Journal of Analytical Science and Technology

RESEARCH ARTICLE Open Access

Antioxidant and antimicrobial activities of water-soluble polysaccharide isolated from Balangu seed (*Lallemantia royleana*) gum

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Abstract

Background: Balangu (*Lallemantia royleana*) belongs to Lamiaceae and is a medicine used in Iranian traditional and folklore medicine in the treatment of various nervous, hepatic, and renal diseases.

Methods: In this study, the influence of molecular weight (MW) was measured on antioxidant and antimicrobial activities of Balangu seed gum fractions. Firstly, Balangu seed gum was fractionated by precipitation method using ethanol on the basis of MW. Two fractions called precipitate (PER) Balangu and supernatant (SUPER) Balangu were obtained as the highest and lowest MW fractions, respectively. Monosaccharide composition was measured by GC-MS. The antioxidant activity was measured by two methods, DPPH and FRAP assay. The antibacterial activities were screened against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and two Gram-negative bacteria (*Salmonella enterica* and *Escherichia coli*) by minimum inhibitory and bactericidal concentration (MIC and MBC), disc, and well diffusion method.

Results: The results showed that the total phenol contents of Balangu, PER-Balangu, and SUPER-Balangu were 76.28 ± 1.41, 43.56 ± 1.72, and 89.46 ± 1.38 μg gallic acid equivalent/mg sample, respectively. Balangu and its fractions were composed of galactose, glucose, arabinose, rhamnose, and xylose. The IC_{50} values of Balangu, PER-Balangu, SUPER-Balangu, and BHT in DPPH assay were 0.46, 0.65, 0.38 and 0.31 mg/ml, respectively. In addition, the reducing power of Balangu, PER-Balangu, SUPER-Balangu, and BHT were 534.92, 431.80, 636.36, and 772.72 (mM Fe^{+2}/g), respectively. Antibacterial test results showed, that for SUPER-Balangu, the minimum inhibitory concentration and the minimum bactericidal concentration against *Staphylococcus* were 3.125 mg/mL and 6.25 mg/mL.

Conclusions: SUPER-Balangu has a great potential as a natural food preservative, antibacterial, and antioxidant agent.

Keywords: Antioxidant activity, Antimicrobial, Balangu seed gum, DPPH, MIC and MBC

Background

Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (Karakoca et al. 2013).

Traditionally, plant compounds are used for treatment of hospital infections in advanced countries. An appropriate method in obviating the common problems of antibiotic side effects is using plant drugs with antimicrobial properties (Golshani and Sharifzadeh 2013). Furthermore, natural antioxidants such as phenolic compounds, flavonoids, and other phytochemicals can act as free radical scavengers. Many reports have indicated that the daily use of foods with high levels of flavonoids may have the potential to decrease the risk of certain cancers, such as colon, breast, and pancreatic cancers (Safaei-Ghomi et al. 2009). These compounds play a crucial role in preventing chronic diseases by retarding the oxidative degradation

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caused by such highly reactive molecules as reactive oxygen species (ROS) (Gharibi et al. 2015).

Antioxidant activity, a common bioactivity of natural-derived polysaccharides, was widely investigated for the prevention and treatment of diseases associated with oxidation damage (Wang et al. 2016). It is widely believed that the antioxidant activity of natural polysaccharides was influenced by raw materials, extraction procedures, and drying methods (Zhu et al. 2012; Zhao et al. 2015).

Balangu (*Lallemantia royleana*) is a medicinal plant, which is widely grown in different regions of European and Middle Eastern countries especially Turkey, Iran, and India. It is used in a wide range of traditional beverages or industrial products in Iran and Turkey (Razavi and Mohammadi 2011). Seeds of *Lallemantia royleana* are dark-brown to black in color and smooth. When moistened with water, the seeds become coated with translucent and voluminous mucilage and hydrocolloids. The taste of the moistened seed is bland, soothing, and spicy (Malavya and Dutt 1941; Mohammad Amini and Razavi 2012).

Traditionally, it is a very common practice that local people use indigenous plants to cure infectious diseases. These indigenous plants or plant products or those that are part of food as dietary components are termed as ethnomedicine. Although there are very few reports on the mechanism of action and phytochemistry of these plant-based phytomedicines, traditional knowledge reports that these plants possess potential to cure infectious diseases. Nowadays, these ethnomedicines have been receiving considerable attention by scientist and pharmaceutical research industries with the aim to investigate for more effective substitute (Karsha and Lakshmi 2010; Dogruoz et al. 2008; Samee et al. 2009). Balangu seed contained 45.25% carbohydrates, 26.60% protein, 18.27% oil, 3.63% ash, and 1.29% crude fiber, and its length and diameter are 3.148 mm and 0.720 mm, respectively (Razavi et al. 2016a, 2016b). The Balangu seed is used by Iranian in medicine due to its nutritional and human health virtues, and its seed also is conventionally used in some drinks, like "Tokhme Sharbati" and bread in Iran and Turkey (Razavi et al. 2016a, 2016b).

In some southern parts of Iran, seed powders have been used as a tonic medication and a remedy for psychopath diseases (Safa et al. 2013). *L. royleana* seeds are reported for the various therapeutics such as treatment of inflammation, respiratory problems, and abscesses (Abdulrasool et al. 2017). Previous studies were focused on its ethno-botany, while medicinal properties of *L. royleana* are not much evaluated on scientific merits. So far, not a single study has been reported with reference to its antibacterial potential (Mohammad Amini and Razavi 2012; Amiri et al. 2012; Akber et al. 2011).

Monosaccharides composition determined by anion-exchange chromatography (HPAEC) showed the major monosaccharides of Balangu seed gum were in following order: arabinose (37.88%), galactose (33.54%), and rhamnose (18.44%). Trace amounts of xylose (6.02%) and glucose (4.11%) were also detected in the polysaccharide composition (Razavi et al. 2016a, 2016b).

To the best of our knowledge, no study about the antioxidant and antimicrobial activities was investigated for Balangu and its fractions. Therefore, the objective of this study is to evaluate the antioxidant and antimicrobial activities of water-soluble polysaccharide isolated from Balangu and its fractions.

Methods

Materials

Seeds of Balangu were purchased from a local market in Bojnord, Iran, in May 2018 and cleaned for dust and broken seeds. Ethanol (96%) was obtained from Pars Alcohol Company (Isfahan, Iran). Other chemicals had analytical grade (Merck Company, Darmstadt, Germany).

Gum extraction

Balangu seed gum was extracted at optimized conditions (water/seed ratio 59:1, pH 7, temperature 85 °C, and entire extraction time 20 min), according to the method described by Mohammad (2007). Segregation of mucilage from the inflated seed was attained through the scraping method. The seeds were crossed through an extractor equipped with a rotating plate which scraped the mucilage layer on the seed surface. The extracted solution was filtered and afterward purified completely by mixing with three volumes of 96% ethanol to precipitate polysaccharide. The precipitated polysaccharide was dissolved in water and dried overnight in an air-forced laboratory oven at 38 °C. The dried gum was then milled (Magic Bullet, Model 6350M, Korea), sieved (mesh 149 μm), packed, and kept in cool and dry condition.

Fractionation

The method of polysaccharide fractionation from Balangu seed gum was set according to Naji-Tabasi et al. (2016) which is on the basis of molecular weight (MW) using ethanol precipitation. For this purpose, Balangu seed gum solution (0.1% *w*/*w*) was prepared at laboratory temperature for about 24 h until complete dissolution. Then, various volumes of ethanol (10–90% *v*/*v*) were added dropwise to the solution at a constant agitation rate on stirrer. The precipitate-Balangu (PER-Balangu) and supernatant-Balangu (SUPER-Balangu) fractions which were known as high- and low-MW fractions were obtained after centrifugation for 10 min at 12,000*g* (Sigma, Germany). The fractions were dried then milled (Magic Bullet, Model 6350M, Korea), sieved (mesh 149 μm), packed, and kept at cool and dry condition.

Determination of total phenolic content

The total phenolic content extract was determined using the Folin-Ciocalteu method (Hayouni et al. 2007). Here, 100 μl of diluted extract (1000 mg/l) was added to Folin-Ciocalteu reagent (1/10, 500 μl), and then, 1.5-ml sodium carbonate (Na_2CO_3) (20%) was added to the mixture. Then, the tubes were incubated at room temperature for 120 min. The absorbance was read at 760 nm using UV-VIS spectrophotometer (Cecil CE 1011; UK). The analysis was done in triplicates. Also, the standard curve of gallic acid in methanol (50–500 mg/l) was prepared (Fig. 1). Total phenolic content was expressed as gallic acid (GA) equivalents (mg GA/g of dried material).

GC-MS analysis

Monosaccharide composition was measured by GC-MS according to the technique given by Fathi et al. (2015). The purified sample (1 ml) was hydrolyzed with trifluoroacetic acid (2 M) for 8 h at 100 °C. The product was recovered using sodium hydride according to the method provided by Wolfrom and Thompson (1951). The acetylation process was performed by adding Ac_2O-Pyridine (1:1 volume ratio) at 25 °C for 20 h. The solution was dried by nitrogen gas and injected with GC-MS (Agilent, Japan, Tokyo, Technology) after the addition of ethyl acetate. The column used was HP 5923 and nitrogen utilized as the carrier gas (flow rate 1.3 mL/min). The primary temperature of the injection was 280 °C. The temperature of the column was kept at 65 °C for 2 min and afterwards increased to 300 °C at 7 °C/min. The temperature remained constant for 15 min. Identification of pixels based on standards and mass spectrometry was carried out.

Antioxidant activity

DPPH radical scavenging assay

The antioxidant activity extracts were assayed according to the method of Kong et al. (2010). The extracts at different concentrations (0.1, 0.2, 0.4, 0.6, 0.8, and 1 mg/ml) were prepared. Thus, 1 ml of the sample was mixed with 0.2 ml DPPH in ethanol. After 30 min, the absorbance was measured at 517 nm. The antioxidant activity was calculated using Eq. 1.

$$\%\text{DPPH scavenging activity} = \frac{\text{Absorbance}_{517}\text{ of control} - \text{Absorbance}_{517}\text{ of sample}}{\text{Absorbance}_{517}\text{ of sample}} \times 100 \quad (1)$$

Concentration of the sample necessary to scavenge 50% of the DPPH radicals was calculated using the Bio-Data Fit online software. Butylated hydroxytoluene (BHT) was used as the reference antioxidant.

Reducing power

The reducing powers of extracts were measured following the method of Malsawmtluangi et al. (2014) using BHT as standard with slight modifications. One milliliter of the sample with various concentrations was mixed with 2 ml of phosphate buffer (0.2 M and 6.6 pH) and 2 ml of 1% potassium ferricyanide (*w*/*v*). The mixture was then incubated at 50 °C for 20 min, and the reaction was stopped by adding 2 ml of 10% trichloroacetic acid. The mixture was centrifuged at 3000*g* for 10 min. About 2 ml of the supernatant was mixed with 2 ml of distilled water and 0.4 ml of 0.1% ferric chloride solution. After 10 min, the absorbance of the resulting solution was measured at 700 nm.

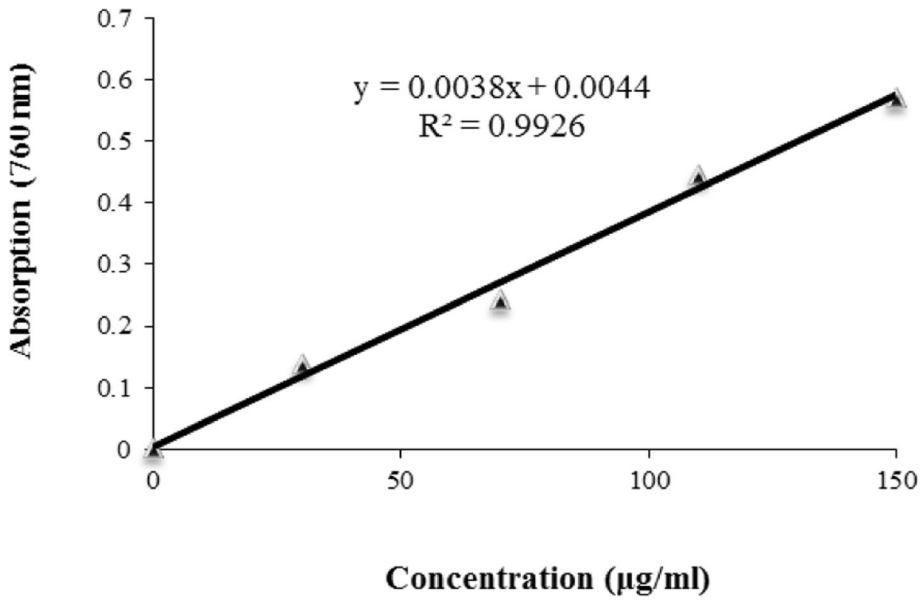

Fig. 1 Standard curve of gallic acid

Organisms and inoculation conditions

The extracts of Balangu and its fractions were individually tested against four bacterial strains, including *Staphylococcus aureus* (PTCC 1431), *Bacillus cereus* (PTCC 1015), *Salmonella enterica* (PTCC 1709), and *Escherichia coli* (PTCC 1399) which were obtained from the Persian Type Culture Collection, Iranian Research Organization for Science and Technology (PTCC, Iran). Bacterial strains were cultured overnight at 37 °C in MH agar (YesilCeliktas et al. 2007).

Antimicrobial activity

Antibacterial activity of the Balangu and its fractions was determined by macro-dilution, disc, and well diffusion methods. All tests were performed in three replicates.

Determination of the minimal inhibitory concentration

Minimum inhibitory concentrations (MIC) were determined by broth macro-dilution method in 96-well plates by Rios et al. (1988) and Duffy and Power (2001) methods.

The initial concentration of the extract was prepared with the aid of bath sonicator with 4 ml solvent and 30% dimethyl sulphoxide in sterile distilled water and one drop of Tween 80. One milliliter of diluted extract was infused into macro-plate with 1 ml of sterile Mueller-Hinton broth (MHB; HiMedia, India) and then diluted (50% with MHB). 0.5 McFarland standard turbidity for microbial suspension equivalent was prepared by suspensions of the growth from brain-heart infusion medium (HiMedia, India). Suspensions were further diluted to obtain a concentration of 10^7 colony-forming units (CFU) per milliliter for the bacteria. Then, 10 μl of diluted inoculums was added to each well of macro-plate. The sterility of the medium was also tested in two wells, and gentamicin was used as the positive control for bacterial strains. Plates were incubated for 24 h at 37 °C for bacteria. The growth of microorganisms was assessed by TTC (2, 3, 5-triphenyl tetrazolium chloride, Sigma, USA) assay. Briefly, 0.5 ml of TTC (5 mg/ml; dissolved in sterile water) was added to each well, and the plates were incubated at 37 °C for bacteria. The results were expressed as the lowest concentration of plant extract that could inhibit any red dye production. Minimum inhibitory concentration (MIC) values were defined as the lowest concentrations of extract that inhibit bacteria after 24 h. All experiments were done in triplicates.

Determination of minimum bactericidal concentrations

The bactericidal effects of the extract were determined according to the method described by Rios et al. (1988). One hundred microliters of clear dilutions in wells of macro-plate was sub-cultured on the Mueller-Hinton agar plates and subsequently incubated at 37 °C for 24 h. Minimal bactericidal concentration (MBC) was recorded from the first tube that showed no growth on solid media.

Antimicrobial activity by disc and well diffusion method

The extract of Balangu and its fractions were tested for antibacterial activity using the disc and well diffusion methods on solid media Mueller-Hinton agar (MHA) plates. The sterile paper discs and wells of 6 mm diameter were placed on the agar plates with the appropriate media, and the bacteria density was adjusted to approximately 10^7 CFU/ml. Then, 50 μl of diluted extract was applied to test paper disc and well in plates, and the agar plates were further incubated for 24 h at 37 °C. Finally, the zones of growth inhibition around the discs were measured. Gentamicin and DMSO were used as positive and negative controls, respectively (Firdaus et al. 2011).

Statistical analysis

The measurements of total phenolic compounds, antioxidant, and antibacterial activities were carried out for three replicates. The results are expressed as mean values ± standard deviation (SD).

Results and discussion

Total phenolic content

Folin-Ciocalteu method is a widely used assay for quantitative determination of phenolic compounds (Tawaha et al. 2007; Pourmorad et al. 2006). Based on the data, the standard curve was plotted and the regression was calculated ($Y = 0.0038X + 0.0044(R^2 = 0.9926)$) (Fig. 1).

The total phenol contents of Balangu, PER-Balangu, and SUPER-Balangu were determined as 76.28 ± 1.41, 43.56 ± 1.72, and 89.46 ± 1.38 μg gallic acid equivalent/mg sample, respectively. Phenolic compounds are secondary plant metabolites that play a key role in the sensory and nutritional quality of fruits, vegetables, and other plants (Ignat et al. 2011).

Monosaccharide composition

Table 1 shows the monosaccharide composition of Balangu and its fractions.

The polysaccharides of Balangu were composed of glucose (3.93%), galactose (28.31%), arabinose (25.64%), xylose (1.49%), and rhamnose (1.24%) (Table 1).

Razavi et al. (2013) found that polysaccharides of Balangu consist of arabinose, galactose, rhamnose, xylose, and glucose which were determined as 37.88%, 33.54%, 18.44%, 6.02% and 4.11%, respectively. Salehi et al. (2018) showed that the polysaccharides of Balangu are composed of rhamnose (10.7%), arabinose (29.7%), and galactose (59.4%).

Behbahani and Imani Fooladi (2018) found that polysaccharides of Balangu consist of galactose, arabinose, rhamnose, xylose, and glucose which were determined as 36.28%, 35.96%, 15.18%, 7.38% and 5.20%, respectively.

Farhadi (2017) showed the Balangu was contained of arabinose (7.55%), rhamnose (5.45%), mannose (6.47%),

Table 1 Monosaccharide composition of the Balangu and its fractions (d.b.%)

Fractions	Glucose	Galactose	Arabinose	Xylose	Rhamnose
Balangu	3.93 ± 0.51^{a}	28.31 ± 0.42^{b}	25.64 ± 0.35^{a}	1.49 ± 0.03^{c}	1.24 ± 0.04^{b}
PER-Balangu	2.32 ± 0.13^{b}	32.11 ± 0.38^{a}	23.02 ± 0.02^{b}	1.93 ± 0.1^{b}	0.35 ± 0.07^{c}
SUPER-Balangu	1.87 ± 0.17^{c}	26.20 ± 0.25^{c}	18.27 ± 0.08^{c}	2.2 ± 0.17^{a}	1.72 ± 0.12^{a}

Means in a column followed by different superscript letters are significantly different at $P \leq 0.05$ by Duncan test

fructose (19.10%), galactose (30.03%), α-D-glucose (2.96%), β-D-glucose (5.88%), glucuronic acid (4.70%), and galacturonic acid (10.18%). In this research, PER-Balangu was composed of glucose (2.32%), galactose (32.11%), arabinose (23.02%), xylose (1.93%), and rhamnose (0.35%). Moreover, SUPER-Balangu was composed of glucose (1.87%), galactose (26.20%), arabinose (18.27%), xylose (2.2%), and rhamnose (1.72%) (Table 1).

Sharifi-Rad et al. (2015) showed that the major compounds aerial parts of essential oils from *L. royleana* in Fars Province, Iran, were trans-pinocarvyl acetate (26.0%), pinocarvone (20.0%), β-pinene (1.5%), (E)-β-ocimene (4.1%), terpinolene (1.1%), linalool (3.4%), trans-pinocarveol (1.6%), 3-thujen-2-one (5.1%), myrtenal (1.5%), verbenone (7.1%), trans-carveol (5.3%), cis-carveol (3.5%), pulegone (4.4%), carvacrol (1.6%), dihydrocarvyl acetate (2.5%), and β-cubebene (2.1%).

Antioxidant activity

In the present study, antioxidant activity was evaluated by using two different methods, namely, DPPH and FRAP. Results were reported as IC_{50}, which is specified as the amount of antioxidant needed to inhibit 50% of DPPH free radicals (Liu et al. 2017).

The IC_{50} value of Balangu, PER-Balangu, SUPER-Balangu, and BHT in DPPH assay were 0.46, 0.65, 0.38, and 0.32 mg/ml, respectively (Tables 2 and 3).

As shown in Table 2, the DPPH radical scavenging activity followed the order of SUPER-Balangu > Balangu > PER-Balangu. DPPH is a synthetic free radical that shows maximum absorption at 517 nm. Antioxidants can scavenge DPPH free radicals by providing a hydrogen atom and converting them to a colorless product resulting in a reduction in absorbance (Antolovich et al. 2002). *Lallemantia royleana* seed mucilage total phenolic content and antioxidant activity (IC_{50}) were equal to 82.56 ± 1.6 μg GAE/mg and 528.54 ± 0.35 μg/ml, respectively (Alizadeh Behbahani and Imani Fooladi 2018).

Table 2 DPPH free radical scavenging activity in different gum concentrations of Balangu, PER-Balangu, SUPER-Balangu, and BHT

Concentration (mg/ml)	Balangu (%)	PER-Balangu (%)	SUPER-Balangu (%)	BHT (%)
0.1	15.95 ± 0.11	8.95 ± 0.32	18.28 ± 0.17	32.5 ± 0.21
0.2	25.60 ± 0.12	15.87 ± 0.14	34.87 ± 0.36	41.32 ± 0.14
0.4	34.85 ± 0.34	38.23 ± 0.34	48.23 ± 0.24	49.97 ± 0.27
0.6	49.85 ± 0.57	65.47 ± 0.10	75.47 ± 0.30	79.38 ± 0.44
0.8	57.84 ± 0.64	69.79 ± 0.42	79.79 ± 0.11	88.43 ± 0.39
1	68.65 ± 0.35	72.65 ± 0.12	88.65 ± 0.41	94.71 ± 0.74

Different flavonoids and phenolic compounds react with free radical to reduce the degradation of membranes by preventing the reaction between free radicals and phospholipids (Bruneton 1995). They can also be used as antioxidants and in vitro as enzyme inhibitors (Smith et al. 1998). Many phenolics, such as flavonoids, have antioxidant capacities that are much stronger than those of vitamin C and E (Prior and Cao 2000). Many studies on medicinal plants confirm the relation between the antioxidant activity and the presence of polyphenolics content (Ibtissem et al. 2012).

Rice-Evans et al. (1996) reported that phenolic compounds have redox properties which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. The redox potential of phenolic compound plays an important role in determining the antioxidant capacity (Yen et al. 2004). Norshazila et al. (2010) reported that extracts with high amounts of total phenolic content also showed a high antioxidant activity.

Our study showed that the plant extracts form a strong hydroxyl radical, scavenging DPPH and superoxide anion, and inhibition of yolk lipid peroxidation in a dose-dependent manner as reported earlier (Ganie et al. 2012; Parray et al. 2011) and its possible mechanism may be due to the presence of the alkaloids and phenolic substances that helps to capture lipid peroxidation chain reactions triggered by reactive oxygen species, reduce the lipid peroxidation chain length, and block or slow down the lipid peroxidation (Huang et al. 2006).

Based on the data of reducing power, standard curve was plotted and the regression was calculated ($Y = 0.0088X + 0.1052 (R^2 = 0.9995)$) (Fig. 2).

The reducing power of Balangu, PER-Balangu, SUPER-Balangu, and BHT were 534.92, 431.80, 636.36, and 772.72 (mM Fe^{+2}/g), respectively (Table 4). The result indicated that the reducing power of SUPER-Balangu was higher than that of Balangu and PER-Balangu.

Table 3 IC_{50} value of Balangu, PER-Balangu, SUPER-Balangu, and BHT

Balangu (mg/ml)	PER-Balangu (mg/ml)	SUPER-Balangu (mg/ml)	BHT (mg/ml)
0.46 ± 0.12	0.65 ± 0.14	0.38 ± 0.15	0.31 ± 0.10

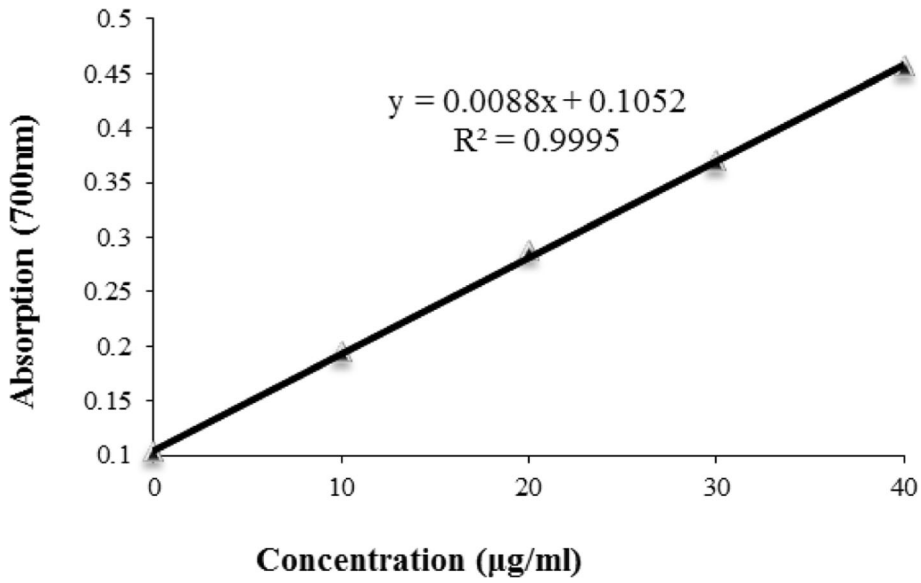

Fig. 2 Standard curve of Fe^{2+} ferric reducing antioxidant power (FRAP) method

Reactive oxygen species (ROS), including oxygen radicals and their reaction products, are known to react with biological molecules, leading to cell and tissue damage. Antioxidant activity is a complex process usually occurring through several mechanisms. Due to its complexity, the evaluation of the antioxidant activity for pure compounds or extracts should be carried out by more than one test method (Aruoma 2003). The lower IC_{50} value indicates a stronger ability of the extract to act as a DPPH scavenger while the higher IC_{50} value indicates a lower scavenging activity of the scavengers as more scavengers were required to achieve 50% scavenging reaction.

Antioxidant activities are known to increase proportionally to the polyphenol content, mainly due to their redox properties (Rasineni et al. 2008). Among the diverse roles of polyphenols, they protect cell constituents against destructive oxidative damage, thus limiting the risk of various degenerative diseases associated with oxidative stress and thus tending to be potent free radical scavengers. Their ability to act as antioxidants is due to their chemical structure and ability to donate/accept electrons, thus delocalizing the unpaired electron within the aromatic structure (Ross and Kasum 2002).

Antibacterial activity

The results presented in Table 5 and Figs. 3 and 4 are zones of growth inhibition around MIC and MBC of Balangu, PER-Balangu, and SUPER-Balangu which were evaluated. Antibacterial test results showed that for SUPER-Balangu, the minimum inhibitory concentration, and the minimum bactericidal concentration against *Staphylococcus aureus* were 3.125 mg/mL and 6.25 mg/mL.

Table 4 Ferric reduction ability of Balangu, PER-Balangu, SUPER-Balangu, and BHT (absorbance at 700 nm)

Balangu (mM Fe^{+2}/g)	PER-Balangu (mM Fe^{+2}/g)	SUPER-Balangu (mM Fe^{+2}/g)	BHT (mM Fe^{+2}/g)
534.92	438.80	636.36	772.72

Table 5 Determination of MIC and MBC of Balangu, PER-Balangu, and SUPER-Balangu

Technique applied	Test bacteria	MIC (mg/ml)	MBC (mg/ml)
Balangu	*Staphylococcus aureus* (PTCC 1431)	25	25
	Bacillus cereus (PTCC 1015)	25	25
	Salmonella enterica (PTCC 1709)	50	50
	Escherichia coli (PTCC 1399)	50	50
PER-Balangu	*Staphylococcus aureus* (PTCC 1431)	50	50
	Bacillus cereus (PTCC 1015)	100	> 100
	Salmonella enterica (PTCC 1709)	100	> 100
	Escherichia Coli (PTCC 1399)	100	> 100
SUPER-Balangu	*Staphylococcus aureus* (PTCC 1431)	3.125	6.25
	Bacillus cereus (PTCC 1015)	12.5	12.5
	Salmonella enterica (PTCC 1709)	25	25
	Escherichia Coli (PTCC 1399)	25	25

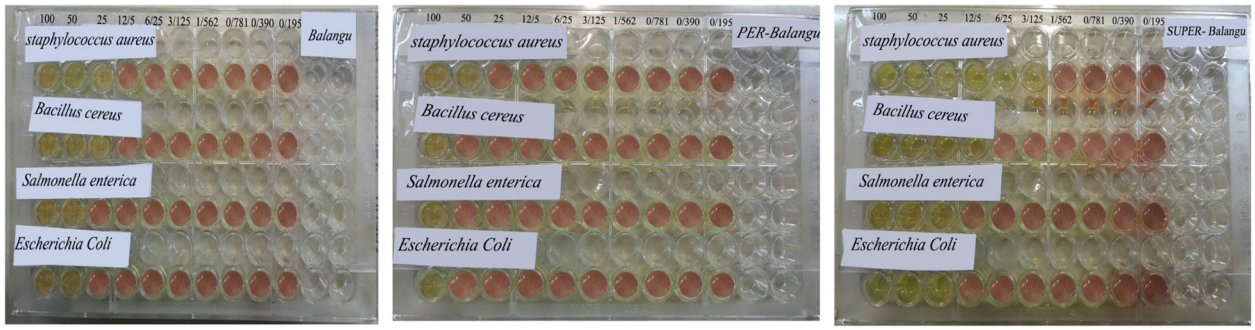

Fig. 3 Determination of MIC of Balangu, PER-Balangu, and SUPER-Balangu

The results showed that plants with high antioxidant properties have high antimicrobial activity too (Fazeli-Nasab et al. 2017), such as the extract of SUPER-Balangu that was the most effective extract on bacteria. In general, phenolic compounds potentially disturb the function of bacterial cell membranes which causes retardation of growth and multiplication of bacteria. Further, phenolic compound is involved in adhesion binding, protein and cell wall binding, enzyme inactivation, and intercalation into the cell wall and/or DNA during inactivation of pathogens (Pereira et al. 2007). Previous studies have suggested that the reactive portion of antimicrobial phenolic compounds may be the free hydroxyl group (Prindle and Wright 1977).

The antibacterial activity of the extracts could be attributed to the high content of phenolic which has been reported to be involved in the inhibition of nucleic acid biosynthesis and metabolic processes (Cushnie and Lamb 2005).

The MIC and MBC values for herbal extracts can vary significantly depending on factors such as chemical composition differences between herbs collected in different countries (differences in the climate, soil composition, age, and vegetative cycle stage) (Masotti et al. 2003), different botanical parts used for extraction (Tilaoui et al. 2015), method of extraction, and differences in strains of microorganisms used (standardized or clinical isolates) (Seukep et al. 2013).

As shown in Tables 6 and 7 and Figs. 5 and 6, antibacterial activities of Balangu, PER-Balangu, and SUPER-Balangu were assessed by well diffusion and disc diffusion methods. Balangu, PER-Balangu, and SUPER-Balangu show antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella enterica*, and *Escherichia coli* food-borne pathogen (Tables 6 and 7).

As shown in Tables 5, 6, and 7, the highest antimicrobial activity followed the order of SUPER-Balangu > Balangu > PER-Balangu. Therefore, it can be concluded that SUPER-Balangu in appropriate combination can act as an effective food preservative.

The disc and well diffusion methods are dependent on the diffusion ability of the substances, and in these methods, antibacterial property is expressed as diameter (mm) of the zone of inhibition (He et al. 2010).

Aromatic plants have great importance for food, cosmetics, and pharmaceutical industries. They have been used since ancient times, and despite many of them were substituted by synthetic ones, the demand for natural

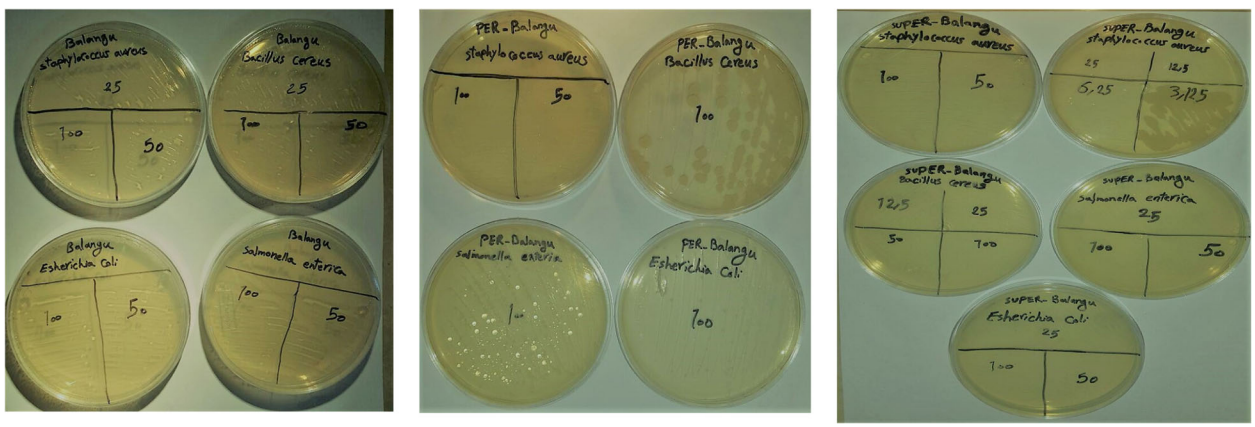

Fig. 4 Determination of MBC of Balangu, PER-Balangu, and SUPER-Balangu

Table 6 Antibacterial activity of Balangu, PER-Balangu, and SUPER-Balangu assessed by well diffusion method

Microorganism	Balangu	PER-Balangu	SUPER-Balangu	Gentamicin	DMSO
Staphylococcus aureus (PTCC 1431)	29 mm ± 0.05	23 mm ± 0.03	34 mm ± 0.04	33 mm ± 0.02	6 mm ± 0.01
Bacillus cereus (PTCC 1015)	26 mm ± 0.03	25 mm ± 0.07	33 mm ± 0.08	32 mm ± 0.05	6 mm ± 0.01
Salmonella enterica (PTCC 1709)	25 mm ± 0.04	24 mm ± 0.05	29 mm ± 0.06	28 mm ± 0.08	6 mm ± 0.01
Escherichia Coli (PTCC 1399)	26 mm ± 0.06	23 mm ± 0.05	28 mm ± 0.02	27 mm ± 0.03	6 mm ± 0.01

Expressed as the size of the growth inhibition zones (mm) as the average of triplicates

products is increasing (Mickiene et al. 2011). The antimicrobial properties of several naturally occurring compounds have been known for decades. Recently, many plants have received attention as sources of antibiotics (Basile et al. 2000).

Most of the studies on the mechanism of phenolic compounds have focused on their effects on cellular membranes. Phenolic compounds attack cell walls and membranes, thereby affecting their permeability and release of intracellular constituents, but they also interfere with the membrane functions (electron transport, nutrient uptake, protein, nucleic acid synthesis, and enzyme activity). Thus, active phenolic compounds might have several invasive targets which could lead to the inhibition of bacterial pathogens (Bajpai et al. 2008).

Oxygenated monoterpenes are lipophilic in nature and act on the cell membrane which causes substantial morphological damage, resulting in a change in permeability and the release of cellular contents (Moosavy et al. 2008). The Gram-positive strains of bacteria that were tested seemed to be more sensitive to the extracts, which are attributed to the absence of an outer lipopolysaccharide layer in Gram-negative bacteria that provides a resistant barrier (Inouye et al. 2001). The antibacterial activity of flavonoids and polyphenols has been attributed to inhibition of synthesis of RNA and DNA (Arora et al. 2013).

The internal stability of the bacterial cells depends on the interaction between a series of physiological factors, and the disturbance of this stability may determine the bacteria's death or the inhibition of its growth. To provide products, reducing the toxicity risk and at the same time obtaining from a new natural and renewable source becomes a growing and economically viable option. The use of vegetal extracts for antibacterial activity is a consummated fact (Nogueirasa et al. 2014).

Sharifi-Rad et al. (2016) showed *L. royleana* essential oil had its best inhibitory effect at a MIC of 1 mg/mL, whereas *Salvia nemorosa* essential oil showed its best inhibitory effects at a higher concentration (8 mg/mL).

Sharifi-Rad et al. (2015) evaluated *L. royleana* essential oil against bacteria and fungi by disk diffusion and microbroth dilution method. Results showed that the essential oil have significant inhibition effects on several bacterial and fungi species such as *S. aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger.*

Viljoen et al. (2002) showed that the two major components of *L. royleana* essential oil are transpinocarvyl acetate (26.0%) and pinocarvone (20.0%). It is assumed that these two components are the main antibacterial agents of *L. royleana* essential oil.

Mahmood et al. (2013) evaluated the antibacterial potential of aqueous and organic extracts (organic solvents are ethanol, methanol, and chloroform) of *L. royleana* seeds against four bacterial strains (*Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) by disc diffusion method. Results showed, except aqueous extracts, all organic extracts of *L. royleana* seeds displayed significant antibacterial activity against all the tested bacteria. The chloroform extract exhibited highest antibacterial activity for all bacterial strains. Therefore, *L. royleana* seeds possess significant antibacterial potential against *S. aureus*, *E. coli*, and *E. cloacae.* Mahmood et al. (2013) reported that Gram-positive *S. aureus* was found to be the most sensitive bacterial strain, showing the maximum inhibition zone as compared to other microorganisms in case of all the extracts. Therefore, it is very obvious from results that *L. royleana* seed extracts have significant antibiotic potential against Gram-positive and Gram-negative bacterial strains which is an indication of presence of broad

Table 7 Antibacterial activity of Balangu, PER-Balangu, and SUPER-Balangu assessed by disc diffusion method

Microorganism	Balangu	PER-Balangu	SUPER-Balangu	Gentamicin	DMSO
Staphylococcus aureus (PTCC 1431)	29 mm ± 0.02	22 mm ± 0.02	33 mm ± 0.05	31 mm ± 0.08	6 mm ± 0.01
Bacillus cereus (PTCC 1015)	28 mm ± 0.06	24 mm ± 0.08	31 mm ± 0.04	29 mm ± 0.05	6 mm ± 0.01
Salmonella enterica (PTCC 1709)	27 mm ± 0.03	21 mm ± 0.05	30 mm ± 0.06	28 mm ± 0.09	6 mm ± 0.01
Escherichia Coli (PTCC 1399)	25 mm ± 0.07	20 mm ± 0.06	29 mm ± 0.04	28 mm ± 0.07	6 mm ± 0.01

Expressed as the size of the growth inhibition zones (mm) as the average of triplicates

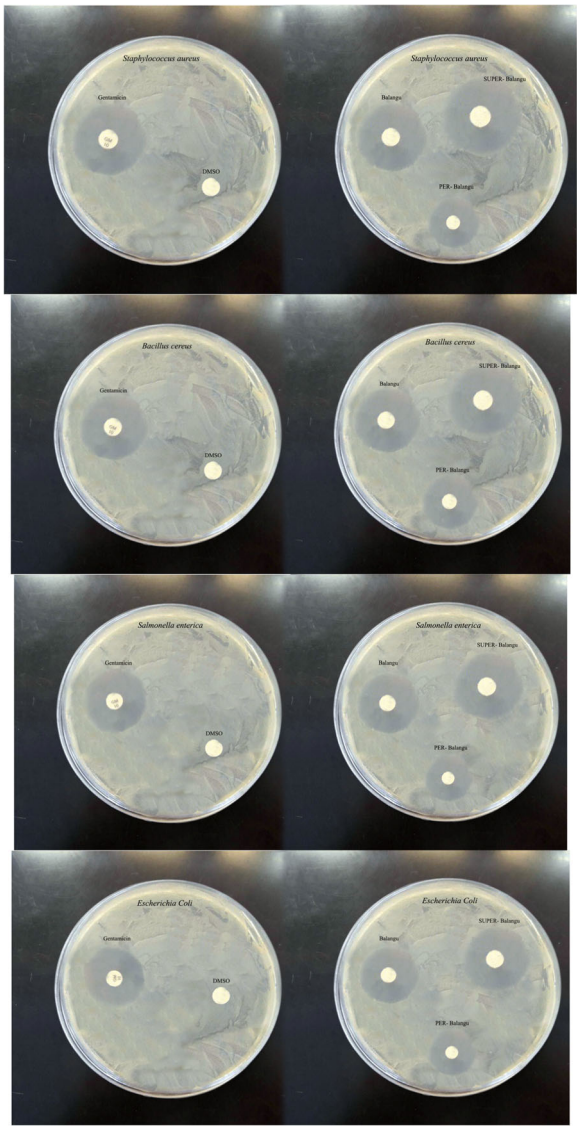

Fig. 5 Antibacterial activity of Balangu, PER-Balangu, and SUPER-Balangu were assessed by well-diffusion method

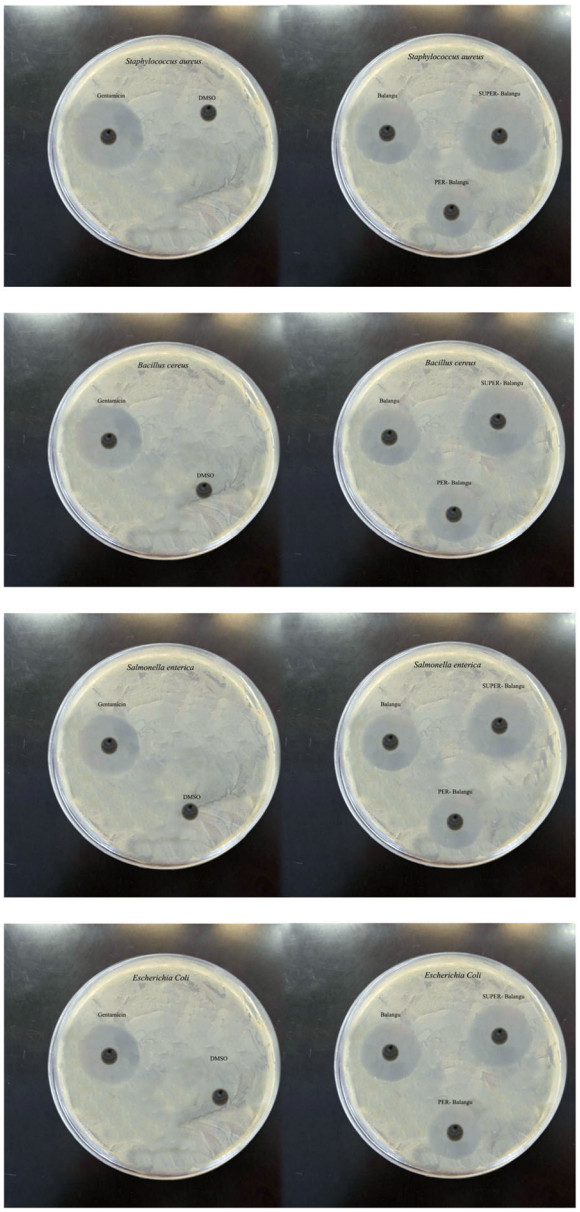

Fig. 6 Antibacterial activity of Balangu, PER-Balangu, and SUPER-Balangu assessed by disc diffusion method*

spectrum antibiotic phytoconstituents (Doughari 2006) which impart respective bioactivity to these plant extracts (Gulfraz et al. 2011). Furthermore, still, there is a requirement to determine the active compounds present in the seeds, to classify the compounds that might be more effective against these human pathogenic bacterial strains, and to use a specific formulation of only those compounds in the drug synthesis (Abraham and Thomas 2012).

Conclusion

The results presented in this study indicated that extracts obtained from the Balangu seed gum possess antioxidant and antibacterial properties. On the basis of the experimental results, it can be postulated that the extracts of the Balangu seed gum have the potent antibacterial properties against some representative food-borne pathogens. Specifically, SUPER-Balangu extract was more active against Gram-negative bacteria which indicated the presence of active compounds. The results of this investigation indicated that SUPER-Balangu had a high potential of antioxidant and antibacterial properties. The findings of this study support this view that some medicinal plants are promising sources of potential antioxidant and could be used as preventive agents for some diseases.

Abbreviations
BHT: Butylated hydroxytoluene; CFU: Colony-forming units; GA: Gallic acid; *L. royleana*: *Lallemantia royleana*; MBC: Minimum bactericidal concentrations; MHA: Mueller-Hinton agar; MIC: Minimum inhibitory concentrations; MW: Molecular weight; PER-Balangu: Precipitate-Balangu; ROS: Reactive oxygen species; SD: Standard deviation; SUPER-Balangu: Supernatant-Balangu

Acknowledgements
The authors are grateful to Research Institute of Food Science and Technology, Mashhad, Iran, for providing their facilities.

Funding
None of the authors did not receive any fund from any person or organization or society.

Availability of data and materials
Not applicable.

Authors' contributions
MS design and carried out the experiment, and AMS and SNT contributed in framing the article. AA supervised the work. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

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Received: 15 January 2019 Accepted: 15 March 2019
Published online: 18 April 2019

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