functional relation to intercellular adhesion. Infection and Inmunity. 60(5):2048-2057.

Mazumder S, Ghosal PK, Pujol CA, Carlucci M, Damonte EB, Ray B.(2002) Isolation, chemical investigation and antiviral activity of polysaccharides from Gracilariacorticata(Gracilariaceae, Rhodophyta). Inter J Biol. Macromol. 31(1-3): 87-95.

Moharib, S.A. (2006).Hypolipidemic effect of dietary fibre in rats.J.Adv. Food Sci. 28,46-53.

Moharib, S.A. and Awad, I.M. (2012).Antioxidant and hypolipidemic activities of Spinach (*Spinocia oleracea*) dietary fibre and polyphenol supplementation in rats fed a high cholesterol diet. Adv.In Food Sci. 34, 14-23.

Moharib, S.A. and El-Batran, S.A. (2008).Hypoglycemic effect of dietary fibre in dibetic rats. Res.J.of Agric..and Biol.Sci. 4, 455-461

Moharib, S. A., Nabila Abd El Maksoud, Halla M. Ragab and Mahmoud, M. Shehata (2014). Anticancer activities of mushroom polysaccharides on chemically induced colorectal cancer in rats. J. of Appl. Pharm. Sci. 4, 054-063.

Moharib, S.A. (2016).Anticancer and antioxidant effects of fructooligosaccharide (FOS) on chemically induced colon cancer in rats. EJPAU 19,1-18 (2016).

Mukherjee PK, Saritha GS, Suresh B (2002).Antimicrobial potential of two different *Hypericum species* available in India. Phytother. Res., 16: 692-695.

Paterson D.L. Resistance in Gram-negative bacteria (2006).Enterobacteriaceae. Am. J. Infect. Control, 119(5): 20-28.

Pongsamart, S. and Panmaung, T. (1998). Isolation of polysaccharides from fruit-hulls of durian (*Durio zibethinus L.*). Songklanakarin J. Sci. Technol. 20(3): 323-332.

Persin, Z., Stana-Kleinschek, K., Foster, T.J., van Dam, J.E.G., Boeriu, C.G. and Navard, P. (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, Vol.84, No.1, (February 2011), pp. 22-32, ISSN 0144-8617.

Pranjal Saikia1, Bhanu P Sahu*1, S K Dash1(2013).Isolation and Characterisation of Some Natural Polysaccharides as Pharmaceutical Excipients International Journal of Pharm.Tech. Research 5, 1196-1206.

Premanath R, Sudisha J, Lakshmi Devi N, Aradhya SM.(2011). Antibacterial and antioxidant activities of Fenugreek (*Trigonella foenumgraecum L.*) leaves.Res J Med Plants 5, 695-705.

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.

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 2011; 4(9): 375-378.

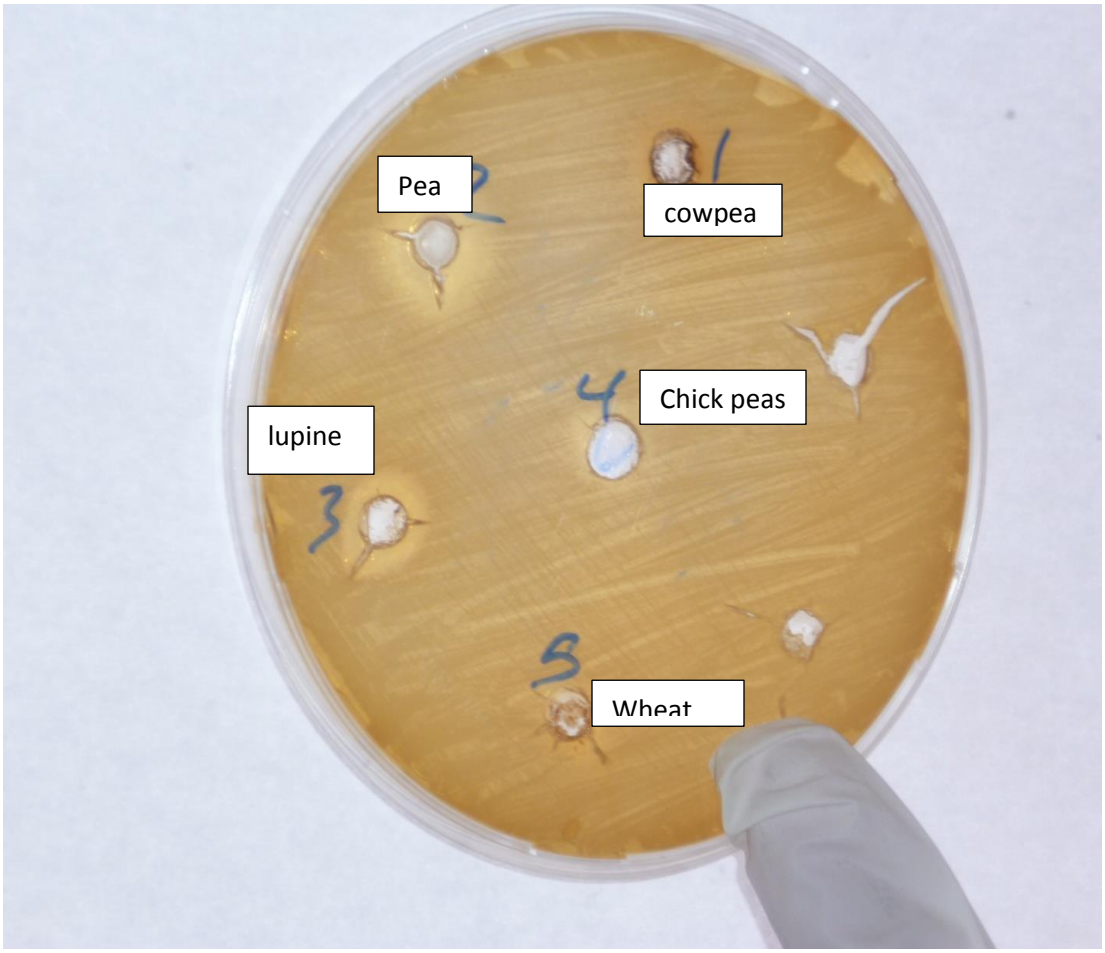
Rashad, M. M.; Moharib, S.A. and Abdo, H. M.(2000). Chemical constituents and nutritive value of 6 local vegetable leaves by-products. J. Agric.Sci. Mansoura Univ. 25, 7229-7238.

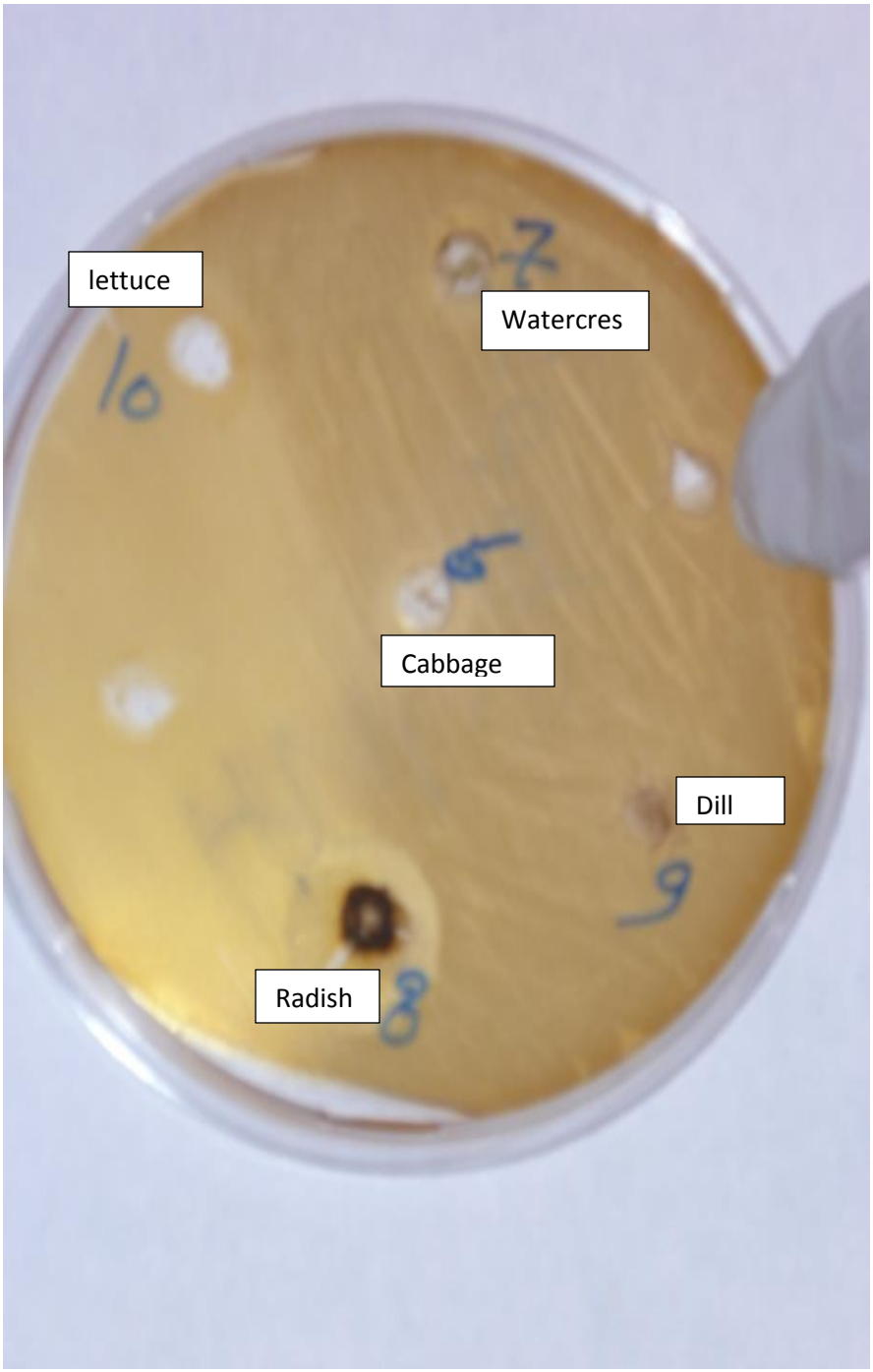
Rawani A, Pal S, Chandra G.(2011). Evaluation of antimicrobial properties of four plants extracts against human pathogens. Asian Pac J Trop Biomed. 1(1): supplement, S71-S75

Singh, R.B. (2013).Nature of seeds polysaccharide obtained from medicinal plant of *Wrightia tinctoria* R. BR. (Roxb). Adv. in appl. Sci.Res.4,115-118.

Sinha S, Astani A, Ghosh T, Schnitzler P, Ray B. (2010).Polysaccharides from *Sargassum tenerrimum*: Structural features, chemical modification and antiviral activity. Phytochem. 71(2-3): 235-242.
Staub, A. M., (1965), Methods in Carbohydrate Chemistry, 5 : 5-7.
Sun Y. (2011). Structure and biological activities of the polysaccharides from the leaves, roots and fruits of *Panax ginseng* C.A. Meyer: An overview. Carbohydrate Polymers, 85, 490–499.
Suresh KD, Pramod RT, Manjunatha M, Rahul KA.(2012). Comparative antioxidant effect of aqueous extracts of curry leaves, fenugreek leaves and butylatedhydroxytoluene in raw chicken patties J of Food Sci and Technol. 49:781-785.
Turkoglu A, Kivrak I, Mercan N, Duru ME, Gezer K, Turkoglu H (2006). Antioxidant and antimicrobial activities of *Morchella conica* Pers. Afr. J. Biotechnol., 5: 1146-1150.
Wang, F.; Li, J. and Jiang, Y. (2010). Polysaccharides from mulberry leaf in relation to their antioxidant activity and antibacterial ability, Journal of Food Process Engineering, 33, 39–50.
Wang, S., Penchala, S., Prabhu, S., Wang, J., Huang, Y. (2010).Molecular basis of tra-ditional Chinese medicine in cancer chemoprevention. Current Drug Discovery Technologies 7, 67-75.
Wilson,C.M.(1959).Quantitative determination of sugars on paper chromatograms. Anal.Chem.31, 1199-1201.
Wu, X., Mao, G., Fan, Q., Zhao, T., Zhao, J., Li, F. and Yang, L. (2012).Isolation, purification, immunological and anti-tumor activities of polysaccharides from *Gymnema sylvestre*.Food Research International, 48,935-939.
Yoichi, T., Yoichi, H., and Shigeru, Y. (1987) An L-arabino-D-galactan and an L-arabino-D-galactan contained proteoglycan from radish (*Raphanus sativus*) seeds. Carbohydr. Res. 161, 113–126. 56

SEE DIAGRAM BELOW




lettuce
Watercres
Cabbage
Dill
Radish