ORIGINAL ARTICLE

Characterization of *Moringa oleifera* leaf polysaccharides extracted by coupling ionic liquid separation system with ultrasound irradiation

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Abstract

Replacement of organic solvents and short time for purification of polysaccharides has gained attention of researchers. Ionic liquid (IL) was optimally utilized to achieve the mentioned target. IL was coupled with ultrasound irradiation to obtain good yield of partially purified *Moringa oleifera* leaf polysaccharides. The yield of purified polysaccharides (75.11%) was close to the predicted (75.78%). Crude polysaccharides were found to be round group-like shape while purified polysaccharides displayed compact flat thick-slice shape under scanning electron microscopy. Using gas chromatography, galacturonic acid was detected as part of monosaccharide composition of crude polysaccharides. Functional groups associated with polysaccharides were confirmed using Fourier transform infrared spectroscopy. Using Congo red assay, polysaccharides were observed to be of nonhelical structure. The crude polysaccharide was more viscous in rheological property and had molecular weight of (304,700 g/mol). Using dynamic light scattering methodology, purified polysaccharides (24,370 g/mol) aggregated in water and possessed an excellent ABTS antiradical ability. Purification of polysaccharides using IL within a short time was feasible and presented useful characteristics needed in formulations by the food and pharmaceutical industries.

Practical application

Feasibility of using ionic liquid aqueous two-phase system to separate biomolecules (amino acids, saccharides) within a short time has been established by researchers. Knowledge on this was applied in the separation of proteins from polysaccharides extracted from *Moringa oleifera* leaf. Knowledge of structure–function relations was thoroughly established for extracted and purified polysaccharides. Characteristics of polysaccharides discovered can be useful to the food and pharmaceutical industries.

1 | INTRODUCTION

Among other options, polysaccharides with immunostimulatory potential, wound healing potential and with effects on hematopoietic and reticuloendothelial systems are being explored as remedies for many infectious and noninfectious which are increasingly being reported (Talmadge et al., 2004). In the food industry, polysaccharides possess the ability to control rheological properties of aqueous phase by acting as thickening, suspending suspension, gelling, and whipping agents.

Ethanol precipitation, ion-exchange chromatography (Chang, Feng, & Wang, 2011), and gel permeation chromatography (Femenia, García-Pascual, Simal, & Rosselló, 2003) are used traditionally in the purification of the target polysaccharides. These techniques demand a lot of time, are very expensive and difficult to scale-up. Aqueous two-

phase system (ATPS) can provide a short processing time and an easy-to-scale-up separation of proteins and impurities from polysaccharides (Hatti-Kaul, 2000). This work therefore sought to use ionic liquid (IL) (1-octyl-3-methylimidazolium chloride, [C_8mim]Cl) and salt (potassium carbonate, K_2CO_3), as an IL ATPS (ILATPS) for the partial purification of polysaccharides from *Moringa oleifera* leaf extracts.

Moringa oleifera Lam. is abundantly cultivated in the tropics and sub-tropics of Asia and Africa (Booth & Wickens, 1988.). It has a remarkable drought resistant attribute, and thus survives in a wide range of soil composition and diverse rainfall conditions (Rajan, 1986). The leaves of this wonder plant contain valuable source of antioxidants due to the presence of chemopreventive phenolic compounds, flavonoids and ascorbic acid (Siddhuraju & Becker, 2003). Polysaccharides from such higher plants have also been revealed to be the fundamental cause of biological activities, either by itself or by inducing complex cascade reactions (Paulsen, 2002).

Tan, Li, Xu, and Xing (2012), using ILATPS to investigate the partitioning behavior of polysaccharides and proteins of the plant *Aloe vera* L., concluded that under optimal extraction conditions, high extraction efficiency can be obtained for purified polysaccharide extracts. Currently, disruptive high power intensity (10–1,000 W/cm^2) or frequency (20–500 kHz) ultrasound is used in food processing for extraction (Mane, Bremner, Tziboula-Clarke, & Lemos, 2015). The nonthermal technology, has numerous advantages which includes reduction of processing time, energy saving and improvement on the shelf-life, and quality of food products (Awad, Shaltout, Asker, & Youssef, 2012; Ercan & Soysal, 2013).

The hypothesis of this work was to establish the possible use of a nonorganic solvent in purification and also monitor the effect of the ILATPS on the purified polysaccharide. The focus of this work included: (a) exploring both single and collaborative impact of irradiation variables on polysaccharides of *Moringa oleifera* leaves, (b) optimizing the irradiation variables using Box–Behnken designs, (3) characterizing extracted crude and purified polysaccharides, and (d) studying the antioxidant properties of extracted polysaccharides.

2 | METHODS

2.1 | Preparation of crude polysaccharide

M. oleifera leaves was obtained from Ghana (Figure 1). The leaves were air-dried, ground, and sifted. Employing ethanol (90% vol/vol) in Soxhlet extraction, bulk of undesirable substances such as pigments, fats, and monosaccharides were detached from the powder. At 40°C, residue obtained after extraction was dried and used as pretreated sample. Under pulsating mode, dry sample was extracted in water at a ratio of 1:20 (wt/vol) using preliminary determined optimum ultrasound operational conditions (power [240 W], frequency [60 kHz], temperature [90°C], and time [90 min]). Crude polysaccharide extracts in water (9.24%) was separated using centrifugation at 4,000 rpm for 15 min (yet to be published). The supernatant was filtered using gauze and then reduced in volume under vacuum condition. Four times the volume of 80% (vol/vol) ethanol was added and made to stand at 4°C overnight. The precipitate was collected by centrifugation at 5,000 rpm for 10 min. Water was added to the precipitates to aid in the removal of the ethanol by the use of the rota-evaporator at 37°C and then lypholized.

2.2 | Polysaccharides purification

Using IL, [C_8 mim]Cl, K_2CO_3 salt, and H_2O (35, 17, and 48% wt/wt, respectively) ILATPS was prepared. An amount of powdered sample was added to the ILATPS. Additional mixture with the same phase constituents deprived of crude polysaccharide was prepared as blank to account for confounders. The mixture was vigorously mixed at 25°C. Purified polysaccharide was transferred into bottom phase of ILATPS using selected temperature, sample concentration, and frequency of an ultrasound under pulsating mode (5 s on, 2 s off) for 30 min. Using Equation (1), polysaccharide content was obtained after separation of two phases.

$$Yield\,(\%) = W1/W2 * 100 \qquad (1)$$

where W_1 (g) is the weight of polysaccharide solution, and W_2 (g) is the weight of crude polysaccharide.

2.3 | Design for purified polysaccharide extraction

Preliminarily, single-factor design was developed with extraction factors X_1 (extraction concentration: 1, 2, 3, 4, and 5 mg/ml); X_2 (extraction frequency: 20, 40, and 60 kHz); and X_3 (extraction temperature:

FIGURE 1 Photo of *Moringa oleifera* obtained from Ghana: (a) leaves, flowers, and pods. (b) Leaves

25, 30, 35, 40, and 45°C), with control for each group. Experiments were fixed under power of 240 W and 30 min time. Experiments were done in triplicate and extraction yield of crude polysaccharide was the dependent variable.

2.4 | Optimization of experimental design

The three aforementioned variables were assigned (X_1, X_2, X_3) (Table 1) and studied based on outcomes from the experiment under Section 2.3. A response surface methodology was then carried out (Table 2).

2.5 | Desalination of polysaccharides

Purified polysaccharide extracted into the salt-rich bottom phase was membrane filtered using a recyclable dialysis membrane (MWCO 8,000–14,000, D44 mm). The extracted polysaccharide was lypholized for further characterization.

2.6 | Chemical composition

2.6.1 | Carbohydrate, protein, and polyphenol analysis

Moringa leaf crude polysaccharides precipitated using 80% (vol/vol) ethanol was annotated, ML-CP80. A portion of the sample ML-CP80 was further purified using IL separation system. The latter sample was also annotated, ML-ILP80. The total carbohydrate and protein of ML-CP 80 and ML-ILP 80 were determined according to DuBois, Gilles, Hamilton, Rebers, and Smith (1956) (DuBois et al., 1956) and Bradford (1976) Methodology (Bradford, 1976) respectively. Using Kumar, Ganesan, and Rao (2008), the phenolic content was established. All experiments were carried out in triplicate.

2.6.2 | Monosaccharide composition (gas chromatography)

The monosaccharides composition was determined using the method described by Wang, Lian, Yu, Wei, and Kang (2017).

2.7 | Spectral analysis

2.7.1 | Fourier transform infrared spectroscopy

The samples infrared spectra were attained as reported by Zhou et al. (2016) using Fourier transform infrared (FTIR) spectrophotometer (Rayleigh, WQF-510A). Briefly, samples were prepared by grinding polysaccharide with KBr (1:100 mg) and then pressed into a translucent pellet and then scanned with a resolution of 4 cm^{-1} in a region of 4,000–400 cm^{-1}.

2.7.2 | Ultraviolet spectroscopy

With the use of ultraviolet–visible (UV–vis) spectrophotometer (UV-1601), the UV spectra of the sample solutions (1.0 mg/ml) were achieved in the 190–400 nm region. Distilled water was used as blank.

2.8 | Structural analysis

2.8.1 | Tertiary structure (Congo red assay)

Tertiary structures of ML-CP 80 and ML-ILP 80 were established as reported by Qiu, Ma, Ye, Yuan, and Wu (2013).

2.8.2 | Scanning electron microscopy

Morphological characteristics of ML-CP 80 and ML-ILP 80 polysaccharides were observed as described by Zhou et al. (2016). Samples were coated with a conductive layer of gold–palladium and observed with a scanning electron microscope (SEM; JSM-7001F, JEOL, Tokyo, Japan).

2.8.3 | Molecular weight (HPSEC)

The molecular weight of samples was obtained as described by Otu, Haonan, Cunshan, and Hongpeng (2018).

2.8.4 | Particle size (dynamic light scattering)

Purified sample had its hydrodynamic diameter measured using dynamic scattering on a Zetasizer nano ZSP (Malvern Instruments, UK), a methodology reported by Ren, Edwards, Perera, and Hemar (2015).

2.9 | Rheological properties

The steady shear and oscillatory tests of ML-CP 80 and ML-ILP 80 were obtained as described by Otu et al. (2018). All measurements were done in triplicates.

TABLE 1 Variables and the corresponding three levels employed in Box–Behnken design

Variable	Units	Symbol	Coded levels		
			−1	0	1
Concentration	mg/ml	X_1	1	2.50	4
Frequency	kHz	X_2	20	40	60
Temperature	°C	X_3	25	30	35

TABLE 2 Experimental design of response surface analysis and its experimental values

Run	X_1 concentration (mg/ml)	X_2 frequency (kHz)	X_3 temperature (°C)	Y extracted polysaccharide (%)
1	2.50	40.00	30.00	37.17
2	4.00	20.00	30.00	70.08
3	4.00	40.00	35.00	75.73
4	2.50	40.00	30.00	34.70
5	1.00	20.00	30.00	32.12
6	4.00	40.00	25.00	65.75
7	2.50	40.00	30.00	34.92
8	1.00	40.00	25.00	29.96
9	2.50	60.00	25.00	31.88
10	2.50	40.00	30.00	36.85
11	4.00	60.00	30.00	73.78
12	1.00	60.00	30.00	31.26
13	2.50	20.00	35.00	36.19
14	2.50	60.00	35.00	42.56
15	2.50	40.00	30.00	35.22
16	1.00	40.00	35.00	30.55
17	2.50	20.00	25.00	35.57

2.10 | Antioxidant activity

2.10.1 | Hydroxyl radical scavenging assay

Hydroxyl radical assay was determined using methodology reported by Jen, Leu, and Yang (1998). Based on Equation (2), the hydroxyl radical scavenging rate was calculated:

$$\text{Scavenging rate} (\%) = [1-(A_1-A_2)/A_0] \times 100\% \qquad (2)$$

Where A_1 is the absorbance of the test sample, A_2 is the absorbance of the background sample, and A_0 is the absorbance of the control sample (without polysaccharides).

2.10.2 | ABTS radical scavenging assay

The ABTS radical scavenging assay was determined using reported methodology by Zhou et al. (2011). The ABTS radical scavenging rate was calculated using Equation (2).

3 | RESULTS AND DISCUSSION

3.1 | Single-factor design

3.1.1 | Effect of extraction concentration

Extraction yield of *M. oleifera* polysaccharides founded on the effect of sample concentration under static conditions (ultrasound frequency: 60 kHz, ultrasound temperature: 30°C) has been shown in Figure 2a.

Increase in polysaccharide yield clearly showed a consistent significant difference ($p < .05$) from 1 to 4 mg/ml. Experimental increase in concentration to 5 mg/ml showed no significant difference ($p > .05$) in polysaccharide yield. In an ILATPS, (C$_4$mim][N(CN)$_2$ + K$_2$HPO$_4$ at 298.14 K, almost all saccharides were preferentially extracted into the bottom phase at a test concentration of 4 mg/ml (Pei, Li, Liu, Wang, & Wang, 2010). The best test concentration in the report by Pei et al. (2010) agrees with the selected extraction concentration in this work. Thus, 4 mg/ml was chosen as the optimal concentration.

3.1.2 | Effect of ultrasound frequency

The study of *M. oleifera* polysaccharides yield based of the effect of ultrasound frequency under static conditions (ultrasound temperature: 30°C, ultrasound concentration: 4 mg/ml) has been depicted in Figure 2b. Significant difference ($p < .05$) in relation with increase in extraction yield was observed from 20 to 60 kHz. The result proposed a possible increase in extraction yield with increase in ultrasound frequency. However, there was a limitation with the maximum operational frequency of the ultrasound used in this work. Thus, the maximum operational frequency of the ultrasound water bath, 60 kHz, was selected as the optimum frequency.

3.1.3 | Effect of ultrasound temperature

The yield of *M. oleifera* polysaccharides founded of the effect of temperature under static conditions (ultrasound frequency: 60 kHz, ultrasound concentration: 4 mg/ml) has been shown in Figure 2c. Constant significant increase ($p < .05$) in extraction yield was also observed from 25 to 35°C but no significant increase ($p > .05$) was observed at

FIGURE 2 Effect of (a) ultrasound extraction concentration, (b) ultrasound frequency, and ultrasound temperature (c) on the yield of Moringa leaves polysaccharide
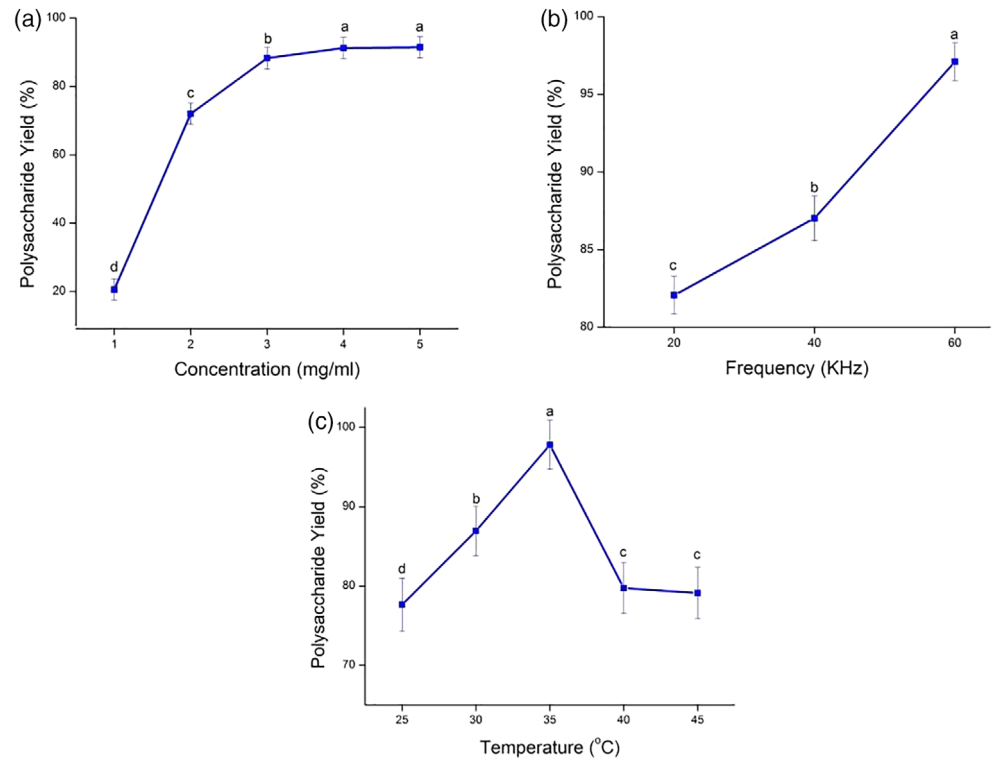

**TABLE 3** ANOVA for response surface quadratic model

Source	Sum of squares	df	Mean square	F value	p-Value Prob > F
Model	4,336.49	9	481.83	600.4	<.0001
X_1	3,877.46	1	3,877.46	4,832.15	<.0001
X_2	7.88	1	7.88	9.82	.0165
X_3	82.04	1	82.04	102.24	<.0001
X_1X_2	5.19	1	5.19	6.46	.0385
X_1X_3	19.18	1	19.18	23.90	.0018
X_2X_3	25.30	1	25.30	31.53	.0008
X_1^2	632.72	1	632.72	788.51	<.0001
X_2^2	4.32	1	4.32	5.39	.0533
X_3^2	0.24	1	0.24	0.30	.5998
Residual	5.62	7	0.80		
Lack of fit	0.32	3	0.11	0.081	.9671
Pure error	5.30	4	1.32		
Cor total	4,342.10	16			
R^2	.9987				
Adj R^2	.9970				

40 and 45°C. Using an IL-ultrasound assisted purification system, secoisolariciresinol diglucoside from flaxseed was purified within a temperature range 12–32°C. The maximum yield was obtained at 22°C (Tan et al., 2015). Beyond a certain temperature, water molecules moves from the top phase to bottom phase. This causes reduction in salt concentration in the bottom phase and subsequently reduces the salting out of polysaccharides (Tan et al., 2012). Thus, 35°C was selected as the optimal experimental temperature.
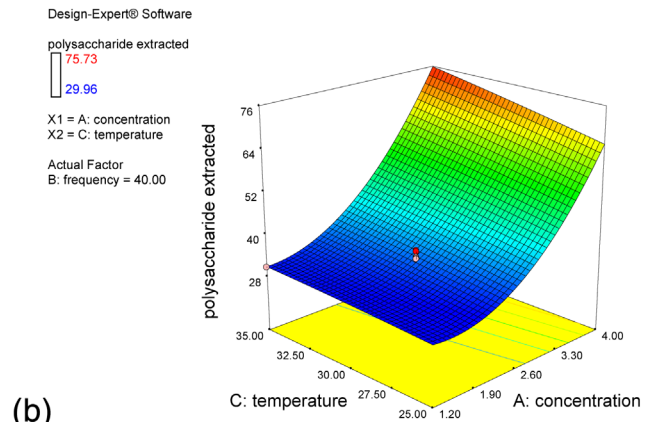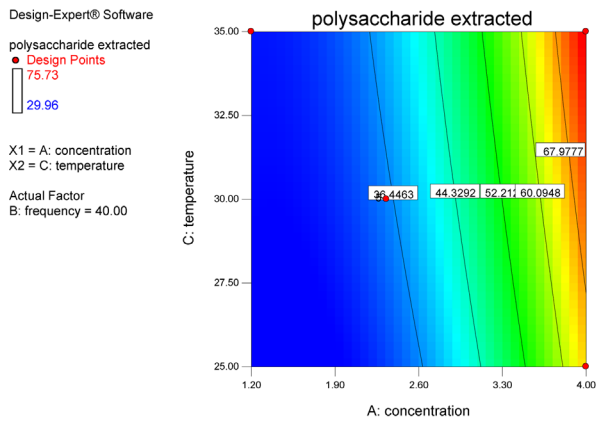
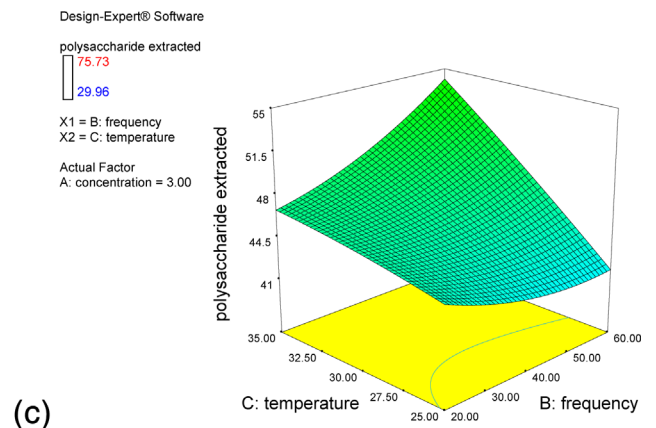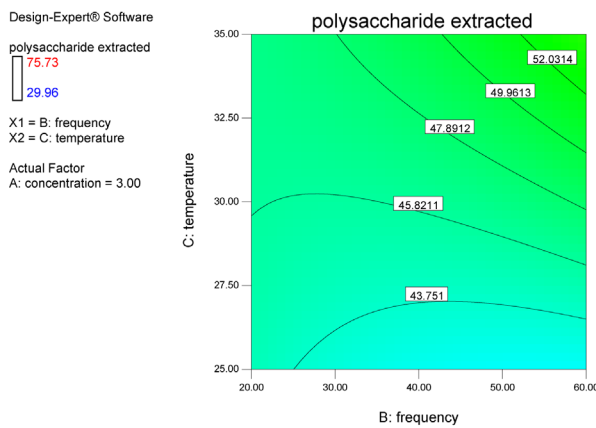## 3.2 | Optimization design

3.2.1 | Fitting the model

According to the analysis of variance conducted (Table 3), the second-order polynomial model for the extraction of purified polysaccharide was statistically significant ($p < .0001$) with ($R^2 = .9987$). The linear parameters (X_1, X_3) and (X_2) were significant at the level of ($p < .0001$)
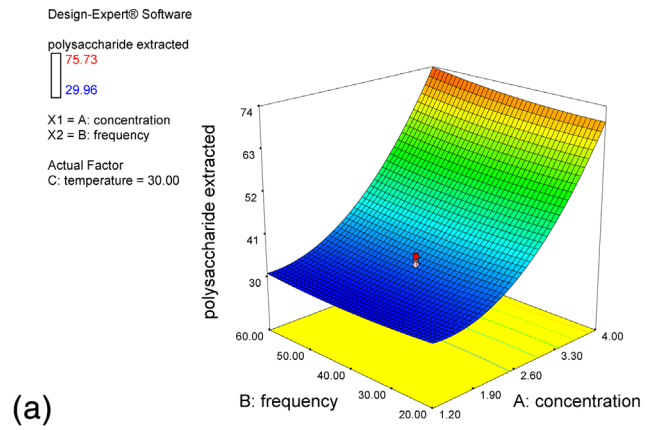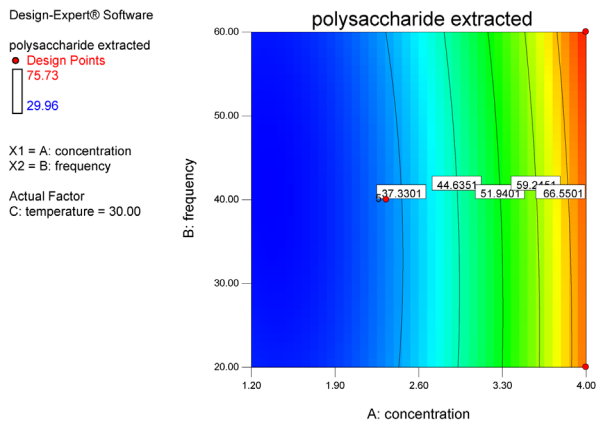
FIGURE 3 Response surface model plot showing the effects of independent variables on Moringa Leaves Polysaccharide yield: (a) concentration (mg/ml) and frequency (kHz), (b) concentration (mg/ml) and temperature (oC), and (c) frequency (kHz) and temperature (oC)

TABLE 4 Predicted and actual experimental values of *Moringa oleifera* leaves polysaccharide (%) extracted under modified optimal extraction conditions

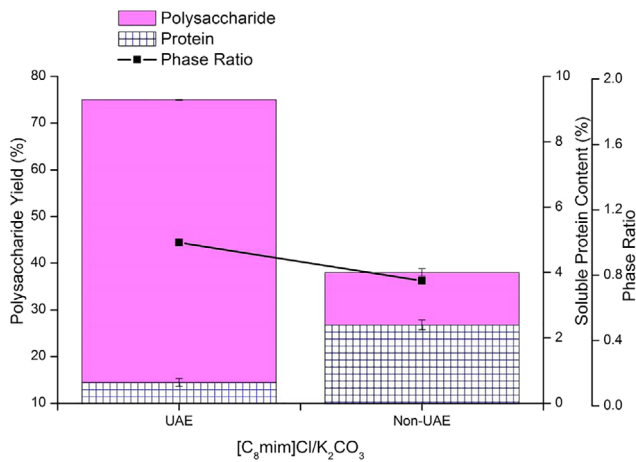|	Extraction variables			
Type	X_1 (mg/ml)	X_2 (kHz)	X_3 (°C)	**Polysaccharide (%)**
Optimum (predicted)	3.89	59.65	33.61	75.78
Modified optimum (experimental)	4	60	35	75.11 ± 0.04

FIGURE 4 Effect of optimum ultrasound application on the extraction yield of polysaccharides and soluble proteins extracted into the bottom phase of the ionic liquid aqueous two-phase system, $[C8mim]Cl/K_2CO_3$

TABLE 5 Preliminary characterization of MLCP 80 and MLILP 80

Composition	ML-CP 80	ML-ILP 80
Carbohydrate (%)	27.20 ± 0.05	10.19 ± 0.07
Protein (%)	3.64 ± 0.06	0.64 ± 0.04
Polyphenol (%)	11.78 ± 0.03	5.95 ± 0.05

and ($p < .0165$), respectively. Quadratic parameters (X_1^2, X_2^2) were also found significant ($p \leq .0001$; $p \leq .0533$), respectively. At the level of ($p < .0385$, .0018, .0008), interaction parameters (X_1X_2, X_1X_3, X_2X_3) were found significant, respectively. The "lack of fit-value" of the model was not significant ($p = .9671$). The significant regression and nonsignificant lack of fit showed that the regression equation is adequate. Quadratic regression equation was obtained using Equation (3):

$$Y = 46.03 + 34.51X_1 + 1.13X_2 + 3.64X_3 + 1.36X_1X_2 + 2.71X_1X_3$$
$$+ 2.52X_2X_3 + 18.60X_1^2 + 1.02X_2^2 - 0.24X_3^2 \quad (3)$$

3.2.2 | Analysis of response surfaces

There was a rise in the polysaccharide yield (Figure 3a,b). Interaction of sample concentrations between (2.50–4 mg/ml) with frequency and temperature started from 40 kHz and 30°C, respectively. Frequencies between 40 and 60 kHz interacted with temperatures between 27 and 35°C for a continuous increase in polysaccharide extraction (Figure 3c). The analysis of variance (Table 3) and response surfaces (Figure 3) indicted that the collaboration effects between ultrasound irradiation frequency, temperature and sample concentration were statistically significant. Thus, it was concluded that effect of irradiation frequency, temperature, and sample concentration were significant on polysaccharides yield.
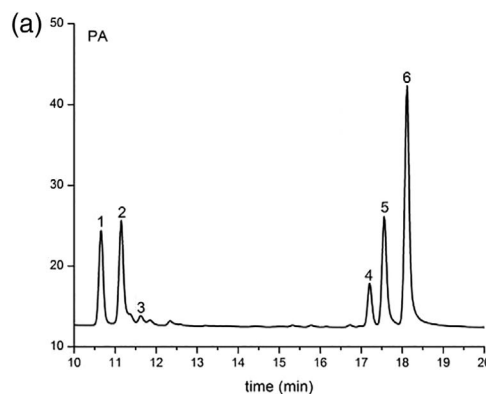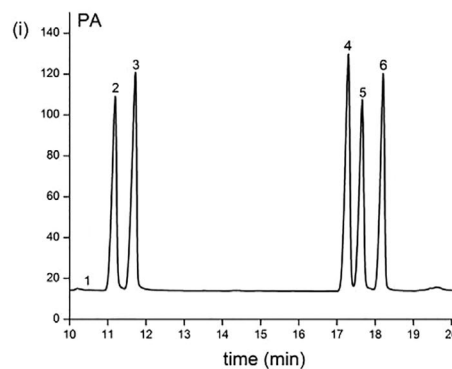

FIGURE 5 Spectrum of monosaccharide composition of: (i) monosaccharides standard (a) ML-CP 80 and (b) ML-ILP 80
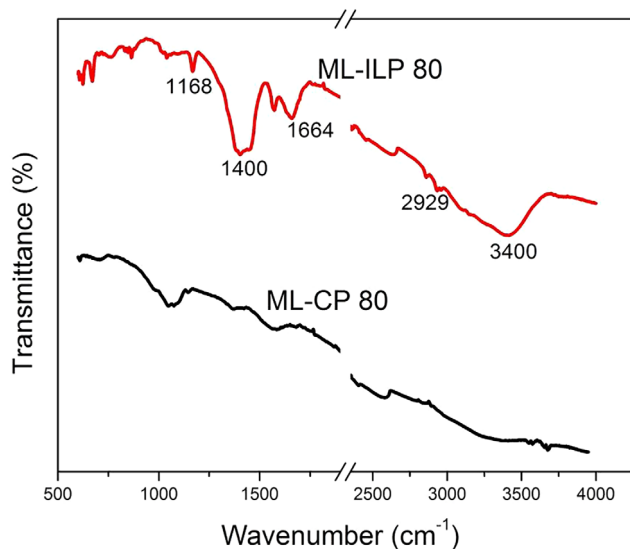
FIGURE 6 Fourier transform infrared (FTIR) spectra of crude ML-CP 80 and purified (ML-ILP 80) polysaccharides
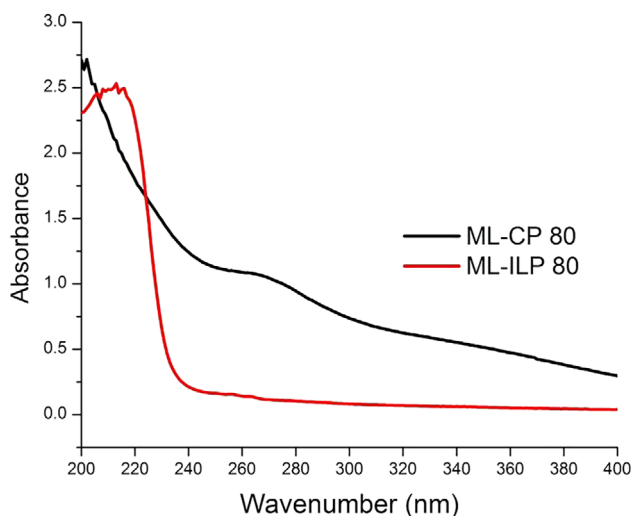

FIGURE 7 The ultraviolet–visible (UV–vis) spectra of crude and ionic liquid aqueous two-phase system (IL-ATPS) purified polysaccharide

FIGURE 8 Helix-coil transition analysis of ML-CP 80 and ML-ILP 80

polysaccharide (38.82%) into the bottom phase salt (K_2CO_3) of the ATPS was comparatively lower (Figure 4). Hence, the model was used to optimize the purification process of crude polysaccharide from *Moringa oleifera* using ILATPS.

3.3 | Chemical composition

3.3.1 | Carbohydrate, protein, and polyphenol analysis

The chemical composition of ML-CP 80 and ML-ILP 80 in terms of the total carbohydrate, protein, and polyphenol content has been displayed in (Table 5). The results indicated that purification process in ILATP resulted in the decrease of chemical components. In a report by Amin, Rashad, and El-Abagy (2007), flavonoid content of onion increased as total sugar content increased (Amin et al., 2007). This agrees with the correlation between the total carbohydrate and polyphenol content observed.

3.3.2 | Monosaccharide composition

The polysaccharides ML-CP 80 and ML-ILP 80 were largely made of D-xylose (53.14 and 52.55%) and D-galactose (33.32 and 38.08%), respectively (Figure 5). D-Glucose (6.11 and 3.27%), D-mannose (1.19 and 1.82%), and L-arabinose (0.04 and 4.29%) were also detected. Galacturonic acid was found in ML-CP 80 (1.94%) but was not detected in ML-ILP 80. A notable reduction of *d*-galacturonic acid was observed for pectin-enriched extract when ultrasound was applied for a longer period (60 min) under pH 2 (Umaña, Dalmau, Eim, Femenia, & Rosselló, 2019). The purification process in this work demanded further application of ultrasound irradiation for

3.2.3 | Verification of models

Optimum extraction was foreseen at 33.61°C temperature, 59.65 kHz frequency, and 3.89 mg/ml sample extraction concentration with results of 75.78% of crude polysaccharide. The UEA process was repeated close to projected values by modifying the temperature from 33.61 to 35°C, frequency from 59.65 to 60 kHz and sample extraction concentration from 3.89 to 4 mg/ml. Summary on the amounts of crude polysaccharide extracted under the projected and actual experimental conditions is shown in (Table 4). The experimental value (75.11%) and predicted values (75.78%) were very close. Furthermore, without the assistance of ultrasound, extracted purified
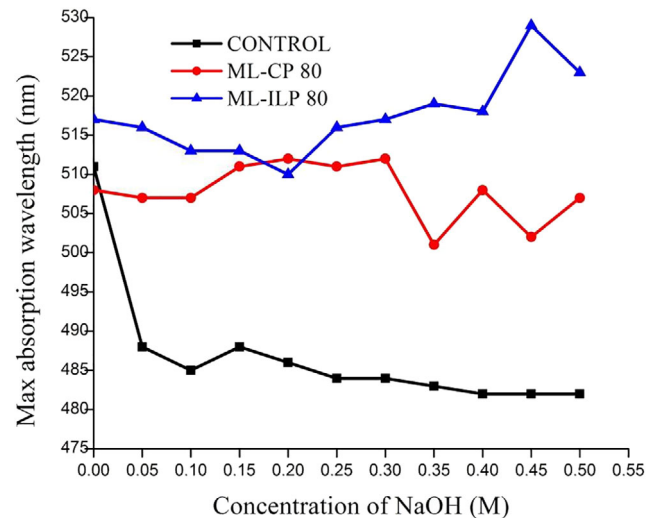
FIGURE 9 Scanning electron microscopy (SEM) of ML-CP 80 and ML-ILP 80
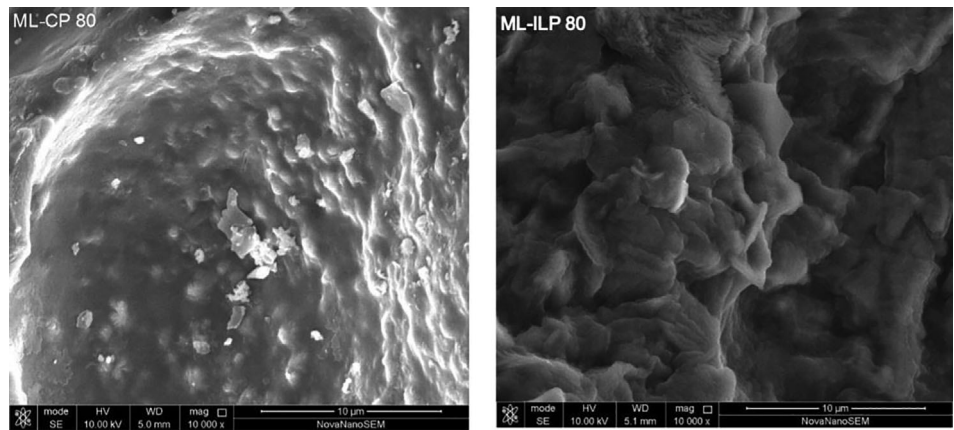
TABLE 6 Molecular weight of polysaccharides from leaves of *Moringa oleifera*

Samples	Molecular weight (g/mol)				
	M_n	M_p	M_w	M_z	M_w/M_n
ML-CP 80	207,000	197,100	304,700	391,700	1.472
ML-ILP 80	16,100	12,500	24,370	189,000	1.514

30 min. This may explain the absence of D-galacturonic acid in purified polysaccharides.

3.4 | Spectral analysis

3.4.1 | FTIR spectra

The functional groups of ML-CP 80 and ML-ILP 80 were studied using the FTIR Spectroscopy (Figure 6). The hydroxyl stretching vibration (Kaur, Lin, Fang, & Wang, 2006) was easily identified by the bands at 3,400 cm^{-1}. This peak is normally associated with carbohydrate compound and therefore confirms that samples to be carbohydrate. The stretching asymmetric vibration of C—H (Kaur et al., 2006) and the stretching symmetric vibration of C—H (Dopico & Tigyi, 2007) at the bands 2,929 and 2,843 cm^{-1}, respectively, was also recognized. These two bands indicate the size of alkyl group present. The carbonyl group displayed a high absorbance at 1,664 cm^{-1} (Sun et al., 2008) followed by the bending stretch of the C—H at the band 1,400 cm^{-1} (Kaur et al., 2006). The bands at 1,168 and 1,046 cm^{-1} suggested peak related to C—C stretching vibration and C—O stretching vibration, respectively (Zou et al., 2013).

3.4.2 | UV spectra

The UV spectra of ML-ILP 80 as shown in (Figure 7) displayed no absorption peaks at 280 and 260 nm, depicting the absence of nucleic acid and extremely low amount of protein (Cai, Xie, Chen, & Zhang, 2013). However, ML-CP 80 recorded some absorbance peaks at
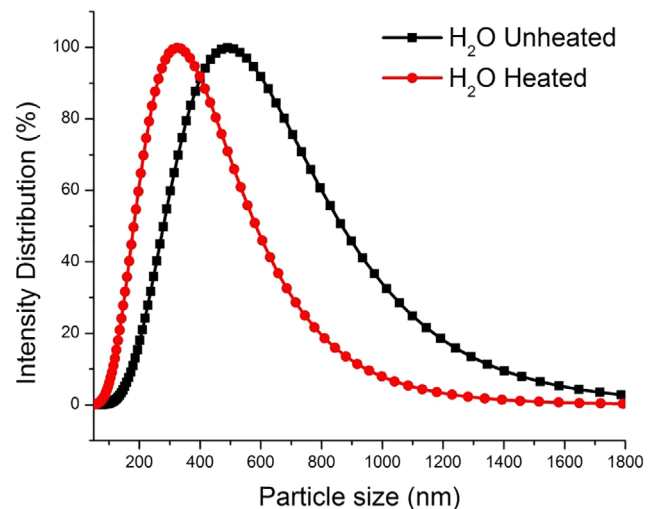

FIGURE 10 Particle size distribution of polysaccharide (ML-ILP 80) in aqueous solution with or without heat

280 and 260 nm, representing the presence of nucleic acid and protein. The results were in accordance of the protein content results (Table 5).

3.5 | Structural analysis

3.5.1 | Tertiary structure

Glucans with β-(1–6)-glycosidic bonds has been established to possess a triple helical tertiary structure. Whenever Congo red react with polysaccharides, the complex formed is characterized by a shift of the absorption of Congo red solutions to longer wavelengths (Villares, Mateo-Vivaracho, & Guillamón, 2012)—Bathochromic shift. Other research studies have also reported that specific transition from triple-helix conformation to single coil conformation possess the tendency to make the maximum absorption decrease to shorter wavelength in Congo Red polysaccharide solution (Semedo, Karmali, & Fonseca, 2015)—hypsochromic shift. Both ML-CP 80 and ML-ILP 80 displayed neither hypsochromic nor bathochromic shift,
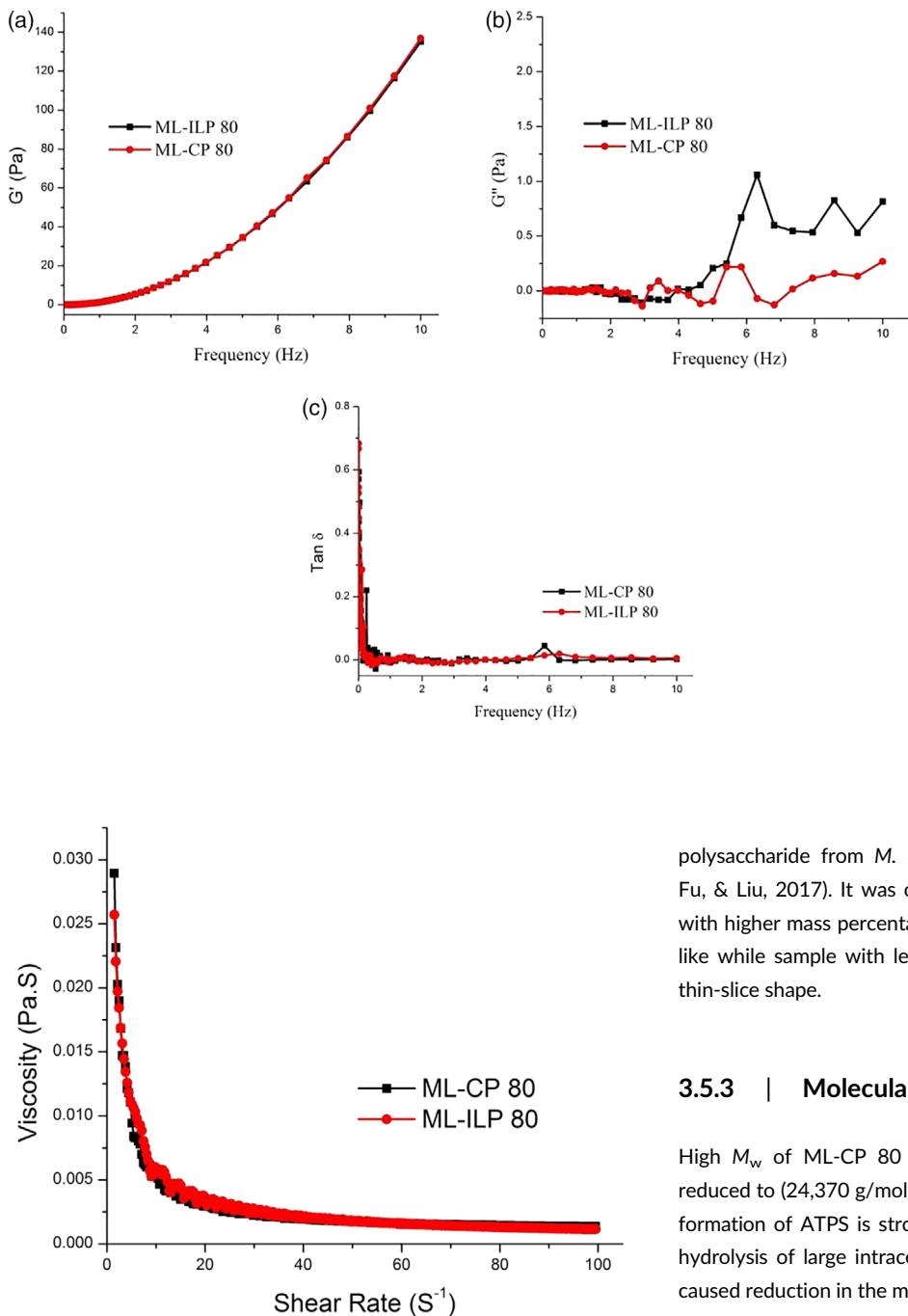
FIGURE 11 The storage modulus G' (a), loss modulus G'', (b) and loss tangent tan δ (c) in dynamic frequency sweep test of crude and ionic liquid aqueous two phase system (IL-ATPS) purified polysaccharide

FIGURE 12 Steady shear flow curves of crude and ionic liquid aqueous two phase system (IL-ATPS) purified polysaccharide

thus both samples demonstrated the absence of triple-helical structures (Figure 8).

3.5.2 | SEM analysis

The surface morphological structure of ML-CP 80 (Figure 9) displayed round group-like shapes, while ML-ILP 80 showed compact flat thick-slice shapes. The observed morphological structures was very similar to the shapes reported for microwave-assisted extracted polysaccharide from *M. oleifera* Lam. leaves (Chen, Zhang, Huang, Fu, & Liu, 2017). It was observed in the report that polysaccharides with higher mass percentage ratio of protein composition was group-like while sample with less protein mass percentage ratio displayed thin-slice shape.

### 3.5.3 | Molecular weight and particle size

High M_w of ML-CP 80 (304,700 g/mol) after purification process reduced to (24,370 g/mol) in ML-ILP 80 (Table 6). The salt used in the formation of ATPS is strongly alkaline in solution, thus slow alkaline hydrolysis of large intracellular and extracellular polymers may have caused reduction in the molecular weight.

The number of particle size distribution of ML-ILP 80 in aqueous solution is illustrated in Figure 10. In water, particle size distribution ranged from 265.39 to 911.18 nm. The main peak was detected at 491.52 nm. Heating the ML-ILP 80 aqueous solution for 1 hr 30 min at 70°C reduced the particle size distribution in terms of area from 172.47 to 615.19 nm with a peak diameter at 325.39 nm. This implies that ML-ILP 80 aggregated in water and that heat detached the large aggregate of the polysaccharide irreversibly.

3.6 | Rheological properties

Storage modulus (G') is concerned with deformation of polymers caused by an external force (Oechsle, Wittmann, Gibis, Kohlus, &

FIGURE 13 Scavenging effects: (a) hydroxyl radicals and (b) ABTS radicals of ascorbic acid, crude and ionic liquid aqueous two phase system (IL-ATPS) purified polysaccharide
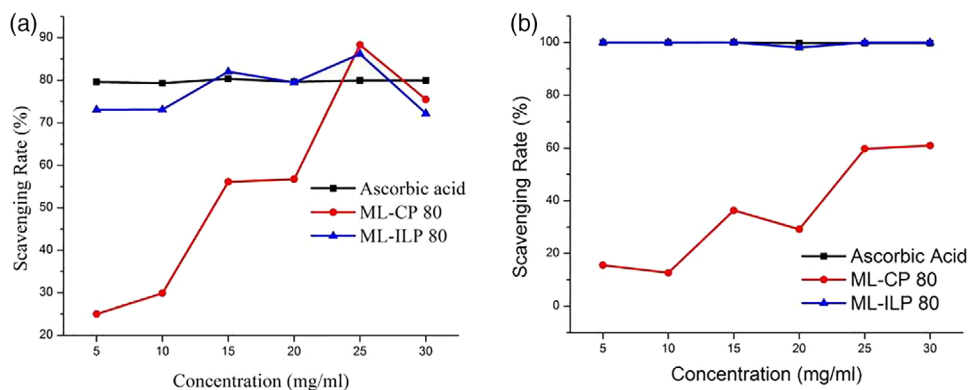
Weiss, 2014). As can be observed from (Figure 11a), both crude and purified polysaccharides extracted showed increase in storage modulus with increase in frequency (1–10 Hz), suggesting strong elasticity. The loss modulus (G''), on the other hand, is concerned with energy dissipation, a typical characteristic of viscous properties. As shown in (Figure 11b), ML-CP 80, with a comparatively higher molecular weight (Table 6) displayed stronger viscosity recording higher G'' values. This agrees with reports by Friess and Schlapp (2001). Figure 11c shows change in tan δ as a function of frequency. The tan δ values of both the crude and purified decreased rapidly under frequency from 0.1 to 0.5 Hz. At a frequency less than 1 Hz, a tan δ < 1 was recorded. Materials with tan δ > 1 shows more damping effect because the loss modulus of the material is greater than the storage modulus, which depicts dissipation of energy, thus viscous component of the complex modulus dominates the material (Korhonen, Hirvonen, & Yliruusi, 2001). This means that both crude and purified polysaccharide proved to be elastic in nature.

The viscosities of ML-CP 80 and ML-ILP 80 dispersions also showed decrease speedily with increase in shear rate (Figure 12), a characteristic of pseudoplastic fluids displaying shear thinning flow behavior.

3.7 | Antioxidant activity

3.7.1 | Scavenging effect on hydroxyl radical

Reactive oxygen species (ROSs) are chemically reactive molecules comprising oxygen which includes; peroxide, superoxide, hydroxyl radical, and singlet oxygen, can damage lipid, DNA, RNA, protein, and thus cause impairment of the physiology of aging, brain dysfunction, liver diseases carcinogenesis, and cardiovascular disorder (Sun, Li, Yang, Liu, & Kennedy, 2010). Among these ROS, hydroxyl radical is much effective in damaging biomolecules (Ke et al., 2009), hence the need for its removal from living system.

The hydroxyl radical scavenging activity of ascorbic acid, ML-CP 80, and ML-ILP 80 at different concentrations (5–30 mg/ml) is shown in (Figure 13a). At a concentration of 15 mg/ml, the scavenging rate of ML-ILP 80 and ascorbic acid was close (above 80%). At a

concentration of 25 mg/ml, the scavenging rate of ML-CP 80 and ML-ILP 80 also showed to be very close (above 85%). The IC$_{50}$ of ascorbic acid, ML-ILP 80, and ML-CP 80 on hydroxyl radical were found to be 5, 13, and 15 mg/ml, respectively.

3.7.2 | Scavenging effect on ABTS radical

Figure 13b shows that ABTS radical scavenging activity of ascorbic acid, ML-CP 80, and ML-ILP 80 at different concentrations (5–30 mg/ml). Aside concentration 20 mg/ml, ML-ILP 80 demonstrated scavenging rate of (100%). Calculated IC$_{50}$ of ascorbic acid, ML-CP 80, and ML-ILP 80 on ABTS radical were 5 mg, 21.80, and 5 mg/ml, respectively. This indicated that ML-ILP 80 had strong ABTS radical scavenging strength as ascorbic acid.

4 | CONCLUSION

The purified polysaccharide extracted into the bottom phase salt, K$_2$CO$_3$ of the aqueous phase two system with the aid of the optimum operating parameters of ultrasound was (75.11%) within 30 min, while purified polysaccharide extracted without UAE was (38.82%) establishing a difference of (36.29%). The molecular bonds within the purified polysaccharide were maintained but with a comparatively lower molecular weight (24,370 g/mol). The purified polysaccharide aggregated in water and showed a close to zero-associated percentage protein (0.64%). The nonhelical structure and dominance in elasticity in terms of its rheological properties was also maintained after purification. However, the ILATPS purified polysaccharide showed higher scavenging ability against ABTS radicals than on hydroxyl radicals. Synergizing ultrasound and ILATPS for polysaccharide purification process can thus be scaled up for an environmentally friendly and faster achievement of targets.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL STATEMENT

Ethics approval was not required for this research.

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