# Modeling of Ultrasound-Assisted Extraction, Chemical Composition, Antioxidant Activity, Rheological Aspects, and Biological Properties of "Barhang-e-Kabir" Mucilage

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**ABSTRACT:** *In this study, response surface methodology (RSM) was used to investigate the influence of independent process parameters including water to seed ratio (g/g), temperature (°C), time (min), and ultrasonic intensity (%) on the extraction yield of "Barhang-e-Kabir". Chemical composition, monosaccharide composition (using HPAEC-PAD), molecular conformation, molecular weight properties, Surface tension, ζ-potential, particle size distribution, Fourier Transform InfraRed (FT-IR) spectroscopy, color measurement, Total Phenol Content (TPC), Total Flavonoid Content (TFC), Antioxidant Activity (AA), antimicrobial and dilute-solution and steady-state behavior were evaluated. The optimum condition to obtain maximum extraction yield (13.1 %) was extraction temperature 70 ° C, extraction time 40 min, water to seed ratio of 1:10, and ultrasonic power of 90 %. Plantago major gum (PMG) had 89.24% carbohydrate, 4.53% ash, 4.11% moisture, and 2.12% protein. Viscometric molecular weight and average molecular weight were found to be 1.13 ×10$^5$ g/mol and 9.9 ×10$^5$ g/mol, respectively. The intrinsic viscosity of PMG was 12.56 dL/g in deionized water at 25 ºC. Steady shear measurement demonstrated that PMG is a shear-thinning fluid with high viscosity at low concentration. TPC, TFC and AA ($IC_{50}$) tests of PMG showed 89.80 ± 1.23 mg GAE/g dry sample, 123.25 ± 1.32 mg g$^{-1}$dry sample, and 470.45 ± 0.35 µg/mL, respectively. Prevention of linoleic acid oxidation in the system of ß-Carotene-linoleic acid was equal to 32.45 %. The results showed that Streptococcus pyogenes and Pseudomonas aeruginosa are the most sensitive and highest resistance strain to PMG, respectively.*

**KEYWORDS:** *Plantago major gum; Ultrasound-assisted extraction; Chemical composition; Molecular conformation; Rheological behavior; Biological activity.*

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## INTRODUCTION

Seed gums play an important role in the sensory and textural properties of food products. Furthermore, these gums can be used as an important source of dietary fiber with various biological activities such as controlling insulin levels and blood glucose and reducing the risk of colon cancer and heart diseases [1]. Due to the enormous application of gums in the food, pharmaceutical, cosmetic and textile industries, it seems that the search for a new source of gums is necessary.

"Barhang-e-Kabir" (*Plantago major*) is a perennial plant, which belongs to the Plantaginaceae family. *Plantago major* leaves have been used as a remedy for wound healing and the diseases related to digestive organs, skin, circulation, and respiratory organs. The seeds of this plant comprise fructose, xylose, glucose, rhamnose, sucrose, and planteose. *Plantago major* seed quickly produces mucilage with high viscosity as it is wetted by water [2].

Hot-water treatment is commonly used for the extraction of polysaccharides [3]. It should be considered that this extraction method is associated with high temperature and long-time of process. High temperatures used in the hot-extraction method can lead to the structural collapse of polysaccharides. Additionally, from an economical point of view, it is necessary to use a mild-extraction method that has high extraction yield. The use of the ultrasonic technique seems to be very promising for extraction with high yield and activities.

Previous research has been introduced on various seeds and the extraction conditions resulting in different amounts of antioxidant activity, total phenolic content, rheological aspects, yield, and functional attributes from one cultivar of the seeds to another. Therefore, in order to achieve quality polysaccharides and the highest yield, it is obligatory to optimize the extraction method. There are multiple factors that improve the extraction. Furthermore, to determine the optimum extraction conditions the verisimilitude of interactions between the autonomous factors should be taken into account. Response Surface Methodology (RSM) has been declared to determine how the independent variables have an interaction effect on dependent variables [4-6].

*Alizadeh Behbahani et al.,* (2017) indicated that the optimum extraction conditions for *Plantago major* gum (PMG) are pH 6.8, temperature 75 ºC, and water to seed ratio of 1:60. They also demonstrated that PMG is a high molecular weight polysaccharide ($1.2 \times 10^6$) with 85.59% carbohydrate, 76.79 mg GAE/g dry total phenol content, and 97.8 mg/g total flavonoid content. The activation energy, ζ-potential, chain flexibility, and droplet size for *Plantago major* gum are $0.78 \times 10^7$ J/kgmol, 946.09, 15.23, and 448.56 nm, respectively [3]. *Niknam et al.,* (2018) the effects of *Plantago Major* Seed (PMS) gum on the rheological properties of the sunflower oil- based emulsions were investigated. Authors reported that all emulsions showed weak gel- like behavior, which showed stable interactions and entanglements in the emulsion structure [7]. *Niknam et al.,* (2019) reported that the intrinsic viscosity of PMS was in the range of 19.211–19.683 dL/g, which indicates the random coil structure of PMS gum in solution [8]. The production of PMG coating and edible film has also been confirmed in various studies [9, 10].

The literature review showed that no data exist concerning the ultrasound-assisted extraction of PMG. The aims of the present research were, therefore, first to optimize the ultrasound-assisted extraction conditions of PMG, which could maximize the yield of extracted gum, and then to investigate molecular conformation, chemical composition, rheological behavior, and surface activity of this polysaccharide to explore its potential application in food, pharmaceutical, cosmetic and textile industries.

## EXPERIMENTAL SECTION

### *Experimental design for gum extraction*

The cleaned PMG were wrapped in plastic bags, sealed, and kept in a dry and cool place. RSM was used to investigate the influence of independent process parameters including water to seed ratio (g/g), temperature (°C), time (min), and ultrasonic intensity (%) on extraction yield (%) of PMG (Table 1). The extraction experiments were done based on a central composite design with four factors and three levels. The experimental range of each factor was determined based on preliminary tests. Data analysis was carried out using Minitab software.

### *Analytical method*

Extraction yield (%) was quantified as the dry weight of the extracted gum relative to seed weight. The amount of carbohydrate was measured by the phenol sulfuric acid method at 490 nm [11]. The moisture, fat, ash, and protein contents of PMG samples were analyzed by AOAC

***Table 1: Independent process parameters and their levels.***

| Treatment/Level | -1 | 0 | +1 |
|---|---|---|---|
| Water to seed ratio (g/g) | 10 | 20 | 30 |
| Temperature (°C) | 40 | 55 | 70 |
| Power (%) | 60 | 75 | 90 |
| Time (min) | 10 | 25 | 40 |

*-1: Low values; +1: High values*

method number 930.15, 985.15, 942.05 and 981.10, respectively [12, 13].

***Monosaccharide composition***

The monosaccharide composition analysis of PMG was carried out as described by *Rhein-Knudsen et al.,* (2017) using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) [14]. 30 mg of freeze-dried sample was hydrolyzed with 72% $H_2SO_4$ for 1h at 30 °C. The resulting mixture was diluted and incubated at 120 ºC for 40 min. An HPAEC-PAD, ICS5000 system (Dionex Corp. Sunnyvale, CA) equipped with a CarboPac™ PA1 column was used to carry out the analysis. D-xylose, D-mannose, L-fucose, L-arabinose, L-rhamnose, D-galactose, D-glucose, D-galacturonic acid, and D-glucuronic acid were used as monosaccharide standards.

***Fourier Transform InfraRed (FT-IR) spectroscopy***

The freeze-dried sample was stored in a vacuum desiccator until the FT-IR analysis. 0.5 mg of PMG was mixed with 100 mg of KBr (spectroscopy grade; Merck), stored overnight in a desiccator over silica gel, then finely ground in an agate mortar and pressed into pellets. The FT-IR spectrum of PMG sample was recorded on FT-IR spectroscopy (AVATAR 370 FT-IR, Thermo Nicolet). The spectrum was obtained at the absorbance mode from 400 to 4000 $cm^{-1}$ at a resolution of 4 $cm^{-}1$ [15].

***Macromolecular characterization***

*Molecular weight measurement*

**Molecular weights by capillary viscosimetry ($M_v$)**

The dilute solution properties of PMG were analyzed using a Ubbelohde capillary viscometer (Cannon Instrument Co., Pa., U.S.A, k = 0.007690 $mm^2/s^2$), which is immersed in a precision water bath to reach the demanded temperature. In order to the preparation of the stock solution, the gum powder was dissolved in deionized water on a magnetic stirrer overnight at 24±2 ºC.

The relative ($\eta_{rel}$) and specific ($\eta_{sp}$) viscosities were determined and used to estimate the intrinsic viscosity of PMG:

Huggins equation [16]:

$$\frac{\eta_{sp}}{C} = [\eta] + k_1[\eta]^2 C \quad (1)$$

Kraemer equation [17]:

$$\frac{\ln \eta_{rel}}{C} = [\eta] + k_2[\eta]^2 C \quad (2)$$

Where $k_1$, $k_2$ and C are the Huggins constant, Kraemer constant, and the polymer concentration, respectively.

Tanglertpaibul & Rao model [18]:

$$\eta_{rel} = 1 + [\eta]C \quad (3)$$

Higiro's equations [19]:

$$\eta_{rel} = e^{[\eta]C} \quad (4)$$

$$\eta_{rel} = \frac{1}{1-[\eta]C} \quad (5)$$

In order to determination of the molecular weight by viscosity, the Mark Houwink Sakurada equation was used. This equation was modified by *Gaisford et al.,* (1986) [20] to take into account the various mannose to galactose ratios of galactomannan gums:

$$[\eta] = 11.55 \times 10^{-6}\left[(1-\alpha)\times M_v\right]^{0.98} \quad (6)$$

$$\alpha = \frac{1}{\left[\frac{M}{G}+1\right]} \quad (7)$$

where [$\eta$] is the intrinsic viscosity (dl/g) and M/G is the mannose/galactose ratio.

**Molecular weights by scattered light intensities**

The weight average molecular weight ($M_w$) of PMG was computed from scattered light intensities using a plot of the excess Rayleigh factor ($\bar{R}_\theta$) at scattering angle of 90° versus PMG concentrations (c). Molecular weight determined using following equations:

$$\frac{KC}{\bar{R}e} = \frac{1}{M_w} + 2A_2c \quad (8)$$

$$K = \left(\frac{2\pi^2}{\lambda_0^4 N_A}\right)\left(1+\cos^2\theta\right)n^2\left(\frac{dn}{dc}\right)^2 \quad (9)$$

$$\bar{R}_\theta = \bar{R}_{\theta,solution} - \bar{R}_{\theta,solvent} \quad (10)$$

in which $k$, $\lambda_0$, $N_A$, $n$, and $\frac{dn}{dc}$ are calibration constant, Avogadro's number ($6.022 \times 10^{23}\, mol^{-1}$), the refractive index of solvent and the specific refractive index increment, respectively [3].

*Estimation of the molecular conformation*

Equation (11) was used to determine *b* parameter from the slope of a double logarithmic plot of specific viscosity against concentration. This parameter can be used as a measure of polysaccharides conformation [19, 21]:

$$\eta_{sp} = aC^b \quad (11)$$

***Steady shear measurements***

PMG solutions with various concentrations (0.5, 0.75, and 1 %w/w) were prepared by dissolving gum powder in deionized water on a magnetic stirrer at 25 ºC overnight to be hydrated completely. Viscosimetric measurements were conducted using a rotational viscometer equipped with a heating circulator at 25 °C [3]. The steady shear behavior of PMG was characterized using the power law equation:

$$\tau = k\dot{\gamma}^n \quad (12)$$

here $\dot{\gamma}$ is the shear rate ($s^{-1}$), $\tau$ is the shear stress (Pa), $n$ is the flow behavior index (dimensionless) and $k$ is the consistency coefficient (Pa $s^n$).

***ζ-potential and particle size measurements***

The zeta potential (ζ) values of the PMG solution (0.1 %) were determined using a Zetasizer Nano ZS (ZEN 3600) instrument (Malvern Instruments Ltd., Malvern, Worcestershire, UK), at 25 ºC and pH = 7. All experiments were carried out on three freshly prepared solutions. The particle of PMG sample was determined using a laser diffraction particle size (Fritsch Particle sizer Analysette 22, Germany) at 25 ºC. The refractive index of the solvent and wavelength of the incident were 1.33 and 633 nm, respectively [3].

***Color measurement***

In order to determine color parameters of PMG samples such as L* (lightness), a* (redness), and b* (yellowness) a Chroma-meter (Konica Minolta, CR-410, Tokyo, Japan) was used [22]. 2.9. Bioactive agents (total flavonoids content (TFC), Total Phenolic Content (TPC), antioxidant activity)

The aluminum chloride method was employed for the determination of TFC of PMG. 1 mL of various concentrations of PMG was blended with 1 mL of methanolic aluminum chloride 2%. After incubation at room temperature in darkness for 15 min, the absorbance value was measured at 430 nm using a spectrophotometer (Sigma3-30k) [23, 24]. The method is a colorimetric assay was carried out for the determination of TPC [25-27].

Antioxidant properties were examined through the methods of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene/linoleic acid inhibition. For antioxidant activity measurement through the DPPH, 3.9 mL of the stock solution of DPPH (0.004 g of DPPH in 100 mL of methanol) was mixed with 0.1 mL of each extract and was kept in a dark place for 30 min. Then, its absorbance was read at 517 nm using a spectrophotometer [26, 28]. The radical scavenging percent of DPPH was calculated using the following equation:

$$\%Scavenging\ activity = \left[\left(Abs_{control} - Abs_{sample}\right)\Big/\left[Abs_{control}\right]\right]\times 100 \quad (13)$$

Potential antioxidant activity was assessed with the oxidation prevention by linoleic acid and inhibition of the development of conjugated hydroperoxides and volatile compounds. The β-carotene/linoleic acid assay was performed [26].

***Antimicrobial effect (disk diffusion method)***

The strains employed in this study included *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli*

ATCC 35218, *Bacillus subtilis* ATCC 6633, *Streptococcus pyogenes* ATCC 19615, and *Staphylococcus aureus* ATCC 25923. Different concentrations of PMG at 100, 200, 300, and 400 mg/mL were prepared. Then the blank discs were immersed in the extract solutions for 15 min. Finally, these discs were immobilized on the medium surface. After that, the Petri-dishes were incubated at 37 °C for 24 The antimicrobial effect was determined in terms of inhibition zone (IZ) diameter (mm) [29, 30].

***Statistical analyzes***

RSM was used to investigate the influence of independent process parameters including water to seed ratio, temperature, time, and ultrasonic intensity on the extraction yield of PMG. Minitab® version 17.1.0 (Minitab Inc. USA) was also employed. To investigate the relation between the independent and dependent variables, the data were subjected to stepwise regression analysis using the Method of Least Squares (MLS). In order to do so, the full quadratic model was fitted to the experimental data. The model is as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i \neq j=1}^{k} \beta_{ij} X_i X_j \quad (14)$$

Where Y denotes the response, $\beta_0$ is the model intercept, $\beta_i$, $\beta_{ii}$, $\beta_{ij}$ represent the regression coefficients for the linear, quadratic, and interactive effects, respectively. $X_i$ and $X_j$ stand for the independent variables and k is the number of them. One-way analysis of variance (ANOVA) for steady shear results was done using a statistical package for the social sciences (SPSS) software (Version 16.0) and the difference at 95% confidence interval was analyzed using Duncan's multiple range test.

## RESULTS AND DISCUSSION

***Modeling of gum extraction and statistical analysis***

A central composite design was used to evaluate the combined effects of independent variables of water to seed ration (g/g), temperature (°C), ultrasonic power (%), and time (min) on yield extraction (%) of PMG. The experimental data and process variables are given in Table 2.

A summary of the analysis of variance, the adequacy, and the goodness of fit of the models are presented in Table 3. To evaluate the statistical significance of the equation, F-test was used. Additionally, a p-value was used to evaluate the significance of each coefficient. The lower value of f-values indicates more significance of the corresponding coefficient, which demonstrates the model is appropriate for use. F-value (1.5) and p-value (0.474) of the lack of fit, the parameter showed that the parameter is insignificant. Yield= 9.488 + 0.269 Temperature + 0.413 Power + 0.961 Time + 0.392 Time×Time – 0.310 Water to seed ratio × Time + 0.262 Temperature×Time

As shown in Table 3, the value of pred R-Squared (0.7096) is in good agreement with that of Adj-R-squared (0.7896). High values of both pred R-Squared and Adj-R-squared demonstrate that the general availability and accuracy of the model used are adequate. The linear effect of extraction temperature, time, and ultrasonic power was significant ($p < 0.05$). The results revealed that the extraction time followed by the ultrasonic power showed the most significant effect on the yield of extraction.

***Interaction between extraction process variables***

The influence of ultrasonic-assisted gum extraction conditions on the yield of PMG is depicted in Fig. 1. It can be observed that the extraction yield changed considerably with extraction time and temperature, and ultrasonic power. As shown in Fig. 1-A, at a constant extraction temperature, the yield had a considerable increase with increasing time of extraction, while that showed a slight increase with increasing ultrasonic power. The effect of ultrasonic power on yield extraction of the gum is the breakdown of seed cells and releasing of the cells' contents into extraction solvent.

Fig. 1-B shows that extraction yield increased with increasing extraction time. The increasing effect of time on extraction yield may be attributed to more exposure of the gum to the release medium and subsequently higher diffusion of the gum from the seeds [31]. In Fig. 1-C, higher yield extraction was observed at more temperature and ultrasonic power, which is due to the reinforcement of the ability of the solvent to solubilize the gum and decrease of the solvent viscosity, leading to better penetration of the solvent into the polymer matrix [5, 32].

The results also demonstrated that at constant ultrasonic power, the yield of extraction had an increasing trend with increasing the water:seed ratio (Fig. 1-D). The reason for that may be attributed to the availability of more liquid which results in reinforcement of the driving force of mucilage out of the seeds [33]. Similarly, *Sepúlveda et al.,* (2007) [34], *Samavati* (2013) [32] and *Bostan et al.,* (2010) [35] indicated that with increasing

***Table 2: Matric of central composite design of independent variables for ultrasonic-assisted extraction of PMG.***

| Water to seed ratio (g/g) | Temperature (°C) | Power (%) | Time (min) | Yield (%) |
|---|---|---|---|---|
| 10 | 40 | 90 | 40 | 10.78 |
| 20 | 55 | 90 | 25 | 10.42 |
| 30 | 40 | 60 | 40 | 9.4 |
| 20 | 55 | 75 | 40 | 10.58 |
| 10 | 40 | 60 | 40 | 10.4 |
| 10 | 70 | 90 | 40 | 13.1 |
| 30 | 70 | 60 | 40 | 10.7 |
| 30 | 55 | 75 | 25 | 10.1 |
| 20 | 55 | 75 | 25 | 9.8 |
| 30 | 70 | 60 | 10 | 8.75 |
| 10 | 40 | 60 | 10 | 8.4 |
| 30 | 40 | 60 | 10 | 8.9 |
| 10 | 70 | 60 | 40 | 10.8 |
| 30 | 40 | 90 | 10 | 9.2 |
| 20 | 55 | 75 | 25 | 9.5 |
| 20 | 70 | 75 | 25 | 9 |
| 30 | 40 | 90 | 40 | 10.6 |
| 20 | 40 | 75 | 25 | 9.2 |
| 10 | 70 | 90 | 10 | 9 |
| 30 | 70 | 90 | 10 | 9.25 |
| 10 | 55 | 75 | 25 | 9.52 |
| 20 | 55 | 60 | 25 | 8.85 |
| 20 | 55 | 75 | 25 | 9 |
| 10 | 70 | 60 | 10 | 8.42 |
| 30 | 70 | 90 | 40 | 11.2 |
| 20 | 55 | 75 | 10 | 9.85 |
| 10 | 40 | 90 | 10 | 8.5 |

***Table 3: Analysis variance of the results of CMD.***

| Variables | | DF | F-value | P-value | Sum of squares | Mean of squares |
|---|---|---|---|---|---|---|
| Model | | 6 | 17.26 | 0.000 | 24.5369 | 4.0895 |
| Linear | | 3 | 29.51 | 0.000 | 20.9764 | 6.9921 |
| | Temperature (°C) | 1 | 5.49 | 0.030 | 1.3014 | 1.3014 |
| | Power (%) | 1 | 12.94 | 0.002 | 3.0669 | 3.0669 |
| | Time (min) | 1 | 70.10 | 0.000 | 16.6080 | 16.6080 |
| Square | | 1 | 3.88 | 0.063 | 0.9204 | 0.9204 |
| | Time (min)*Time (min) | 1 | 3.88 | 0.063 | 0.9204 | 0.9204 |
| 2-way interaction | | 2 | 5.57 | 0.012 | 2.6401 | 1.3200 |
| | Water to seed ratio(g/g)*Time (min) | 1 | 4.49 | 0.019 | 1.5376 | 1.5376 |
| | Temperature (°C)*Time (min) | 1 | 4.69 | 0.043 | 1.1025 | 1.1025 |
| Lack of fit | | 18 | 1.50 | 0.474 | 4.4119 | 0.2451 |
| Pure error | | 2 | | | 0.3267 | 0.1633 |
| Total | 26 | 29.2755 | | | | |

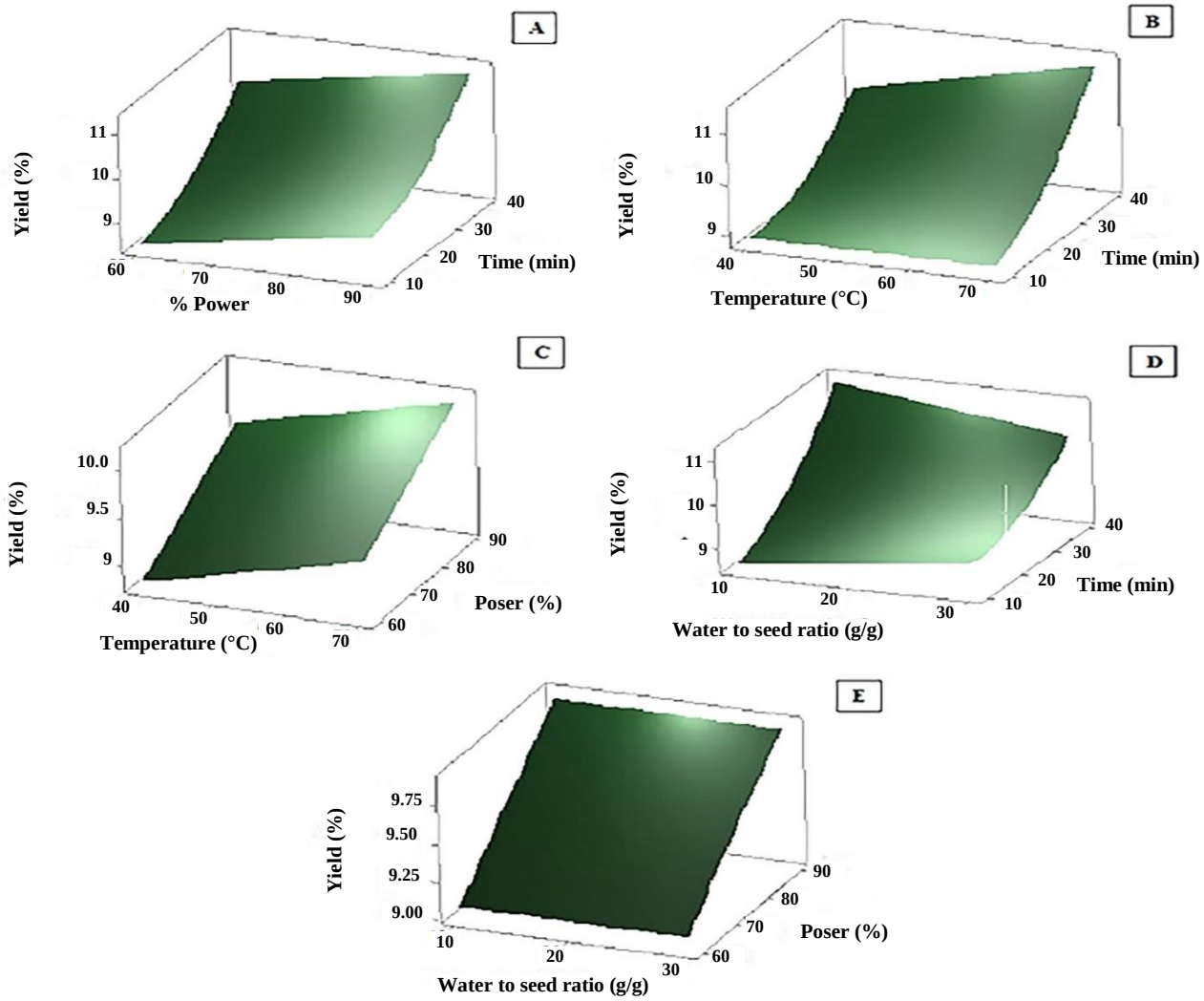


***Fig. 1: The surface plots of extraction yield depicting the effect of extraction temperature, extraction time, and ultrasonic power. Where (A) is extraction time and ultrasonic power; (B) is extraction time and temperature; (C) is temperature and ultrasonic power; (D) extraction time and water to seed ratio; and (E) water to seed ratio and ultrasonic power.***

water:seed ratio, the extraction yield of Opuntia spp. seed, *Abelmoschus esculentus*, and sage seed gum increased.

Based on numerical and graphical optimization procedures, the optimum condition to obtain maximum extraction yield (13.1 %) was extraction temperature 70 ° C, extraction time 40 min, and water to seed ratio of 10:1 (g/g), ultrasonic power of 90 %. To evaluate the adequacy of the model, the gum was extracted in the optimum condition determined by the RSM optimization. The comparison between the predicted and experimental values yield was found to be 11.58 and 11.36, respectively, exhibiting that the experimental results were very near to the predicted values. Therefore, the experimental data were in good consistent with the predicted values. Comparatively, the extraction yield value of PMG is close to that of sage seed gum (12.2%) [35] and lower than that reported for *Opuntia* mucilage (19.4%) [34].

### *Chemical composition*

The chemical composition of PMG is tabulated in Table 4. PMG had 89.24% carbohydrate, 4.53% ash, 4.11% moisture, and 2.12% protein. Hence, the majority of this gum is carbohydrate. Carbohydrate content of the gums is commonly used to evaluate their purity.

***Table 4: Chemical composition and monosaccharide composition PMG.***

| Composition (%) | | PMG |
|---|---|---|
| Carbohydrate | | 89.24±0.50 |
| Protein | | 2.12±0.52 |
| Ash | | 4.53±0.50 |
| Moisture | | 4.11±0.54 |
| Fat | | Trace |
| Monosaccharides | | |
| | Arabinose | 5.32±1.06 |
| | Galactose | 21.59±1.13 |
| | Xylose | 3.22±0.63 |
| | Mannose | 59.59±3.83 |
| | Rhamnose | 0.96±0.19 |
| | Glucose | 2.12±0.17 |
| | Galacturonic acid | 1.75±0.12 |
| | Glucuronic acid | 5.45±1.02 |

The carbohydrate content of PMG was higher than those of commercial gums like guar gum (71.1%) [36] and gum ghatti (78.365) [37] and comparable to the data reported for locust bean gum (85.1-88.7%) [38], which confirmed the purity of this gum. Based on the large extraction yield of PMG, it can be introduced as a viable substitution for some commercial gums. The carbohydrate content of PMG extracted by ultrasound-assisted extraction (89.24 %) is considerably higher than that reported for classical extraction (at a temperature of 75 ºC, water: seed ratio 1:60, and pH 6.8) [3]. The reason for the higher carbohydrate content of the gum obtained from ultrasound-assisted extraction is attributed to the mechanical action of ultrasound on cell walls leading to an increment of extractability of the polysaccharides [39].

The monosaccharide composition of PMG measured by HPAEC-PAD is presented in Table 4. The results demonstrated the presence of mannose (59.59%), galactose (21.59%), arabinose (5.32%), xylose (3.22%), glucose (2.12%), rhamnose (0.96%), galacturonic acid (1.75%), and glucuronic acid (5.45%). Trace amounts of xylose, glucose and rhamnose refer to a more complex polysaccharide structure. Galactose and mannose make up about 81% of total carbohydrate content. Thus, it can be suggested that PMG can be considered to belong to the galactomannan polysaccharide. Since seed galactomannans are non-toxic, non-expensive, and eco-friendly, they have preferred gums [40]. Commercial functionalities of galactomannans are mainly dependent on the mannose/galactose ratio [41]. Comparatively, the mannose/galactose ratio of PMG (2.76) is more than the values reported for guar gum (1.62) [36] and fenugreek (1.1) and lower than that of locust bean gum (3.5-4) [38]. The gums with a higher mannose/galactose ratio indicate more solubility [43], and thus PMG can be introduced as a good stabilizing and viscosity-enhancing agent.

Based on most pharmacopeias, the moisture content of natural gums should be at about 15.0% due to its deterioration effect on the quality and shelf-life of the gums [44]. The moisture content of PMG (4.11%) is well within the limit. The moisture content obtained here is comparable to that reported for the gum extracted by the conventional method (3.69%) [3]. A similar observation has been reported by *Farahanky et al.,* (2013) who indicated that the ultrasonic process had no significant effect on of moisture content of *salvia macrosiphon* seed gum [45].

The protein content of PMG (2.12 %) was equal to those cited for xanthan gum (2.125%) [46], and lower than those reported for guar gum (8.19%) [36] and locust bean gum (5.2-7.4%) [38]. Proteins contain both hydrophobic and hydrophilic side chains which impart their surface activity. It is therefore expected that this gum has the ability of surface tension reduction. Ash content of PMG was 4.53 % which is higher than the data reported for locust bean gum (0.7-1.5 %) [38], lower than that of guar gum (11.9 %) [36]. Comparatively, the protein and ash contents of PMG were lower than those reported for the gum extracted by the conventional method [3]. This difference is because of the high shear forces used to the hydrated seeds crushing and breaking their hard cores. But, in ultrasound-assisted extraction, the gum layers are isolated layer by layer, and after removing all mucilage layers, the hard seed cores are exposed to waves to be isolated [45].

***FT-IR analysis***

FT-IR was used to investigate the changes in the structure of PMG due to sonication. The FT-IR spectrum of PMG is depicted in Fig. 2. The FT-IR spectrum of this gum illustrates all typical peaks and bonds of polysaccharides. The diagnostics peaks below 700 $cm^{-1}$ are

related to the skeleton vibration of hydrocolloids with two modes: angle deformation arising from heavy atoms (*COC-CCO*) and internal rotations around *C-O* [47]. The wavenumbers between 800 to 1200 $cm^{-1}$ which are known as "fingerprint" areas for carbohydrates, can be used to elucidate the structural differences in different hydrocolloids [48]. Furthermore, this region is commonly used to identify the major functional groups in polysaccharide structure [49]. The peaks observed at 848 and 898 $cm^{-1}$ indicate the presence of α and β linkages in the PMG structure [50]. The absorption at 898 $cm^{-1}$ is attributed to β-D-mannopyranose unites [51].

The wavenumber between 950 to 1200 $cm^{-1}$ corresponds to the stretching vibration of alcoholic *C-O* in *C-O-H* bands in polysaccharides structure [52]. The signal at 1647 $cm^{-1}$ is due to dissociated carboxylate groups present in the structure of uronic acid, whereas the main one at 1074 $cm^{-1}$ is caused by o-acetyl groups [50]. As mentioned before, PMG has, on average, 5.45% glucuronic acid and 1.75% galacturonic acid. Therefore, it can be found that polyelectrolyte nature of this gum mainly originates from carboxyl functional groups of these two acids.

In the wavenumbers between 1200 to 1500 $cm^{-1}$, appear peaks are related to the coupling of the deformation vibrations of *HCH*, *CCH*, *HOC*, and *COH* [53]. The peak observed at 1647 is assigned to *C-OO* groups and valence vibration.

The absorption at 2929 $cm^{-1}$ is attributed to *C-H* stretching, symmetric and asymmetric of the free sugar [48]. Additionally, *Kacurakova et al.,* (2000) reported that this peak may arise from doubles overlapping with a hydroxyl group [54].

The broad absorbance peak at 3000 to 3500 $cm^{-1}$ is arisen from hydroxyl stretching absorption because of intra and inters molecular hydrogen bonds [55], and also may be caused by hydrogen bonding involving the hydroxyl groups in the structure of glucopyranose ring [56]. Generally, the comparison of FT-IR spectrum obtained here with that presented in the previous study *Alizadeh Behbahani et al,* (2017) [3] indicated that ultrasound did not change the primary structure of PMG. However, the absorption of around 1745 $cm^{-1}$ only was detected in the gum extracted by ultrasound-assisted method, which may be due to increased GalA content during sonication [57].

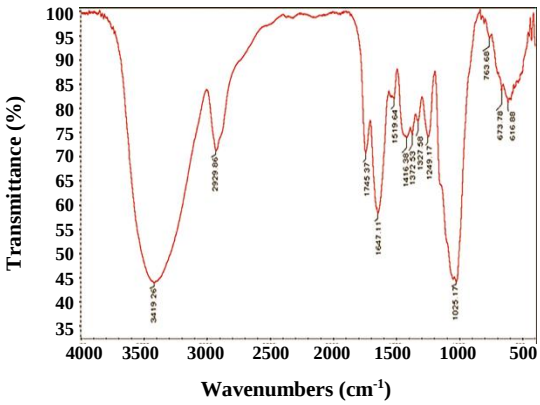


***Fig. 2: FT-IR spectrum of PMG.***

### *Dilute solution properties*

*Intrinsic viscosity*

The intrinsic viscosity values of PMG estimated by different models are given in Table 5. It is evident that Tanglertpaibul-Rao's equation has more determination coefficient ($R^2$) and less Root Means Square Error (RMSE), indicating the appropriateness of this model to describe dilute solution properties of PMG. PMG has an intrinsic viscosity of 12.56 dL/g in deionized water at 25 ºC. This value was lower than those reported for some galactomannans like Fenugreek (15.10 dL/g), Guar (15.80 dL/g), Tara (14.55 dL/g), and Locust bean gum (14.20 dL/g) [58].

An increase in solution temperature was accompanied by a decrease in the intrinsic viscosity of PMG, revealing that the coil dimension of PMG molecules decreased. Furthermore, this trend may arise from a decrease in hydrogen bond stability between polymer and solvent molecules [59]. This falling trend also could be due to a reduction of the radius of gyration and as a resulting increment of flexibility and compactness of the molecules, causing a decrease in the intrinsic viscosity. As evident in Table 5, the intrinsic viscosity of PMG has high-temperature sensitivity, so each 20 °C temperature elevation starting from 25 °C, causes a reduction in intrinsic viscosity of about 16% and 18.40%, respectively.

*Molecular conformation*

log $\eta_{sp}$ against log concentration × intrinsic viscosity, known as a master curve, was plotted to elucidate molecular entanglement in PMG solution. A slope of about 1.4 shows that no molecular entanglements and coil overlapping

***Table 5: Intrinsic viscosity values determined by five models for PMG at selected temperatures.***

| Temperature (°C) | Huggins | | | Kraemer | | | Tanglertpaibul and Rao | | | Higiro 1 | | | Higiro 2 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [η] | $R^2$ | RMSE | [η] | $R^2$ | RMSE | [η] | $R^2$ | RMSE | [η] | $R^2$ | RMSE | [η] | $R^2$ | RMSE |
| 25 | 11.52±1.01 | 0.49 | 0.25 | 10.25±0.20 | 0.91 | 0.18 | 12.56±0.50 | 0.99 | 0.00 | 7.98±0.45 | 0.94 | 0.01 | 4.81±0.35 | 0.97 | 0.03 |
| 45 | 7.22±0.15 | 0.71 | 0.30 | 8.01±0.56 | 0.52 | 0.18 | 10.55±0.35 | 0.99 | 0.01 | 9.41±1.10 | 0.96 | 0.01 | 9.17±1.20 | 0.98 | 0.01 |
| 65 | 11.04±0.49 | 0.89 | 0.07 | 10.78±0.75 | 0.95 | 0.08 | 8.60±0.10 | 0.99 | 0.00 | 3.04±0.50 | 0.98 | 0.01 | 3.01±0.50 | 0.98 | 0.00 |

happen while, in a concentrated regime, the slope increase to 3.3 [60]. As can be seen in Table 3, the slopes of master curves at tested temperatures are in the range of 0.71 to 1.13, indicating PMG solutions were in a dilute regime without coil overlapping.

Additionally, the values of Berry number (C[η]) for PMG 0.20-0.89, exhibiting again that no coil-overlapping happened.

The slope of a power-law model was used to elucidate the molecular conformation of PMG. A slope of more than one revealed that the molecular conformation of polysaccharide is a random coil [61], while when this parameter is less than one, demonstrates a rod-like conformation. The magnitude of this parameter at 25 and 45 ºC were 1.13 and 1.01, respectively, indicating the molecular conformation of PMG is rod-like. However, the value of this parameter at 65 ºC was 0.71. Therefore, it can be concluded that temperature can result in alteration of the PMG conformation

*Molecular weight*

The macromolecular weight of hydrocolloids is considered an important parameter due to its effect on functional characteristics [62]. Therefore, the molecular properties of PMG were determined. The viscometric molecular weight ($M_V$) of PMG was found to be $1.13\times10^6$ g/mol. This value is comparable to those of other galactomannan gums like guar gum ($2.07\times10^6$ g/mol) [36]

***Table 6: Berry number and b value of PMG at various temperatures.***

| Temperature (°C) | C[η] | *b* |
|---|---|---|
| 25 | 0.31-0.89 | 1.13±0.01 |
| 45 | 0.20-0.85 | 1.01±0.01 |
| 65 | 0.21-0.79 | 0.71±0.01 |

and Descurainia Sophia seed gum ($2.1\times10^6$ g/mol) [48]. The average molecular weight of this gum measured by scattered light intensities was $9.9\times10^5$ g/mol. Comparatively, this value is near to those reported for other galactomannans like locust bean gum ($1.64\times10^6$ g/mol) [63] and guar gum ($1.45\times10^6$ g/mol) [64]. The molecular weight of hydrocolloids has a considerable effect on their viscosifying and gelling properties. For example, due to the high molecular weight of xanthan gum, it imparts high viscosity even at low concentrations [65]. Due to the high molecular weight of PMG, it is expected that this gum has a high gelling and viscosity-enhancing ability. Additionally, *Martinez et al.*, (2005) [66] indicated that high molecular weight polysaccharides do not have much tendency to be adsorbed at the water-air interface, but they can lead to a considerable improvement in the stability of protein foams, and thus PMG can be used to stabilize the protein foams.

The molecular weight of PMG was slightly lower than that reported by *Alizadeh Behbahani et al.*, (2017) [3] which is attributed to the backbone breaking of long

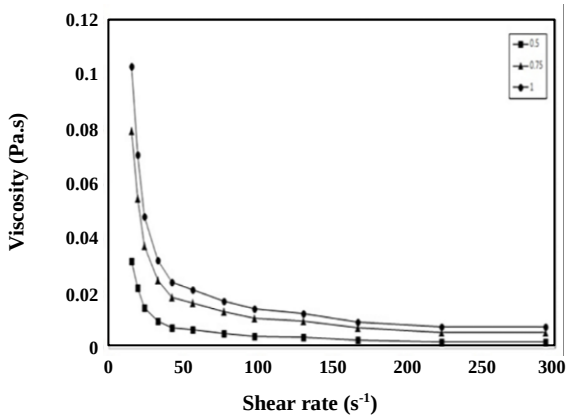


***Fig. 3: Viscosity-shear rate profile of PMG as a function of gum concentration.***

chains in the polymer after ultrasonic treatment [67]. From a nutritional point of view, the reduction in molecular weight can be an advantage in the case of indigestible fibers [68].

***Steady shear properties***

Fig. 3 illustrates the effect of different concentrations of PMG solution at 25 °C in the range of shear rates from 1 to 300 $s^{-1}$. A non-Newtonian shear-thinning behavior was observed for the PMG solutions with different concentrations. With increasing PMG solution, the viscosity of the gum solution decreased, which is typical behavior of biopolymers because of their polymeric structure and high molecular weight. The decreasing viscosity observed results from the rearrangement of macromolecular chains in the direction of flow with the input force and consequently less interaction between adjacent polymer chains [69]. The reduction of apparent viscosity at a high shear rate may be associated with a decrease in the number of chain entanglements [70]. From an industrial point of view, the reduction of apparent viscosity with shear rate can be regarded as an advantage in unit operations including pumping [49]. As shown in Fig. 3, an increase in gum concentration led to an improvement of apparent viscosity. The observed effect is attributed to the more solid contents at a higher concentration which results in reinforcement of the apparent viscosity, mainly because of higher molecular entanglements and interfacial film formation [71].

The viscosity of ultrasound-assisted PMG was lower than obtained from than conventional method *Alizadeh Behbahani et al.*, (2017) [3] which was related to the reduction of the average molecular weight of the gum after ultrasonic treatment. Additionally, ultrasonic treatment typically has a negative effect on the gelling ability of the gums due to reducing their average molecular weight [68].

***Particle size distribution and zeta potential***

Zeta potential can be used as a useful parameter for investigating the extent and kind of electrostatic interactions where charged macromolecules are present at the same time. Zeta potential of PMG with a concentration of 0.1 % (at pH = 7) was 17.56 mV, exhibiting this gum is an anionic biopolymer. The anionic nature of PMG is due to the presence of galacturonic acid (1.75%), and glucuronic acid (5.45%) which was confirmed by the monosaccharide analysis described above. Uronic acids contain acidic functional groups carbonyl and carboxylic acid) that are ionizable at pH above acid dissociation constant. Due to the anionic nature of PMG, it can be introduced as an appropriate polymer for encapsulation of phytochemicals using the coacervation technique. Comparatively, the zeta potential of PMG was lower than those of basil seed gum (-63.2 mV) [72], okra extract at pH=3, and *perfoliatum* seed gum (-43.7 mV) [73] and close to that reported for peach gum at pH=7 (-20 mV) [74].

The measurement of particle size is important due to its effect on viscofying ability of the gums [75]. The value of particle size in 0.1% PMG solution was, on average, 400.32 nm, which is more than those reported for food grade guar gum (54-500 μm) and near to that of (225.36 nm) [51], indicating the PMG solution may be more stable than food grade guar gum.

***Surface tension***

The surface tension of PMG solutions at various concentrations is presented in Fig. 4. The decrease in surface tension is concentration-dependent: with increasing gum concentration from 0.01 to 0.5%, the surface tension of the samples decreased from 80.05 to 79.33 mN/m, but with further increasing gum concentration, the surface tension of the solution increased. This trend is consistent with those observed for most gums. *Chaires-Martínez et al.*, (2008) [76] indicated that the surface tension of locust bean gum decreased with increasing the concentration from 0.1 to 0.3%, but it increased at higher gum concentrations. *Razavi et al.*, (2014) [77] also reported that an increase in sage seed gum concentration from 0.01 to 0.25% led to a decrease in surface tension, but it increased

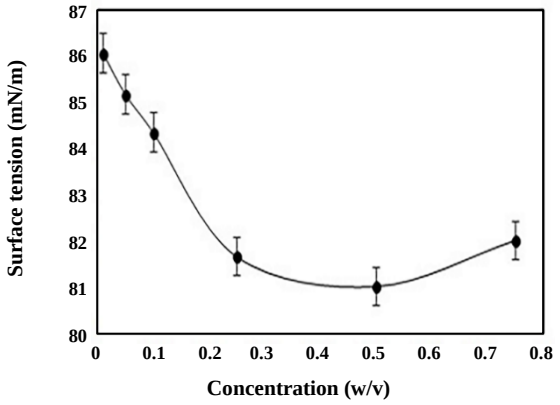


***Fig. 4: The surface activity of PMG with different concentrations.***

at higher concentration. The increment of surface tension at high concentrations could be attributed to the excessive development of viscosity, which made surface tension determination difficult [78]. The surface activity of the gums could arise from the presence of hydrophobic functional groups (acetyl and methoxy groups), proteins, and fractions with small molecular weight which were confirmed by FT-IR and compositional analyses described above [78]. Comparatively, the surface activity of PMG was close to those reported for locust bean gum, fenugreek gum, xanthan, and guar gum, but was considerably lower than those of gum ghatti [37] and Arabic gum [79] which is due to the low amount of protein in PMG composition. The gum dispersions with lower surface activity have a better wetting ability and therefore, result in the formation of granules with higher quality [80]. Generally, it can be proposed that PMG gum can be used in oil/water emulsions due to its ability to reduce surface tension.

***Color measurement***

The values of L*, a*, and b* in powder PMG samples were measured to demonstrate the color properties of PMG. PMG powders, lightness, redness, and yellowness values were found to be 29.07 ± 0.22, 5.69 ± 0.09, and 8.79 ± 0.12, respectively.

***Bioactive agents***

TPC, TFC and AA ($IC_{50}$) tests of PMG showed 89.80 ± 1.23 mg GAE $g^{-1}$dry sample, 123.25 ± 1.32 mg/g dry sample, and 470.45 ± 0.35 µg/mL, respectively. Prevention of linoleic acid oxidation in the system of ß-Carotene-linoleic acid was equal to 32.45 %. This value was somewhat lower than that of the synthetic antioxidant of Butylated hydroxytoluene. The antioxidant activity of the PMG was directly related to its phenolic and flavonoid compounds. Comparatively, the TPC, TFC, and antioxidant activity contents of PMG were higher than those reported for the gum extracted by the conventional method [3]. In general, the number of hydroxyl groups in the antioxidants' structure is not mainly responsible for their functionalities. The position of hydroxyl groups, the presence of other functional groups, such as double bonds, and the combination of hydroxyl and ketone groups, will play an important role in the antioxidant activity. The reason for the difference in the results of the antioxidant activity and phenolic compounds of PMG in several studies can be related to the climatic conditions, the method of drying, and different extraction techniques [3, 10]

Many studies have shown that large TPC is the main reason for the high antioxidant activity of some gums and extracts because there is a positive correlation between TPC and the antioxidant activity of plants [10, 81].

***Antimicrobial effect***

The antimicrobial effect of PMG measured by the disk diffusion method is presented in Table 7. The results showed that the Gram-positive *Streptococcus pyogenes* was the most sensitive strain to PMG. The highest resistance to PMG was observed for Gram-negative *Pseudomonas aeruginosa*. An increase in gum concentration was accompanied by an increase in mean inhibition zone diameter increased. Phenolic compounds can make a destructive role on the bacterial cell membrane by interacting with the membrane proteins through their hydroxyl groups. *Alizadeh Behbahani et al.,* (2017) [81] reported that Barhang seed mucilage screening antimicrobial activity in 2 mg/mL, inhibits *Streptococcus pyogenes* growth. However, 2 mg/mL concentration of Barhang seed mucilage, had no significant antimicrobial effect on *Bacillus cereus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Escherichia coli* and it is not able to prevent the growth of bacteria on culture. *Alizadeh Behbahani* and *Imani Fooladi*, (2018) [30] investigated the antibacterial activity of *Lallemantia royleana* seed mucilage on *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, and *Candida albicans* "*in vitro*".

***Table 7: Mean inhibition zone diameter (mm) of PMG concentrations for some pathogenic bacteria.***

| Microorganisms | 100 | 200 | 300 | 400 |
|---|---|---|---|---|
| Bacillus subtilis | $7.50 \pm 0.50^{a}$ | $9.00 \pm 0.28^{b}$ | $10.70 \pm 0.50^{c}$ | $12.10 \pm 0.54^{d}$ |
| Streptococcus pyogenes | $8.00 \pm 0.50^{a}$ | $9.60 \pm 0.50^{b}$ | $11.40 \pm 0.54^{c}$ | $13.20 \pm 0.28^{d}$ |
| Staphylococcus aureus | $7.00 \pm 0.54^{a}$ | $8.30 \pm 0.54^{a}$ | $9.90 \pm 0.28^{b}$ | $10.70 \pm 0.50^{b}$ |
| Escherichia coli | $7.00 \pm 0.50^{a}$ | $8.00 \pm 0.54^{a}$ | $9.50 \pm 0.28^{b}$ | $10.10 \pm 0.52^{b}$ |
| Pseudomonas aeruginosa | - | $7.00 \pm 0.54^{a}$ | $8.80 \pm 0.54^{b}$ | $9.50 \pm 0.50^{b}$ |

*Values are expressed as mean ±standard deviations, n = 3; different letters (a, b, c and d) in each row show significant differences at $p \leq 0.05$.*

They reported that 2 mg/mL *Lallemantia royleana* seed mucilage, inhibited *B. subtilis* and *S. pyogenes* from growing. However, 2 mg/mL *Lallemantia royleana* seed mucilage, had no significant effect on *B. cereus*, *E. coli*, and *P. aeruginosa*. It is not able to prevent the growth of these bacteria in culture.

## CONCLUSIONS

We have investigated the ultrasonic-assisted extraction of PMG. PMG had 89.24% carbohydrate, 4.53% ash, 4.11% moisture, and 2.12% protein. The following findings can be drawn from the results observed in this research: The optimum condition to obtain maximum extraction yield (13.1 %) was the extraction temperature of 70 ° C, extraction time of 40 min, water to seed ratio of 10:1, and ultrasonic power of 90 %. The physicochemical properties observed for PMG were different from those previously reported. Various factors such as extraction and purification techniques, age of the plant, and growing conditions can change the compositional properties of the gums. Hence, the observed differences may be arising from different extraction techniques. Ultrasonic was able to reduce the molecular weight of PMG and increase its purity. Viscometric molecular weight and average molecular weight were found to be 1.13 $\times 10^5$ g/mol and 9.9 $\times 10^5$ g/mol, respectively. The intrinsic viscosity of PMG was 12.56 dL/g in deionized water at 25 ºC.

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