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Original article

Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases

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ABSTRACT

Prevention of food spoilage and food poisoning pathogens is usually achieved by use of chemical preservatives which have negative impacts including: human health hazards of the chemical applications, chemical residues in food & feed chains and acquisition of microbial resistance to the used chemicals. Because of such concerns, the necessity to find a potentially effective, healthy safer and natural alternative preservatives is increased. Within these texts, Plant extracts have been used to control food poisoning diseases and preserve foodstuff. Antimicrobial activity of five plant extracts were investigated against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* using agar disc diffusion technique. Ethanolic extracts of *Punica granatum, Syzygium aromaticum*, *Zingiber officinales* and *Thymus vulgaris* were potentially effective with variable efficiency against the tested bacterial strains at concentration of 10 mg/ml while extract of *Cuminum cyminum* was only effective against *S. aureus* respectively. *P. granatum* and *S. aromaticum* ethanolic extracts were the most effective plant extracts and showed bacteriostatic and bactericidal activities against the highly susceptible strains of food borne pathogenic bacteria (*S. aureus* and *P. aeruginosa*) with MIC's ranged from 2.5 to 5.0 mg/ml and MBC of 5.0 and 10 mg/ml except *P. aeruginosa* which was less sensitive and its MBC reached to 12.5 mg/ml of *S. aromaticum* respectively. These plant extracts which proved to be potentially effective can be used as natural alternative preventives to control food poisoning diseases and preserve food stuff avoiding healthy hazards of chemically antimicrobial agent applications.
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1. Introduction

Food poisoning is considered as one of the most common cause of illness and death in developing countries (Doughari et al., 2007; Pirbalouti et al., 2009; Sapkota et al., 2012). Most of food poisoning reports are associated with bacterial contamination especially members of Gram negative bacteria like *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* (Solomakos et al., 2008; Pandey and Singh, 2011). Other Gram positive bacteria including *Staphylococcus aureus* and *Bacillus cereus* have been also identified as the causal agents of food borne diseases or food spoilage (Braga et al., 2005). Prevention of food spoilage and their etiological agent is traditionally achieved by the use of chemical preservatives (Yamamura et al., 2000; Shan et al., 2007). Despite of the proven efficiency of these chemical preservative in prevention and outbreak control of food poisoning diseases, their repeated applications has resulted in the accumulation of chemical residues in food and feed chain, acquisition of microbial resistance to the applied chemicals and unpleasant side effects of these chemicals on human health (Akinyemi et al., 2006; Bialonska et al., 2010). Because of such concern, efforts have been focused on developing a potentially effective, healthy safer and natural food preservatives. Within these contexts is the utilization of plant extracts as antimicrobial agents for food preservation (Nasar-Abbas and Kadir, 2004; Hara_Kudo et al., 2004; Mathabe et al., 2005). These plant extracts considered as natural sources of antimicrobial agents, regarded as nutritionally safe and easily degradable (Cowan, 1999; Duffy and

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Power, 2001; Berahou et al., 2007; Chika et al., 2007). The antimicrobial activity exhibited by plant extracts against food poisoning bacteria has been demonstrated by several researchers (Delgado et al., 2004; Alzoreky and Nakahara, 2003; Verma et al., 2012; Akinpelu et al., 2015). Gupta et al. (2010) investigated antibacterial activity of five ethanolic and aqueous plant extracts against *S. aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* and their results showed that the ethanolic extracts of four plants (*Achyranthes aspera, Cynodon dacynodon dactylon, Lantana camara* and *Tagetes patula*) were effective against all tested microorganisms with MIC's ranged from 25 to 125 mg/ml. Sapkota et al. (2012) studied antibacterial effect of guava leaves, garlic and ginger against some human microbial pathogens and they ascertained that ginger was only effective against *S. aureus* while guava and garlic were effective against all tested microorganisms. Akinpelu et al. (2015) investigated antibacterial potential of crude and butanolic extracts of *Persea americana* against *Bacillus cereus* implicated in food poisoning. The extracts exhibited antibacterial activity at concentrations of 25 and 10 mg/ml with MBC of both extracts ranged between 3.12 and 12.5 mg/ml respectively. Moreover, antimicrobial activity of different natural substances such as medicinal plant extract have been investigated against food borne bacteria. For example; Ahmad and Beg (2001), Kokoska et al. (2002), Ateb and Erdo_Urul, (2003), and Rios and Recio (2005) tested the suppression of food borne bacteria and their diseases by medical plant extracts. The extract of three medicinal plants used in Nigerian folk medicine showed a highly antibacterial activity against some food borne pathogens. All extracts exhibited a strong antimicrobial activity against *Salmonella enteritidis, E. coli* and *S. aureus* but in variable degree and with different MIC's depending upon the plant extract and pathogenic organism. (Ahmad et al., 1998; Akinyemi et al., 2006). In addition, Sher (2009), Venkatesan and Karrunakaran (2010) and Pirbalouti et al. (2010) investigated antimicrobial activity of eight medicinal plants against *E. coli*, *Bacillus cereus* and *Listeria monocytogenes*. The most effective extracts were those obtained from *Myrtus communis* and *Thymus daenensis* with MIC values ranged between 0.039 and 10 mg/ml. Antimicrobial activity of *Punica granatum* against food poisoning bacteria was proved by several investigators (Prashanth et al., 2001; Negi and Jayaprakasha, 2003; Voravuthikunchai et al., 2005; Naz et al., 2007; Nuamsetti et al., 2012). Antibacterial activity of *Punica*, *Citrus* and *Allium* extracts against food borne spoilage bacteria was investigated by Verma et al. (2012). All plant extracts was potentially effective against *S. typhi, E. coli, B. cereus* and *S. aureus* implicated in food spoilage but the extract of *Punica granatum* was the most effective extract with concentration of 500 mg/ml. Ethanolic *P. granatum* peels extracts was found to be potentially effective against *Micrococcus luteus, S. aureus*, *Bacillus megaterium* and Gram negative bacteria like *E. coli* and *P. aeruginos* in concentration ranged between 30 and 50 μg/ml. (Duman et al., 2009; Sadeghian et al., 2011; Dey et al., 2012). Antimicrobial activity of ethanolic *Punica granatum* extract and its fractions showed a highly antibacterial activity against Gram positive (*S. aureus* and *B. cereus*) and Gram negative bacteria (*E. coli* and *S. typhi*) causing food poisoning and these extracts can be used for prevention of food borne diseases or as preservative in food industry (Alzoreky, 2009; Mahboubi et al., 2015). Spices extracts used as food additives were potentially effective against some food poisoning bacteria and their antibacterial activity was investigated by several researchers (Ozcan and Erkmen, 2001; Nevas et al., 2004; Parekh and Sumitra, 2007; Abdulrahman et al., 2010). Cinnamon extract was found to be the most effective spice against all tested strain while the weakest antimicrobial activity was displayed by cumin, ginger and clove respectively. Antimicrobial activity of clove (*S. aromaticum*) against Gram negative bacteria and food borne pathogens was investigated by Sakagami et al. (2000), Mahfuzul_Hoque et al. (2007), Saeed and Tariq (2008), and Pandey and Singh (2011). Some researchers reported that ethanolic clove extract was potentially active against *S. aureus*, *Vibrio parahaemolyticus* and *P. aeruginosa* while it was inactive against *E. coli* and *Salmonella enteritidis* (Mahfuzul_Hoque et al., 2007). Other researchers ascertained activity of clove oil against all tested pathogenic bacteria while *Vibrio cholera*, *S. typhi* and *Klebsiella pneumonia* were found to be resistant to aqueous clove extract (Saeed and Tariq, 2008; Saeed et al., 2013). Moreover, the methanolic clove extract was reported to be potentially effective against *S. aureus*, *P. aeruginosa* and *E. coli* with MIC ranged from 0.1 to 2.31 mg/ml (Pandey and Singh, 2011). Antimicrobial activity of cumin seeds (*Cuminum cyminum*) extract was reported to be potentially effective against several strains of Gram positive and Gram negative bacteria implicated in food poisoning with variable MIC's (Arora and Kaur, 1999; Shan et al., 2007; Chaudry and Tariq, 2008). MIC's of cumin extract effective against *E.coli, P. aeruginosa, S. aureus* and *B. pumilus* were ranged between 6.25 and 25 mg/ml (Dua et al., 2013) while a higher concentration ranged between 20 and 60 mg/ml were previously reported by Sheikh et al. (2010). Seven ethanolic and aqueous plant extracts were investigated against some clinically pathogenic bacteria. Ethanolic *Punica granatum* extract was effective against all tested bacterial pathogens with MIC of 0.2 mg/ml. *Zingiber officinales* extract was also effective against *P. aeruginosa* and *K. pneumonia* while *Thymus kotschyana* was potentially effective against *S. aureus* and *E. coli* (Qader et al., 2013). Rasooli et al. (2006) investigated antimicrobial activity of some thyme essential oils against food borne pathogens (*Listeria monocytogenes*). Most of food poisoning diseases have been associated with bacterial contaminations particulary members of Gram negative bacteria like *E. coli*, *S. typhi* & *P. aeruginosa* and Gram positive like *S. aureus* & *B. cereus*. Research concerning the efficiency of *Syzygium aromaticum*, *Thymus vulgaris*, *Punica granatum*, *Zingiber officinales* and *Cuminum cyminum* against the previous etiological food spoilage bacteria are scanty in Arabian area. Therefore, the present study aimed to evaluate antibacterial activity of these plant extract against food poisoning diseases caused by *S. aureus, B. cereus, E. coli, S. typhi* and *P. aeruginosa in vitro*.

2. Materials and methods

2.1. Plants extraction preparation

Plant materials of five plant species included in this study (Table 1) were collected from local market of Riyadh, Saudi Arabia. The collected plants were watery washed, disinfected, rinsed with distilled water and finally dried in shade. The dried plant material of each plant species was grounded into fine powder to pass 100 mm sieve. 50 g of the fine powder was soaked in 200 ml of ethanol with stirring for 48 h., filtered through double layers of muslin, centrifuged at 9000 rpm for 10 min and finally filtered again through Whatman filter paper No. (41) to attain a clear filtrate. The filtrates were evaporated and dried at 40 °C under reduced pressure using rotatory vacuum evaporator. The extract yields were weighted, stored in a small bottles in fridge at 5 °C and their yield percentages were calculated using the following formula: Extract yield% = R/S × 100 (where R; weight of extracted plants residues and S; weight of plant raw sample).

2.2. Antibacterial activity of the plant extracts

2.2.1. Bacterial strains

The antibacterial potency of each plant extract was evaluated using five bacterial strains causing food poisoning diseases. Two

Table 1
The ethnobotanical data of employed plant species and their extract yield percentage.

Plant species	Family	Local name	Common name	Plant part used	Extract pH	Extract yield (%)
Cuminum cyminum	Apiaceae	Kammun	Cumin	Seeds	6.2	3.12
Punica granatum	Lythraceae	Romman	Pomegranate	Peels	4.7	9.74
Syzygium aromaticum	Myrtaceae	Koronfil	Clove	Flowers	5.3	4.38
Thymus vulgaris	Lamiaceae	Za'ater	Thyme	Leaves	6.8	6.54
Zingiber officinale	Zingiberaceae	Zanjabil	Ginger	Rhizome	7.1	5.26

strains of Gram positive (*Staphylococcus aureus* and *Bacillus cereus*) and three strains of Gram negative (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*) bacteria. The bacterial strains were provided from the culture collection of Botany and Microbiology Dept. King Saud University, Riyadh, K.S.A.

2.2.2. Inoculums preparation

Each bacterial strain was subcultured overnight at 35 °C in Mueller-Hilton agar slants. The bacterial growth was harvested using 5 ml of sterile saline water, its absorbance was adjusted at 580 um and diluted to attain viable cell count of 10^7 CFU/ml using spectrophotometer.

2.2.3. Antibacterial activity of plants extract

The disk diffusion method is used to evaluate antimicrobial activity of the each plant extract. The plant extract residues (50 mg) were re- dissolved in 2.5 ml of ethanol, sterilized through Millipore filter (0.22 μm) then loaded over sterile filter paper discs (8 mm in diameter) to obtain final concentration of 10 mg/disc. Ten ml of Mueller-Hilton agar medium was poured into sterile Petri dishes (as a basal layer) followed with 15 ml of seeded medium previously inoculated with bacterial suspension (100 ml of medium/1 ml of 10^7 CFU) to attain 10^5 CFU/ml of medium. Sterile filter paper discs loaded with plant extract concentration of (10 mg/ml) were placed on the top of Mueller-Hilton agar plates. Filter paper discs loaded with 5 μg of Gentamycin was used as positive control. The plates were kept in the fridge at 5 °C for 2 h. to permit plant extracts diffusion then incubated at 35 °C for 24 h. The presence of inhibition zones were measured by Vernier caliper, recorded and considered as indication for antibacterial activity.

2.2.4. Determination of minimum inhibitory concentrations (MIC's) of the effective plants extract

MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24 h. of incubation. The most effective plant extracts which exhibiting a strong antibacterial activity at 10 mg/ml was manipulated to determine their MIC using disk diffusion method and evaluate their efficiency in controlling bacterial strains causing food poisoning diseases. Different concentrations of the effective plant extract (1.25, 2.5, 5.0, 10.0, 12.5 and 15.0 mg/ml) were prepared separately by dissolving 50 mg in 2.5 ml of ethanol, sterilized through Millipore filter and loaded their requisite amount over sterilized filter paper discs (8 mm in diameter). Mueller-Hilton agar was poured into sterile Petri dishes and seeded with bacterial suspensions of the pathogenic strains. The loaded filter paper discs with different concentrations of the effective plant extract were placed on the top of the Mueller-Hilton agar plates. The plates were kept in the fridge at 5 °C for 2 h. then incubated at 35 °C for 24 h. The inhibition zones were measured by Vernier caliper and recorded against the concentrations of the effective plant extracts.

2.2.5. Determination of minimum bactericidal concentrations (MBC's) of the effective plants extract

Streaks were taken from the two lowest concentrations of the plant extract plates exhibiting invisible growth (from inhibition zone of MIC plates) and subcultures onto sterile Tryptone soya agar (TSA) plates. The plates were incubated at 35 °C for 24 h. then examined for bacterial growth in corresponding to plant extract concentration. MBC was taken as the concentration of plant extract that did not exhibiting any bacterial growth on the freshly inoculated agar plates.

3. Results and discussion

3.1. Plants extraction yield

The ethanobotanical data of the employed plants and their extract percentage yield are illustrated in Table 1. The extract of 50 g of dried plant materials with ethanol yielded plant extract residues ranged from 1.56 to 4.87 g. The highest yield of plant extract was obtained from *Punica granatum* (4.87 g) followed by *Thymus vulgaris* (3.27 g) while *Cuminum cyminum* give the lowest extract yield respectively.

3.2. Antibacterial activity of plants extract

Five plant species were investigated to evaluate their antibacterial activity against food poisoning bacteria including two strains of Gram positive bacteria (*B. cereus* & *S. aureus*) and three strains of Gram negative bacteria (*E. coli, S. typhi* & *P. aeruginosa*) using disc diffusion method. Evaluation of antibacterial activity of these plant extracts was recorded in Table 2 and illustrated in Fig. 1. The results revealed that all plant extracts were potentially effective in suppressing microbial growth of food poisoning bacteria with variable potency. *P. granatum* was the most effective extract retarding microbial growth of all tested pathogenic bacteria at concentration of 10 mg/ml while extract of *C. cyminum* was effective only against *S. aureus*. Other plant extracts showed variable antimicrobial activity against food poisoning bacterial strains. *S. aromaticum* exhibited inhibitory effect against four of the pathogenic strains (*B. cereus*, *S. aureus*, *E. coli* & *P. aeruginosa*) whereas *Z. officinales* was effective against three of them (*B. cereus*, *S. aureus* & *P. aeruginosa*) and *T. vulgaris* was effective against *S. aureus* and *P. aeruginosa.*

Results of antimicrobial activity of the five plant extracts can suggested that *S. typhi* was the most resistant strain to plant extracts followed by *E. coli* while *S. aureus* and *P. aeruginosa* were the most susceptible strains to the extracted plants respectively. Moreover, *P. granatum* and *S. aromaticum* extracts were the most effective extracts and showed a strong antibacterial activity against food poisoning bacteria. Hence, experiments were conducted to determine their minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against the most susceptible bacterial strains (*S. aureus* and *P. aeruginosa*).

3.3. Minimum inhibitory concentrations (MIC's) of the effective plants extract

The MIC and MBC of the most effective plant extracts (*P. granatum* and *S. aromaticum*) were employed by disc diffusion method to evaluate their bacteriostatic and bactericidal properties. The

Table 2
Antimicrobial screening test of ethanolic plants extract (10 mg/ml) against some bacterial strains of food poisoning diseases.

Plant species	Inhibition zones (mm)				
	Gram (+ve) pathogenic bacteria		Gram (−ve) pathogenic bacteria		
	B. cereus	*S. aureus*	*E. coli*	*S. typhi*	*P. aeruginosa*
Cuminum cyminum	0.0 ± 0.0	9.5 ± 0.74	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Punica granatum	16.3 ± 0.57	18.5 ± 0.13	14.2 ± 0.61	9.7 ± 0.22	16.1 ± 0.46
Syzygium aromaticum	14.6 ± 0.37	15.8 ± 0.41	11.9 ± 0.34	0.0 ± 0.0	13.4 ± 0.11
Thymus vulgaris	0.0 ± 0.0	17.6 ± 0.31	0.0 ± 0.0	0.0 ± 0.0	14.7 ± 0.25
Zingiber officinales	8.3 ± 0.46	15.4 ± 0.23	0.0 ± 0.0	0.0 ± 0.0	11.2 ± 0.17
Gentamycin (5 μg)	16.8 ± 0.37	20.5 ± 0.24	15.6 ± 0.53	18.7 ± 0.61	13.1 ± 0.35

Data are means of three replicates (n = 3) ± standard error.

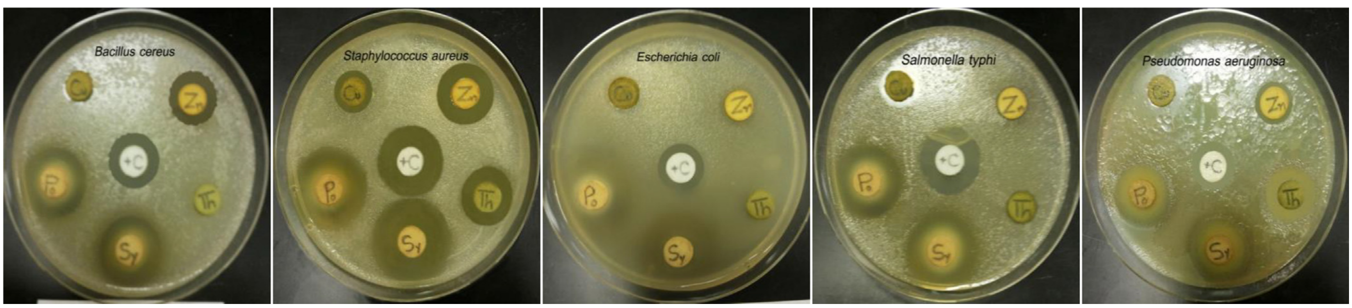

Fig. 1. Growth inhibition of some food poisoning bacterial strains caused by plant extracts. Cu, Cumin; Po, pomegranate; Sy, Clove; Th, Thyme; Zn, Ginger and +C, positive control.

Table 3
MIC's of the most effective plant extract against *S. aureus* and *P. aeruginosa.*

Plant ext.	Conc. mg/ml	Inhibition zones (mm)	
		Gram (+ve) pathogenic bacteria *S. aureus*	Gram (−ve) pathogenic bacteria *P. aeruginosa*
P. granatum	1.25	0.0 ± 0.0	0.0 ± 0.0
	2.50	9.6 ± 0.65	8.3 ± 0.95
	05.0	14.8 ± 0.83	13.2 ± 1.1
	10.0	18.7 ± 0.75	16.4 ± 0.56
	12.5	21.4 ± 0.46	18.5 ± 0.36
	15.0	23.7 ± 0.35	22.6 ± 0.74
S. aromaticum	1.25	0.0 ± 0.0	0.0 ± 0.0
	2.50	0.0 ± 0.0	0.0 ± 0.0
	05.0	11.4 ± 0.37	9.1 ± 0.80
	10.0	15.3 ± 0.85	12.8 ± 0.45
	12.5	16.6 ± 0.13	14.7 ± 0.52
	15.0	19.3 ± 0.65	17.5 ± 0.35

Data are means of three replicates (n = 3) ± standard error.

concentration effect of the effective plant extracts were reported in Table 3 and illustrated in Fig. 2. The inhibitory effect of *P. granatum* extract started at 2.5 mg/ml with inhibition zones of 9.6 and 8.3 mm against *S. aureus* and *P. aeruginosa* while extract of *S. aromaticum* suppressed bacterial growth of these strains at concentration of 5 mg/ml with inhibition zones of 11.4 and 9.2 mm respectively.

3.4. Minimum bactericidal concentrations (MBC's) of the effective plants extract

The MBC was confirmed by absence of bacterial growth of the tested strains streaked form inhibition zone corresponding to their lowest MIC's. *P. granatum* extract showed potentially bactericidal activity against the tested pathogenic bacteria (*S. aureus* and *P. aeruginosa*) with MBC of 5 mg/ml while MBC of *S. aromaticum* extract reached to 10 mg/ml except *P. aeruginosa* which was less sensitive and its minimal bactericidal concentration reached to 12.5 mg/ml. The results of MIC and MBC of the effective plant extracts suggested that *P. granatum* and *S. aromaticum* can be used to control and prevent food borne bacteria and food poisoning diseases. Bacterial strains included in this study were chosen for their importance in food spoilage and food poisoning. *S. aureus* considered as the one of the most common source of food borne disease while *B. cereus, E. coli, S. typhi* and *P. aeruginosa* produce toxins and other metabolites that induce human gastroenteritis diseases. *P. granatum* extract suppressing microbial growth of all tested bacterial strains followed by extract of *S. aromaticum* which appear to be potentially effective against four bacterial strains and less effective against *S. typhi.*

These results are in accordance with those of Verma et al. (2012), Qader et al. (2013) and Mahboubi et al. (2015). A great variation in MIC of *P. granatum* extract demonstrated in several investigation may be due to considerable variation in their method of

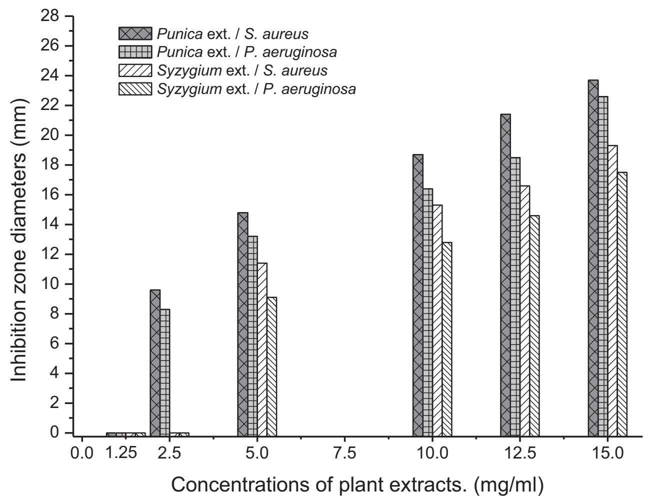

Fig. 2. MIC's of the effective plant extracts against *S. aureus* and *P. aeruginosa.*

extraction, constituents as well as bacterial strains used. Also, variation in MIC of different plant extracts may arise from variation in their chemical constituents and volatile nature of their constituents. On the other hand, *S. aromaticum* extract was found to be effective with concentration of (10 mg/ml) against *B. cereus, S. aureus, E. coli* and *P. aeruginosa* suppressing their growth with inhibition zones of 14.6, 15.8, 11.9 and 13.4 mm respectively. These results are in accordance with that of Mahfuzul_Hoque et al. (2007) and Pandey and Singh (2011). Cumin was found to be ineffective in controlling the other bacterial strains and these results were contrasted with that of Dua et al. (2013) who reported cumin with potentially effective with MIC ranged from 6.25 to 12.5 mg/ml .On the other hand, a higher concentration of cumin extract reached to 60 mg/ml may be required to be effective against food spoilage bacteria and these results were coincident with that previously reported by Sheikh et al. (2010). Several researchers investigated the efficiency of plant extracts and their effective compounds as antimicrobial agents to control growth of food borne and spoilage bacteria. Some researchers have suggested that antimicrobial components of the plant extracts (terpenoid, alkaloid and phenolic compounds) interact with enzymes and proteins of the microbial cell membrane causing its disruption to disperse a flux of protons towards cell exterior which induces cell death or may inhibit enzymes necessary for aminoacids biosynthesis (Burt, 2004; Gill and Holley, 2006). Other researchers attributed the inhibitory effect of these plant extracts to hydrophobicity characters of these plants extracts which enable them to react with protein of microbial cell membrane and mitochondria disturbing their structures and changing their permeability (Friedman et al., 2004; Tiwari et al., 2009). The present study suggested that plant extracts which proved to be potentially effective can be used as natural preservatives to control food poisoning diseases and preserve food avoiding application of healthy hazards of chemical preservatives.

4. Conclusion

Food spoilage is often caused by the growth of many pathogenic bacterial strains. Prevention of food spoilage in food industry and food stuff is mainly based on the application of chemical preservatives. The adverse effects of these chemical preservatives on human health increases the demand to search for potentially effective, healthy safer and natural food preservative. The plant extracts which proved to be potentially effective as (*P. granatum* and *S. aromaticum*) can be used as natural alternative preventives to control food poisoning diseases and preserve food stuff avoiding healthy hazards of chemically antimicrobial agent applications.

Declaration of conflict of interest

None.

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