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Original article

Optimization of aqueous-assisted extraction of polysaccharides from pumpkin (*Cucurbita moschata* Duch) and their biological activities

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ABSTRACT

The pumpkin pulp contains a greater composition of edible polysaccharides and has reported with excellent biological applications. This research pertains to optimize the extraction of polysaccharides from the fleshy portion of the pumpkin using aqueous assisted extraction (AAE). The result showed that the optimal extraction condition of pumpkin polysaccharide was as follows: extraction temperature at 55 °C, pH 4.5, and enzyme concentration of 4000 μ/g for 80 min. Under the optimal extraction condition, the yield of pumpkin polysaccharide via AAE (15.4) was significantly higher. The biological activities of extracted polysaccharide including α-amylase inhibition (57.41% at 1000 μg/mL) and anti-inflammatory (50.41% at 25 μg/mL) activity increased significantly. Additionally, the antioxidant activities of extracted pumpkin polysaccharides including IC_{50} values of DPPH and ABTS were 59.87% and 58.74%, respectively. The pumpkin polysaccharide has maximum inhibitory effects against bacterial strains especially for *Escherichia coli* than that of fungal strains. It is suggested that the aqueous assisted extraction of is a cost-effective promising method to decrease the processing time as well as enhancing extracted polysaccharide yield – times.

1. Introduction

Polysaccharides, an important biomolecule found in the living organism and the most abundant form of carbohydrates in plants. The plant-based polysaccharides have been documented for their various bioactive approaches comprising antimicrobial, antidiabetic, anti-inflammatory, antioxidant activity, anti-cancer and so on. The polysaccharides can counteract free radicals produced in the living organism and shield the tissue, thus helping to prevent various ailments triggered by cell injury. Health-related benefits and therapeutically use of such bio-polysaccharides have attracted keen interest among the biologist. Polysaccharides are capable like protein to interact with a living organism and influence on alteration in their biological activities. The composition, structure, molecular weight and conformation of pumpkin polysaccharide make it difficult to characterize; hence their role as medicine is yet under research. The potential interactions of polysaccharides greatly vary due to its conformation. This polysaccharide active ingredient modulates the immune system by stimulating macrophages (Schepetkin and Quinn, 2006), reduce inflammation (Wu et al., 2010) and anti-tumour effect (Wasser, 2002). Many studies emphasized on the hydrophilic polysaccharides which charged negatively such as with OH groups and oxygen atoms and they can be free radical scavengers and metal chelators, prominent to lipid peroxidation inhibition. Therefore, plant-derived polysaccharides can be a good source of valuable medicine.

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Cucurbita moschata Duch (Cucurbitaceae) commonly known as pumpkin. Pumpkin fruit is considered to be one of the main vegetables, especially in the classical farming sector across the whole world (Maran et al., 2013). The plants of this family have a rich source of chemicals containing, tetracyclic triterpenes, saponins, proteins, fibres, polysaccharides and minerals (Abuelgassim and Showayman, 2012; Fu et al., 2006). The 100 g of pumpkin powder comprises about 4 g of fat, 21 g of carbohydrates and around 300 mg of calcium (Zhang and Guo, 2011). Among the nutritional composition, polysaccharides fascinated more consideration. The saccharides include xylose, arabinose, glucose, galactose and gluconic acids are the major components of pumpkin polysaccharides (PPs). These are macromolecular compounds characterized by water-insoluble, soluble in organic solvents and possess significant biological applications (Song et al., 2012; Yang et al., 2007). PPs normally possess unsaturated oil, with a predominant presence of oleic and linoleic acid, provide high oxidative consistency of pumpkin seed oil for perseverance or industrial application and lower free radical assembly in healthy diets (Caili et al., 2006). PPs has been concerned in providing several health applications such as hypertension, mitigate hypercholesterolemia and arthritis. Compliance linked to the reduced bladder by PPs also been reported to diabetes by endorsing hypoglycemic commotion (Li et al., 2010). The unripened melon is edible as a better choice of a source as a vegetable. The ripened fruit has a sweet taste, hence it used to make sweetmeat, drinks or can be integrated into baked goods. The sugars composition present in pumpkin pulp is most important for diet nutrition, particularly for the diet of diabetics' patient.

Extraction methodologies are essential to synthesis bioactive compounds. The utmost active compounds and low unanticipated constituents were intended for extraction. Several aspects such as kind of extraction method, time, temperature, pH and the extracted material are the key features. The low extraction temperature is frequently considerable because the bioactive compound can be degraded or lost during extraction. Common methods used for the extraction of polysaccharide from plant sources encompass maceration, mechanical pulverizing, heat reflux, ultrasound and acidic hydrolysis. All of these approaches of abstraction is time-consuming as well as require relatively high temperatures, extensive machinery, or cause environmental contamination (Chen et al., 2012; Ptichkina et al., 2008). In contrast, aqueous mediated technology appears to be eco-friendly and more efficient in terms of polysaccharide production. However, extraction of the PPs through aqueous assisted methods was also not often reported. Though some studies revealed enhancement of the extract's properties including biological activity using PPs, there is little information focusing on the AEE of crude polysaccharide, especially from the pumpkin. Therefore, this study aimed to investigate and optimize the AEE of polysaccharide from pumpkin using four independent variables including extraction temperature, extraction time, pH and enzyme concentration through a response surface methodology (RSM). The aim was to optimize extraction condition while obtaining high biological activities of obtained polysaccharides.

2. Materials and methods

2.1. Materials

The tropical pumpkin *(Cucurbita moschata)* was bought from indigenous vegetable market Uthangarai, Krishnagiri, Tamil Nadu, India. The peels were removed by scrubbing from the pulpy portion and desiccated in a laboratory air drier at 40 °C for 7 days and pulverized. The sample preserved in desiccators at laboratory condition until further use.

2.2. Aqueous assisted extraction of polysaccharides

The powder form of dried pumpkin pulp was used for extraction of polysaccharide followed by the method reported by (Qian, 2014) with slender revisions. 100 g of the pumpkin was mixed with four volumes of 95% ethanol and shaken in a magnetic stirrer for 1 h. The solvent mixture was filtered by using filter paper to separate soluble and insoluble fraction. 300 mL of 80% ethanol was added to the insoluble fraction of pumpkin and stirred for 30 min. The procedure was repeated twice. The precipitate was separate, washes with acetone three times, and air-dried. For the extraction of polysaccharide, 10 g of alcohol insoluble fraction with four volumes of distilled H_2O and boiled at 100 °C for 1 h and then centrifuged. Three volumes of ethanol were added to the collected supernatant and centrifuged at 10,000 rpm for 10 min. The precipitated polysaccharide washed with acetone, air-dried and stored for further studies.

2.3. Recovery of PPs

Hydrolysates were strained into Whatman No.1 filter paper and concentrated to ~ 15% (W/V) and finally, the protein was removed by precipitating with the 5 volumes of ethanol, filtered and free dried. The percentage of yield of PPs was determined using the equation below,

$$Yield = \frac{W2}{W1} \times 100$$

Where, W_1 is the pumpkin powder weight and W_2 is the weight of recovered PPs

2.4. Characterization

In order to create a suspension at a concentration of 1% (W/V), the aqueous extracted PPs were immersed in distilled H_2O. To assess the effect of pH, the pH of the suspension was attuned to 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5. Various concentrations of enzymes (1000, 2000, 3000, 4000, 5000 and 6000 μ/g) were added to this suspension for the cellulose effect. The reactor was maintained at different temperatures (40, 45, 50, 55, 60 and 65 °C) in a thermostatic water bath. The protein and the total sugar content of the extracted polysaccharides were quantified conferring to the standard procedure (Hou, 2004).

2.5. Optimization of PPs by RSM

The single factor experiment was followed to disclose the impact of time, temperature, pH and enzyme concentration on the yield of PPs. One parameter remains constant in all experiments during a single factor experiment.

Box-Behnken's design was employed on the basis of single-factor results to optimize the aqueous - aided extraction condition of PPs. A four-factor-three-level (**A** = extraction time, **B** = extraction temperature, **C** = pH, and **D** = enzyme concentration) test was accomplished to determine their collective influence. On the basis of results of single factor experiments, the values of range and cen-

Table 1
Factors and Ranges of Box-Behnken experiment design.

	Level of Ranges		
Factors	**−1**	**0**	**+1**
A-Extraction time (min)	40	80	120
B-Extraction temperature (°C)	45	55	65
C-pH	3.5	4.5	6.5
D-Enzyme concentration (μ/g)	2000	4000	6000

tre point were determined for all the independent variables studied (Table 1). Expression of extracted polysaccharide was expressed as follows by second-order polynomial equations;

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=1+i}^{4} \beta_{ij} X_i X_j$$

Where Y is the dependent variable; β_0, β_i, β_{ii}, and β_{ij} the linear, quadratic and interactive coefficients, respectively; X_i and X_j are independent variables (i # j).

2.6. α- amylase inhibitory effect

The α-amylase inhibitory activity of PPs was assessed at different concentrations within the range from 62.5 to 1000 μg/mL. About 25 μL of 0.5 μg/mL of porcine alpha-amylase was added to this reaction mixture and incubated at 25 °C for 10 min. To this, 25 μL of 0.5% starch solution was added and incubated at 25 °C for 10 min. In order to stop the reaction, 50 μL of 96 mM 3, 5 DNS were added. The absorbance was read at 540 nm using a microplate scanner.

$$\%inhibition = control - test/control \times 100$$

2.7. Anti-inflammatory activity

Different concentrations of PPs extracted ranged between 62.5 μg/mL – 500 μg/mL was used for the study. In the 2 mL reaction mixture, 0.06 mg trypsin, 1 mL 20 Mm Tris-HCl buffer (pH 7.4) and 1 mL PPM were added at each concentration and kept in incubation at 37 °C for 5 min. Then 1 mL of 0.8% (w/v) casein was added to the solution and was again kept in incubation for another 20 min. In order to stop the reaction, 2 mL of 70% perchloric acid was added and vortexed at 3,000 rpm for 10 min. The absorption was measured using UV–VISIBLE Spectrophotometer at 200 nm (SL119, Systronics) against buffer as blank.

The percentage of inhibitory activity of proteinase was measured using the standard formula,

$$\%inhibition = 100 - [(ODofthetestsoution - ODofproductcontrol) / ODoftestcontrol \times 100]$$

2.8. Anti-oxidant activity of PPs

Antioxidant activity of aqueous extracted PPs was evaluated with DPPH scavenging activity (Song et al., 2010), ABTS radical scavenging activity, Hydroxyl scavenging activity (Li et al., 2013) and phenol activity (Chen and Huang, 2019).

2.9. Anti-microbial activity of PPs

The antimicrobial activity of polysaccharides derived from *C. moschata* plant extracts was investigated using a disk diffusion process. The efficiency of PPs was tested against three bacterial (*Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa)* and fungal strains (*Aspergillus flavus, Aspergillus fumigates,* and *Aspergillus niger,*). For each disk, PPs of 2.5, 5 and 10 mg/disk were loaded with Ampicillin (10 mg) as the positive control. To evaluate the antimicrobial effect, inhibition zone near the disks was recorded and evaluated.

2.10. Statistical analysis

All data are viewed as mean ± SE statistical analysis using R data analysis. A multifactor ANOVA with a multiple-range posterior test was used to detect important differences.

3. Experimental results

AAE of Polysaccharides from pumpkin has been identified, the extraction conditions have been optimized and their application, such as anti-diabetic, anti-inflammatory, antioxidant and antimicrobial PPs activity, has been determined.

3.1. Effect of time on the extraction of PPs

In this investigation, the effect of various extraction time (20, 40, 60, 80, 100 and 120 min) on the yield of PPs was examined while maintaining pH-4.5, enzyme 4000 μ/g and extraction temperature 55 °C. As observed in Fig. 1a, the average yield of PPs was 15.4 ± 0.19% when the extraction period was 80 min and thereafter decreased.

3.2. Effect of temperature, pH and enzyme amount on the extraction of PPs

The aqueous aided extraction was done at various temperatures (40, 45, 50, 55, 60 and 65 °C) to evaluate the effect of temperature on the yield of PPs. The duration of extraction, pH and enzyme amount was set at 80 min, 4.5, and 4000 μ/g respectively. Fig. 1b showed that the yield of PPs increased gradually with an increase in temperature, finally reaching a maximum of 55 °C, and the PPs yield decreased at a higher temperature.

The effect of different pH (3.0, 3.5, 4.0, 4.5, 5.0 and 5.5) on PPs yield was scrutinized when the other observational factors (time, temperature, enzyme concentration) were fixed at 80 min, 55 °C and 4000 μ/g respectively. The yield of PPs increased with an increase in pH and peaked when the pH was 4.5 (Fig. 1c). The yield of PPs extracted was found to decrease beyond pH 4.5.

The AAE was executed at different enzyme amount (1000, 2000, 3000, 4000, 5000 and 6000 μ/g) on the yield of PPs while setting the factors as follows: extraction temperature of 55 °C, pH 4.5 and extraction time 80 min. Fig. 1d disclosed the yield of PPs increased by increasing the volume of enzyme concentration up to 4000 μ/g and then started to decrease. The amount of enzyme can also play a significant role in the extraction of polysaccharides.

3.3. Experimental design and analysis of variance

3.3.1. Building response surface model

The experimental design and experiential responses were illustrated in Table 2. The overall design entailed of a total of 29 experimental points and the factors were assessed using Design-Expert software. As a result, the value of extracted polysaccharide yield could be represented by the succeeding polynomial equations of the second order:

$$Y = 3.19 + 0.38A - 0.14B + 0.034C + 0.096D + 0.50AB - 0.12AC + 0.14AD + 0.13BC - 0.20BD + 0.06CD - 70A^2 + 0.70B^2 + 0.085C^2 + 0.68D^2$$

Where Y was the PPs yield, A, B, C and D were the extraction time, extraction temperature, pH and enzyme concentrations correspondingly.

The statistical implication of the regression comparison was tested by F-value and p-value, and the variance analysis (ANOVA) for the quadratic response surface model was précised in Table 3. The high model F-value (719.64) and the low p-value (<0.001) showed that the model was extremely important. The lack of fit F-value with the value of 0.24 indicated the adequacy of the model for predicting variations. The assessment of the coefficient (R^2 = 0.9986) of the quadratic regression model showed that only

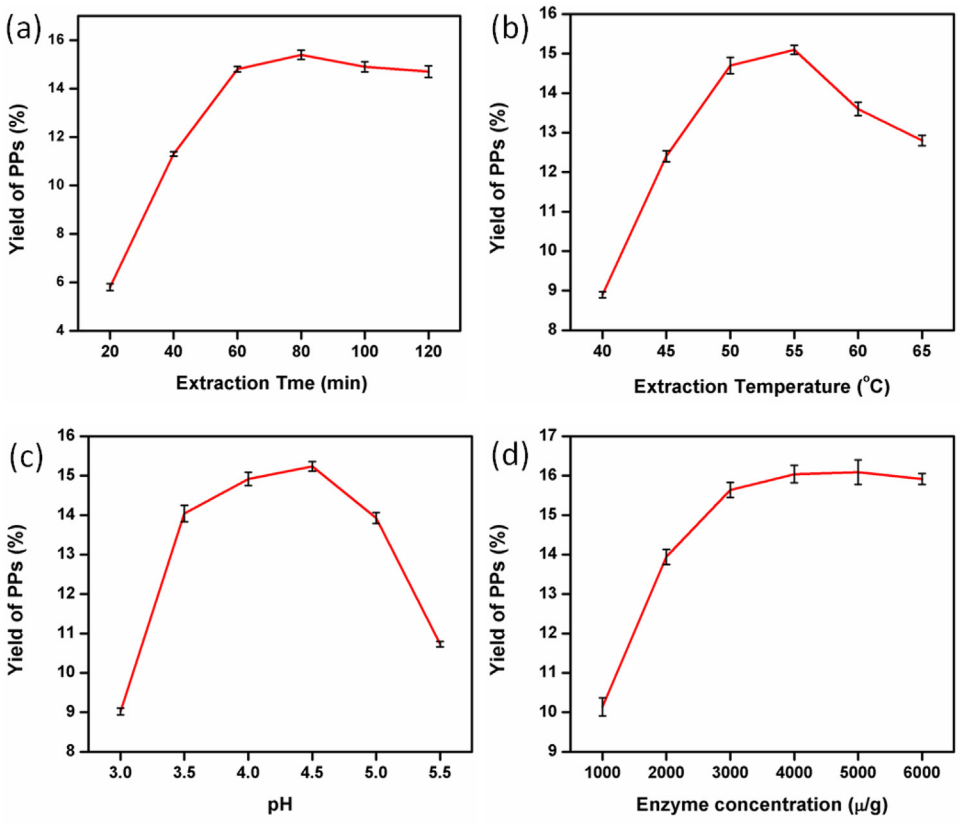

Fig. 1. Effect of different experimental variables on the yield of PPs. (a) extraction time; (b) extraction temperature; (c) pH; (d) enzyme concentration.

Table 2
Box- Behnken design and observed responses.

Run	**A**-Extraction time	**B**-Extraction Temperature	**C**-pH	**D**-Enzyme concentration	Yield
	(min)	(°C)	(pH)	(µ/g)	(%)
1	40	65	4.5	4000	13.8
2	80	45	4.5	2000	14.2
3	120	55	4.5	6000	15.2
4	40	45	4.5	4000	13.7
5	80	55	4.5	4000	15.3
6	80	55	4.5	4000	15.3
7	120	55	4.5	2000	14.7
8	120	45	4.5	4000	14.5
9	80	55	4.5	4000	15.4
10	80	65	4.5	6000	14.8
11	40	55	5.5	4000	13.2
12	40	55	4.5	6000	14.3
13	80	65	5.5	4000	13.7
14	40	55	4.5	2000	13.8
15	80	55	5.5	6000	14.2
16	80	55	5.5	2000	13.7
17	80	55	3.5	2000	14.6
18	80	55	4.5	4000	15.4
19	120	55	5.5	4000	14.1
20	80	45	3.5	4000	14.4
21	80	55	4.5	4000	15.4
22	120	55	3.5	4000	14.9
23	40	55	3.5	4000	14.1
24	80	65	4.5	2000	14.3
25	120	65	4.5	4000	14.6
26	80	45	4.5	6000	14.7
27	80	55	3.5	6000	15.1
28	80	45	5.5	4000	13.6
29	80	65	3.5	4000	14.5

Table 3
Analysis of variance for the second-order polynomial model.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	0.0003	14	0.0000	719.64	**
A-Extraction time	0.0001	1	0.0001	2125.26	**
B-Extraction Temperature	7.346	1	7.34	29.46	**
C-pH	0.0001	1	0.0001	2164.90	**
D-Enzyme concentration	0.0000	1	0.0000	689.81	**
AB	7.999	1	7.999	0.0321	
AC	2.640	1	2.640	10.59	*
AD	2.190	1	2.190	0.8781	*
BC	8.347	1	8.347	0.0335	
BD	2.693	1	2.693	0.0108	
CD	2.283	1	2.283	0.9154	*
A^2	0.0001	1	0.0001	2052.17	**
B^2	0.0001	1	0.0001	2043.65	**
C^2	0.0001	1	0.0001	2834.91	**
D^2	8.065	1	8.065	323.43	**
Residual	3.491	14	2.494		
Lack of Fit	1.330	10	1.330	0.2461	
Pure Error	2.1627	4	5.404		
Cor Total	0.0003	28			
R^2			0.9986		
Adjusted R^2			0.9972		

Notes ** $p < 0.01$; *$p < 0.05$.

1% of the total deviations could not be explicated by the model. The adjusted coefficient determination (R^2_{adj} = 0.9972) was also significantly high, indicating a higher possibility of similarity between the actual and expected values. Around the same time, the coefficient of the variance (C.V.) with the low value of 4.75 explicitly indicated that the dispersal of data points was about the mean and had a strong consistency.

Table 3 also revealed that the linear correlations (A, B, C, D), the quadratic concept coefficient (A^2, B^2, C^2, D^2), and the bridge- product coefficients (AB, AC, AD, BC, BD, CD) suggestively impacted the yield ($p < 0.05$ or $p < 0.01$). In particular, extraction time was the single most substantial parameter followed by extraction temperature and pH.

3.3.2. Estimation of the model precision

The distinction between the actual and expected values as illustrated in Fig. 2a. It shows that the distribution of both actual and predicted model values was arranged in a straight line. This indicates the consistency of predicted model value with that of the actual estimated value. Fig. 2b showed the distribution of the Normal probability plot of standardized residual on a straight line without severe deviance, indicating the good fitting effect of the model. In Fig. 2c, the dispersal of data lies in the satisfactory ranges (±3.9).

3.3.3. Optimization of experimental procedures

The response surface design was represented in Fig. 3. The evaluation of interactions present between two independent variables and their determination to obtain optimum value is quite simple. Fig. 3 a, c, e revealed extraction time (A), extraction temperature (B) and pH (C) possess significant impacts on PPs production. Meanwhile, the interfaces between extraction time with extraction temperature and pH with extraction time and extraction temperature exerted a significant effect on polysaccharide yield. While the enzyme concentration (D) produced relatively weaker influences on extraction yield (Fig. 3 b,f) and their interaction with both extraction time and extraction temperature and pH was not significant (Fig. 3d). Based on Fig. 3, the optimal conditions of PPs extraction could be deduced that extraction time 85.92 min, extraction temperature at 55.20 °C, pH at 4.55 and enzyme concentration of 3993.96 μ/g with a maximum response of predicted by this mode under these circumstance was 14.60.

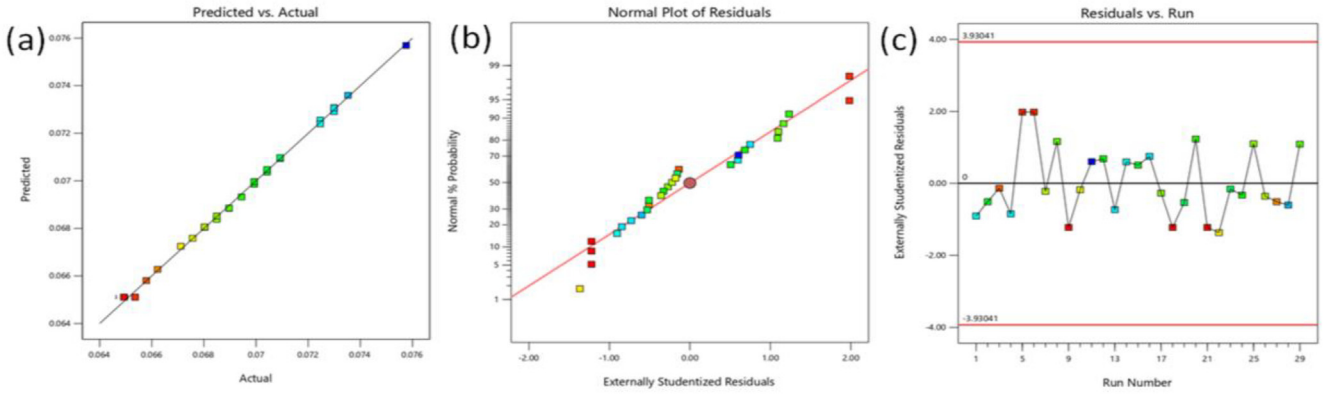

Fig. 2. Estimation of the model precision. (a) Plot of predicted Vs actual value; (b) The normal % probability plot; (c) Plot of internally studentized residual versus actual run.

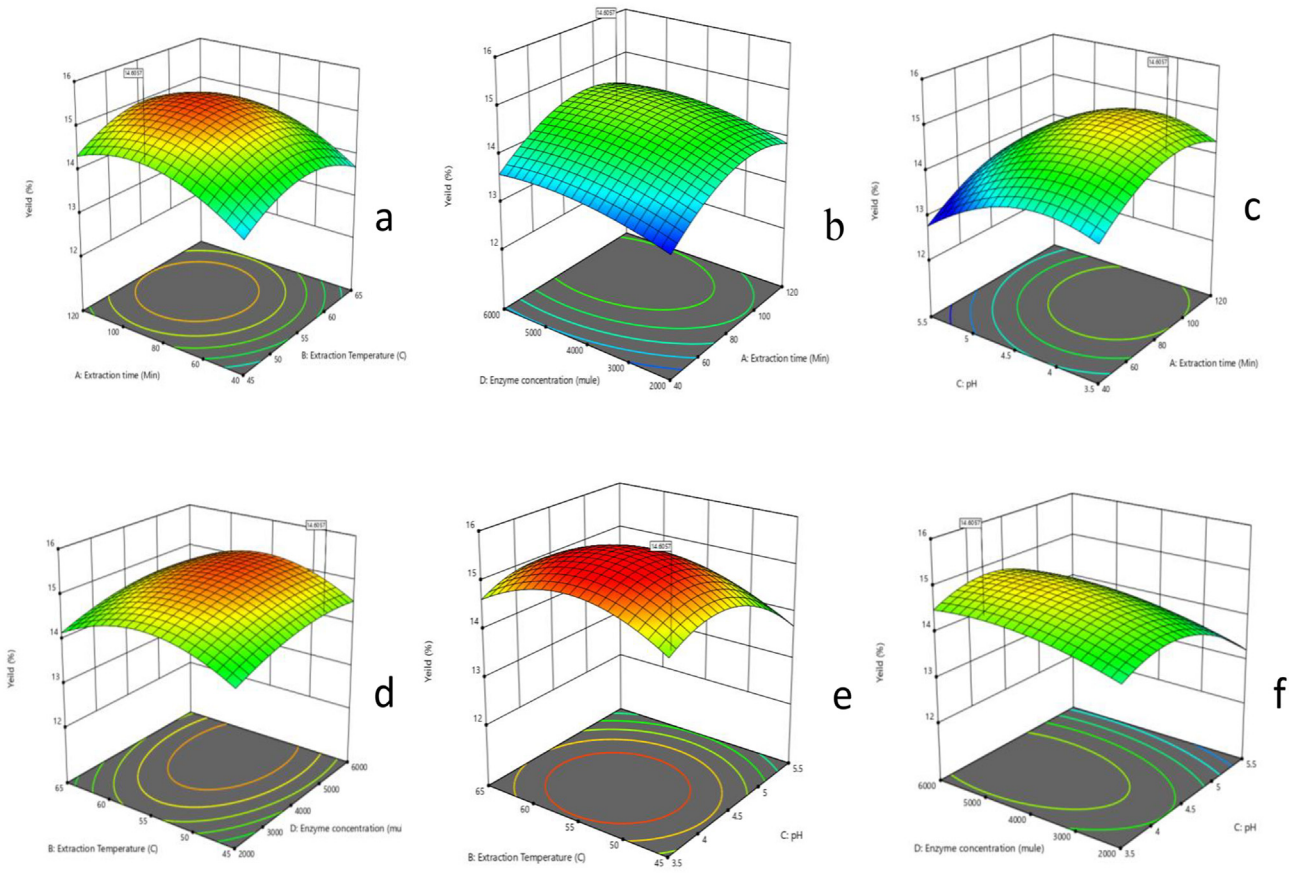

Fig. 3. Response Surface (3D) showing the interactions between different extraction parameters. (a) Extraction time and extraction temperature; (b) Extraction time and enzyme concentration; (c) pH and extraction time; (d) extraction temperature and enzyme concentration; (e) Extraction temperature and pH; (f) pH and enzyme concentration.

3.4. Product characterization

The ash, moisture, protein, starch and total sugar content were 0.8, 1.5, 0.18, 58.0 and 62.0 %, respectively (Table 4).

3.5. PPs with α- amylase inhibitory effect

In this study, the *In-vitro* α -amylase inhibition effect of PPs were studied in a concentration ranged between 62.5 and 1000 μg/mL. The IC_{50} value was recorded at 1000 μg/mL. The highest percentage of inhibition of 57.41% is exhibited at 1000 μg/mL. The percentage inhibition of all the extract was dose-dependent (Fig. 4). α- amylase hold a significant position in hydrolysis and integration of carbohydrate.

3.6. In vitro anti-inflammatory activity

The *In vitro* anti-inflammatory efficiency of polysaccharides derived from pumpkin was studied using proteinase inhibitory action. The PPs demonstrated important anti-proteinase effects at a various concentration as shown in Fig. 5. The PPs extract was effective in all the concentration taken and the IC_{50} value was observed in 25 μg/mL. The highest percentage of proteinase inhibition observed was 50.41% at 25 μg/mL followed by 50.61% at 50 μg/mL.

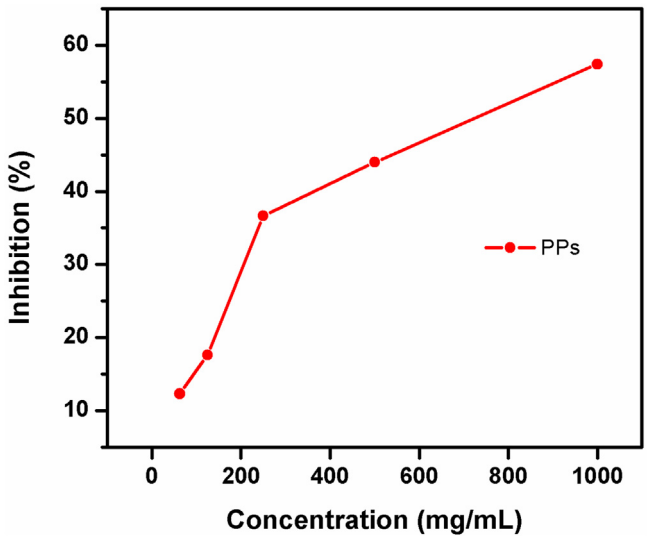

Fig. 4. Effect of pumpkin polysaccharides on *In-vitro* α-amylase activity.

Table 4
Characterization of aqueous extracted pumpkin polysaccharide.

Product characterization	Content%
Ash	0.80
Moisture	1.50
Protein	0.18
Starch	58.00
Total sugar	62.00

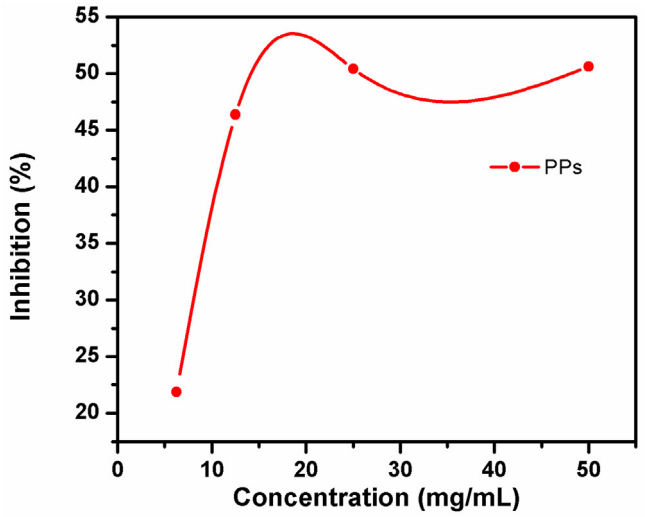

Fig. 5. Effect of pumpkin polysaccharides on *In-vitro* anti – inflammatory activity.

3.7. Anti-oxidant activity

The total phenolic content of PPs was assessed using standard gallic acid equivalent to phenols. The total phenolic acid content of PPs was 0.74%. The Hydrogen peroxide scavenging efficiency of PPs was estimated and equated with ascorbic acid the IC_{50} value observed was 0.40%. The DPPH radical scavenging efficiency of AAE PPs was assessed with ascorbic acid as standard and results are specified in Fig. 6. The inhibition percentage at different concentration (10 – 60 μg/mL) of PPs along with standard was calculated and plotted a graph to find out IC_{50} values. The IC_{50} value was 59.87% at 30 μg/mL. The ABTS radical scavenging activity of PPs was valued and analyzed with ascorbic acid and results are given in Fig. 7. The percentage of inhibition at different concentration (10–60 μg/mL) of PPs as well as standard was calculated and plotted a graph to find out IC_{50} values. The IC_{50} value was 58.74 at 40 μg/mL. The hydroxyl radical scavenging activity of PPs shows a dose dependent increase with respect to standard. The IC_{50} value was recorded at 60 μg/mL (Fig. 8).

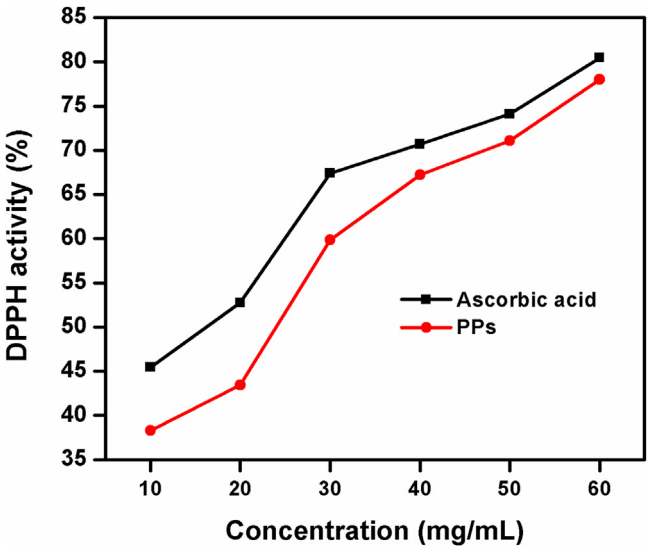

Fig. 6. DPPH radical scavenging activity of PPs.

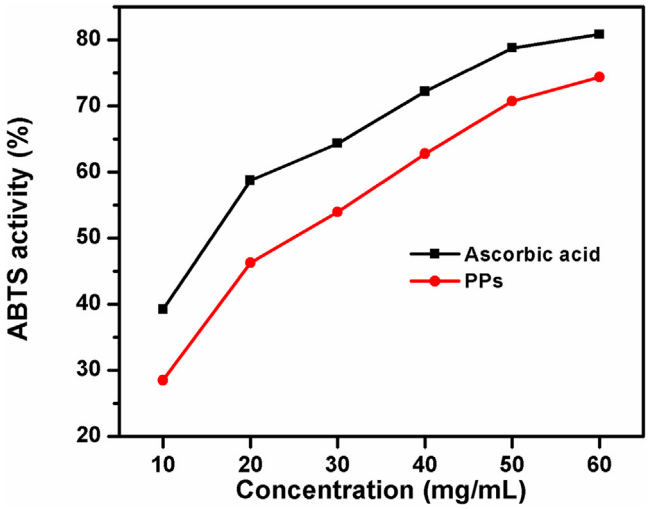

Fig. 7. ABTS radical scavenging activity of PPs.

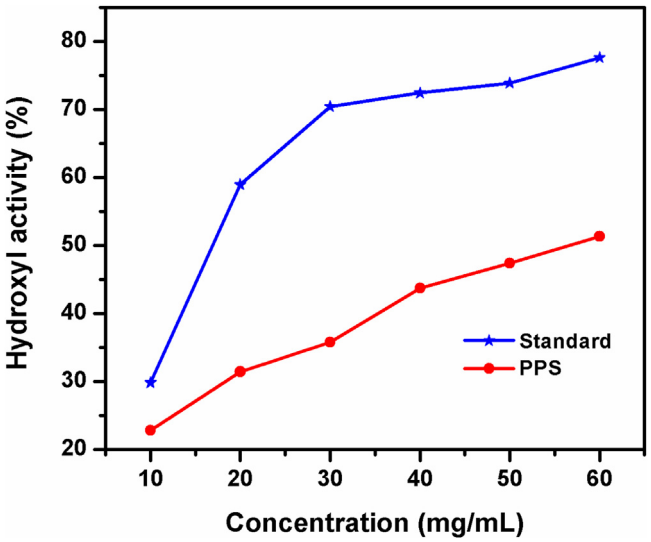

Fig. 8. Hydroxyl radical scavenging activity of PPs.

3.8. Anti-microbial activity

The antimicrobial activity of PPs indicates better antibacterial activity especially in comparison to antifungal activity (Fig. 9). The highest inhibition zone was found in *E. coli* (12 ± 0.1) accompanied by *S. aureus* (10 ± 0.2). The PPs shows the least effect on all the fungal strains studied.

4. Discussion

Pumpkins are known to contain a large source of biologically active macromolecules, including proteins, polysaccharides, sterol and *para*-aminobenzoic acid (Yadav et al., 2010; Adams et al., 2011, 2014; Patel, 2013). Pumpkin has also been shown with anti-diabetic properties several times as the bulk of these bioactive chemicals are found in the fruit (Behera et al., 2011). A typical serving of pumpkin is higher in fibre and low in carbs. While pumpkin has a high glycemic index, it has a low glycemic load and has a substantial influence on blood sugar level. The research shows that pumpkin has many potential benefits specific to people with diabetes (Perkins-Veazie, 2010).

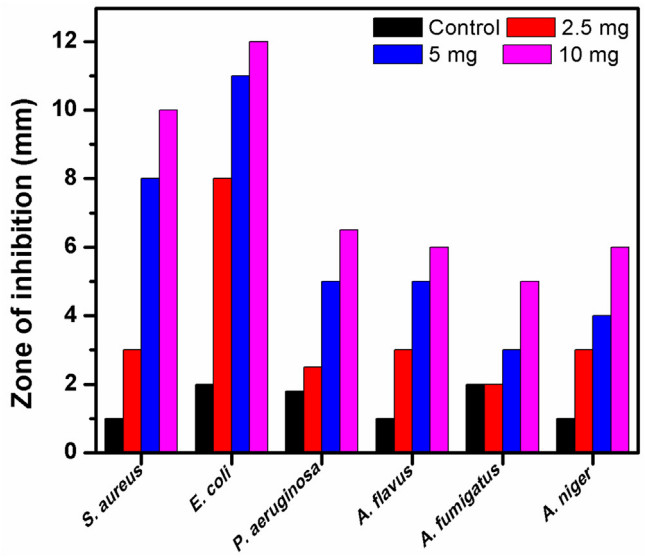

Fig. 9. Antimicrobial activity of PPs.

4.1. Effect of time, temperature, pH and enzyme amount on the extraction of PPs

In this investigation, the yield of PPs was observed highest at extraction period of 80 min while maintaining pH – 4.5, enzyme 4000 μ/g and extraction temperature 55 °C and thereafter decreased. This is because the longer the time, the more polysaccharide tends to be the balance in a certain period. However, when the extraction period was further extended, the amount of polysaccharide decreased. The decrease in PPs yield by increasing the extraction time to 80 min was due to the thermal degradation of the extracted PPs. The results indicated that longer extraction time could lead to the deterioration of polysaccharides and a consequent decline in yield of PPs (Wang et al., 2018). As a result, 80 min was taken as the optimal extraction time.

The extraction of time, pH and enzyme amount was set at 80 min, 4.5, and 4000 μ/g respectively to observes the maximum yield of PPs at maximum of 55 °C temperature, beyond the yield was decreased as shown in Fig. 1. It may be due to a decrease in the surface tension and viscosity of the solvents and an increase in the pressure of small bubbles at higher temperatures. The temperature is a critical parameter the influences the activity of various biological mechanisms in plants and animals (Bosco et al., 2000). At higher extraction temperature, the biomolecules may reduce by either degradation or oxidation (Dang et al., 2013). These results indicate that the decline in the yield of PPs at higher extraction temperature could degrade polysaccharides and increases the dissolution rate of polysaccharides. Based on this result, 55 °C was used for further experiment.

The PPs yield was seemed to be decreased beyond pH at 4.5. This applies to the structure of polysaccharide and protein isoelectricity. At low pH, the hydrogen ion concentration is high and stimulates the hydrolysis of insoluble polysaccharide composition in the solvent. The higher pH induces the promotion of protein dissolution and inhibits the polysaccharide dissolution. On the basis of this, pH at 4.5 was chosen for the next extraction process.

4.2. PPs with α- amylase inhibitory effect

The *In-vitro* α -amylase inhibition effect of PPs showed a dose-dependent percentage of inhibition (Fig. 4). The α- amylase holds a significant position in hydrolysis and integration of carbohydrate. The inhibitory effect of α- amylase results degradation of composite sugar including starch will delay and further extend all over carbohydrate digestion duration. Observance of significant results shows that the molar masses of the polysaccharide is particularly important. Thus, oligosaccharides from this polymer would, therefore, have a stronger inhibition of the activity α- amylase at the lowest polymerization level. Song et al., (2012) reported substantial, non-competitive suppression of alpha glycosidase in tested enzymatic reaction at 0.7–0.9 mg/mL. According to Kwon et al., (2007), the anti-diabetic property of pumpkin in terms of beta-glucosidase and alpha-amylase is due to the richness of phenolic phytochemicals. The angiotensin I-converting enzyme suppressing reactions of pumpkin show its hypotensive effects. Furthermore, Quanhong et al., (2005) isolated protein-bounded polysaccharides result in the hypoglycaemic activity of pumpkin.

4.3. In vitro Anti-inflammatory activity

Aqueous assisted extracted PPs shown to hold greater hypoglycemic properties (Zhang, 2004). This is probably because of the rich composition of pectin in pumpkin fruits (Fissore et al., 2007). The conception of pumpkin controls the glycemic content and to reduce the necessity of insulin when a patient with diabetes consumes fibre – rich food (Guillon and Champ, 2000). The process of inflammation is also linked with the free radical generation which contributes to the formation of edema. The production of excessive reactive oxygen species (ROS) leads to oxidative damage and induce peroxidation of lipids in the membrane. The significant anti-inflammatory effects of PPs (Fig. 5) can be an alternative pharmacological drug to manage inflammatory disorders.

4.4. Anti-oxidant activity

From the Figs. 6-8, it was observed that the extracted PPs showed comparatively significant anti-oxidant activity when compared to standard. Superoxide radicals are toxic agents generated by a wide range of biological and photochemical reactions. The role of polysaccharides in radical scavenging processes was linked to hydrogen atoms or electron donation, and the hydrogen-donating potential was affected by variations in monosaccharide configuration, molecular weight and composition. In the present study, the high restraint values of PPs may be due to their effective hydrogen-donating potential. It should be noted, according to the above, that the strong anti-oxidant properties of PPs in this study should provide investigational confirmation for the folkloric use of pumpkin as a promising natural anti-oxidants source given the processes aqueous extraction method of polysaccharides.

4.5. Anti-microbial activity

The PPs extracted via aqueous assisted method results better antibacterial activity when compared to anti-fungal activity as shown in Fig. 9. Similar findings have been reported by Wan et al., (2012) that the PPs may effectively impede the growth of *E. coli*. The antibacterial potential of polysaccharide is examined by the adhesion of cellular components such as cell wall, cytoplasmic membrane or DNA, mainly by bacterial glycoprotein receptors. PPs had the greatest inhibition effect against bacterial strains compared to fungal strains. The inhibitory action of the PPs may be due to the high polysaccharide quality of the extracts. The defensive response is, therefore, a result of the creation and transition of antibiotic resistance plasmids and the possession of endogenous resistance mechanisms. The efficacy of PPs against fungal strains is comparatively less than that of bacterial strains. The fungal cell has acted as a defensive shield against stress and has also acted as a signal transmitting receptors. The fungal cell wall consists mainly

of manno proteins, chitins, and α- and β- linked glucans and serves many functions, including providing cell rigidity, many of which serve as binding receptors for the host defense molecule or activate the host immune response via interactions with membrane-bound receptors. The mechanical strength of the fungal cell wall allows it a vital surface and defense against a variety of threats and serves as vital to survival under stress. This may be the explanation for the negligible inhibitory impact of fungal strains.

5. Conclusion

The pumpkin fruit is an important vegetable due to their rich source of pectin-type dietary fibre and biological effect. The optimum of AAE was determined as follows: extraction temperature of 55 °C, extraction time of 80 min, pH at 4.5 and enzyme concentration of 4000 μ/g. This optimal extraction condition resulted in a higher yield of polysaccharides (15.4%), which was closed to the predicted value. Experiments were accompanied to assess the effects of PPs on anti-diabetic, anti-inflammatory, antioxidant and antimicrobial activity. Results showed significant α-amylase inhibitory and anti-inflammatory activities for the extracted PPs. Moreover, the anti-oxidant activities were more prominent when compared to the control. PPs showed better anti-bacterial effect when compared to anti-fungal treatment. It is recommended that AEE be an effective method for the extraction of high-yield crude polysaccharides in shorter processing time with suitable biological efficiency. Future studies are needed on investigation and characterization the extracted polysaccharides in terms of the structure and effects of polysaccharide interactions which employing sophisticated measurements.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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