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Functional characterization and evaluation of multi-biological activities of water soluble polysaccharide from *Periploca laevigata*.

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Abstract

The water-soluble polysaccharide was isolated from *Periploca laevis* root barks and purified. The structural, surface, functional properties, and biological activities of *P. laevis* polysaccharide (PLP1) were investigated. Scanning electron microscopy (SEM), X-ray diffraction crystallography (XRD), were used for structural characterization of PLP1. SEM analysis suggested that the polysaccharide had irregular particle size with a large number of cavities, mostly seen as aggregates, and fibrous in nature. The samples had peaks at approximately 22°, and 38° 2θ degrees in the XRD pattern, which indicated both crystalline and amorphous structure. Functional properties of PLP1 were investigated based on water and oil holding capacity, solubility, emulsification activity, and foaming ability. Interestingly, PLP1 exhibited, a noticeable ability to emulsify corn and olive oil. Experimental analysis indicated that PLP1 able to decrease surface tension of water of 67.5 mN/m to 43.7 mN/m. On other trend, bioactivities evidenced that PLP1 is a potent protease and lipase inhibitor. The cell viability proved the cytoprotective effect of PLP1 on cell treated with hydrogen peroxide H₂O₂, suppressing apoptosis and ameliorating oxidative lesions. The experimental data and multi functionality provides enough evidence and open many new possibilities for biomedicine and food applications.

Key words: Water soluble polysaccharide; Functional properties; Cytotoxicity; .

56 **1. Introduction**

57 Polysaccharides are biopolymer constituted by repeating subunits linked together by
58 glycosidic bonds. These macromolecules, are frequently existed in plants and algae, play a
59 prominent role in the development of green products including pharmaceuticals, packaging
60 materials and foods [1,2]. Recently, the water-soluble polysaccharides (WSP) have
61 developped a great deal of interest cause of their bioactivities such as antibacterial,
62 hypoglycemic, and hypolipidemic potential [3,4]. In fact, the functions of this
63 macromolecules are strongly linked to their chemical composition, tertiary structure, linkage
64 type, branch degree, and molecular weight.

65 Oxidation of polyunsaturated fatty acids, resulting from storage conditions and/or
66 during processing, is among the major factors that lead to the allteration of food quality and
67 the aproduction of toxic compounds [5]. likewise, it has been demonstrated that antioxidants
68 are important in the pre-theurapie of cancer, biological effect in living tissues, cardiovascular
69 and neurodegenerative diseases, where the Reactive Oxygen Species (ROS) are the main
70 effectors [6]. Among the synthetic antioxidants the most most commonly implicated in
71 stabilization of foods, are butylated hydroxyanisole (BHA) and butylated hydroxytoluene
72 (BHT) which are very effective in the prevention of oxidative damages [7]. However, the use
73 of these chemical compounds that undoubtedly induced genotoxicity and DNA damages, has
74 become restricted [8]. From the health point of view, governmental authorities and
75 consumers are concerned to checked food additives for their potential harmful effects [9].
76 Therefore, it is extremely important to develop green antioxidant to overcome the limits of
77 synthetics compounds. Due to its high stability and biosafety plant-derived polysaccharides
78 has often served as an effective natural antioxidants in pharmaceutical and food fields [10].

On the other hand, microbial contamination associated with food borne diseases is the major challenge of the third world and developing countries, and even in developed nations [11]. The consumption of foods contaminated with some microorganisms is closely related to the appearance of serious human health risk. Thereby, The subsistence and multiplication of microorganisms in foods may lead to obtaining food products with high toxicity and low quality [12]. In this approach, several researchers were challenged to discover green molecules characterized by number of advantages in comparison to other food additives and preservatives including the high quality and the low cost, which are important issues faced by the food industry.

P. laevigata that belongs to the Asclepiadaceae family, is very abundant species in North Africa. In Tunisia, this plant is widely grown in the steppes of desert, arid and semi-arid areas. It has been extremely used as medicinal plant in both modern and traditional medicine. Further, It is reported that *P. laevigata* polysaccharides have high antioxidant and antibacterial activities [3]. However, there is no systematic study on the functional properties and anti-lipase and anti-proteases activities of *P. laevigata* polysaccharides.

2. Materials and Methods

2.1. Chemicals and enzyme

Bovine haemoglobin, trichloroacetic acid (TCA), were purchased from Sigma Chemical Co. (St. Louis MO, USA). The lipase used was lipase type VII from *Candida rugosa* (EC 3.1.1.3) from SIGMA. The trypsin and pepsin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals and reagents used were of analytical grade. Dulbecco's Modified Eagle's Medium (DMEM), Dulbecco's, Phosphate Buffer Saline (DPBS), antibiotic

mixture (streptomycin, penicillin), and trypan blue solution were purchased from Lonza (Cologne GmbH, Germany).

2.2. Functional properties

2.2.1. Solubility

The solubility of PLP1 was determined according to the method of Mirhosseini et al. [13]. Based on this method, 0.2 g of PLP1 was resuspended in 20 ml distilled water. The prepared sample was placed at room temperature for 30 minutes and homogenized at intervals. Afterwards, the sample was centrifuged for 15 minutes at 3000 rpm. 10 ml of the upper clear solution was removed and transferred to an oven at 105 °C. Then after, the supernatant was completely dried and weighed. Solubility of PLP1 was calculated using the following equation:

$$\text{Solubility (\%)} = \left(\frac{C2}{C1} \right) * 100$$

C1: Initial weight; C2: Final weight

2.2.2. Water-holding capacity (WHC)

Using the method [14] to evaluate the WHC of polysaccharide, 0.250 g of PLP1 was placed in a centrifuge tube and dispersed in 25 mL of distilled water. The obtained solution was stirred, left at room temperature for 30 min and followed by centrifugation for 15 min at 3000 rpm. The supernatant was carefully decanted, and the sample was reweighed.

WHC was expressed as g of absorbed water per g of PLP1.

2.2.3. Oil-holding capacity (OHC)

OHC was recovered by the method of Lin et al. [14]. PLP1 (0.5 g) was dispersed in 10 ml of corn or olive oil to determine OHC. The mixture was kept at room temperature for 1 h and then centrifuged at 6000 rpm for 30 min. The upper phase was removed carefully and the

centrifuge tube was drained for 30 min on a Whatman N° 1 filter paper after tilting to a 45° angle.

OHC was reported as g of oil bound per g of the PLP1.

2.2.4. Emulsifying activities

PLP1 emulsifying activities was assayed as reported by Freitas et al.[15]. Different oils (6 ml) were added to PLP1 aqueous solution (4 ml, 2% (w:v)) in a test tube (10 mm diameter × 70 mm) and stirred in the vortex at 2400 rpm for 2 min (at room temperature). After 1, 24 and 168 h, respective emulsification indexes E1, E24 and E168, were calculated as follows:

$$Et = \left(\frac{he}{ht} \right) * 100$$

where he (mm) is the emulsion layer height and ht (mm) is the mixture overall height after t hours.

2.2.5. Microscopic assessment of emulsions

Emulsion samples were examined under a light microscope. Photographs were taken after 24 h of emulsion storage at 4 °C through a 40 × objective lens.

2.2.6. Foaming properties

Foam capacity (FC) and stability (FS) of PLP1 were determined according to the method of Shahidi et al [16]. Twenty milliliters of PLP1 solution at different concentrations (0.5%, 1%, and 2%; w/v) were homogenized using a vortex to incorporate the air. The whipped sample was immediately transferred into a graduated cylinder, and the total volume was immediately measured after whipping at 0 min, and 60 min.

Foam capacity was expressed as volume increase percentage after homogenization at 0 min, which was calculated according to the following equation:

$$FC (\%) = \left(\frac{V_T - V_0}{V_0} \right) * 100$$

Foam stability was calculated as the foam remaining volume of after 60 min.

Where VT is the total volume after whipping (ml); V0 is the volume before whipping; Vt is the total volume after leaving at room temperature.

$$FS (\%) = \left(\frac{V_t - V_0}{V_0} \right) * 100$$

2.3. Surface tension measurements

The surface tension of PLP1 (1 to 5 g/100 mL) was evaluated according to the method of Wilhelmy plate using a manual digital surface tensiometer (GIBERTINI Elettronica™ TSD Digital Tensiometer).

2.4. X-ray diffraction (XRD)

X-ray powder diffraction patterns of PLP1 were recorded at room temperature on an X-ray diffractometer (D8advance, Bruker, Germany). Samples were scanned between diffraction angles of 5 to 80° 2θ with a step size of 0.05° and accounting time of 5 s/step.

2.5. Scanning electron microscopy

The morphological features of the PLP1 were visualized using a scanning electron microscope (Cambridge Scan-360 microscope). Briefly, in order to make the dried PLP1 conductive, the sample was mounted on a metal stub and sputtered with gold. The images of sample were taken at an accelerating voltage of 3.0 kV.

2.6. Bioactivities of PLP1

2.6.1. Lipase inhibition

The lipase activity was measured titrimetrically at pH 7.2 and 37°C with a pH-Stat (Metrohm, Switzerland) using olive oil emulsion. One lipase unit corresponds to 1 μmol of fatty acid released per minute.

A preincubation of lipase with inhibitor was used in order to determine the lipase inhibitory activity of PLP1. This method aimed to test, in an aqueous medium and in the

absence of the substrate, the possible reactions between the lipase and the inhibitor. Lipase was pre-incubated at room temperature for 1 h with different concentrations of the inhibitor dissolved distilled water. The reaction medium contained the correspondent inhibitor concentration and 100 μ L of lipase. A negative control (distillated water) was incubated in the same medium than the test of inhibition without the inhibitor. The lipase inhibition (% Inhibition) was calculated as compared to the initial activity measured in the absence of inhibitor.

2.6.2. Protease inhibition

The trypsin inhibitor activity was determined using casein (1%) as substrate [17]. Enzyme solution (40 μ g of trypsin) was pre-incubated with different concentration of PLP1 in a total volume of 2 ml at 37 °C for 10 min in 0.01 M Tris-HCl buffer, pH 8.0. Absorbance of the mixture was measured at 280 nm and the residual activity was determined. Trypsin inhibitory unit is defined as the number of trypsin units inhibited under the assay conditions.

The inhibition of pepsin activity was performed according to Anson method [18], using haemoglobin as a substrate at pH 3.0 and 37 °C for 15 min. To 100 μ l substrate was added 50 μ l of pepsin in 0.1 M glycine-HCl buffer, and 350 μ l of PLP1 in water at different concentration. Pepsin inhibitory unit is defined as the amount of enzyme units inhibited in the absorbance at 280 nm per minute under the assay conditions.

The activity of the enzymes (trypsin and pepsin) assayed without PLP1 was taken as control (100% activity).

2.7. In vitro antioxidant effects of PLP1 on H9c2 cells

2.7.1. Cell culture

Embryonic rat cardiomyocyte derived cell line H9c2 was obtained from America Tissue Type Collection (CRL-1446). Cells were cultured in DMEM medium supplemented with 10%

fetal bovine serum, 100 U/ml penicillin, and 100 lg/ml streptomycin in 75 cm² tissue culture flasks at 37 °C in a humidified atmosphere of 5% CO₂. They were fed every 2–3 days, and sub-cultured when reaching 70–80% confluence in order to prevent the loss of the differentiation potential. Cells were then differentiated into cardiomyocytes by methods described by Ménard et al [19].

2.7.2. Cell viability

Cell viability was determined using an MTT assay. H9c2 cells were plated in 96-well plates at 200 H9c2 cells/well. After cell differentiation, cells were treated with water-soluble polysaccharide (PLP1) at various concentrations (0.5, 1, and 2 mg/ml) or with DMSO used to dilute the PLP1. To evaluate the cytoprotective assay, cells were exposed to H₂O₂ (0.1 mM) as well as PLP1 at the same time for 12 h at 37 °C. At 12 h after the treatment, the medium in the wells was replaced with 5 mg/ml MTT solution, and the cells were incubated for 2 h at 37 °C. Following incubation, the MTT solution was discarded, and 100 µl DMSO was added to dissolve the precipitate completely at room temperature. The optical density was then measured at 540 nm. The cell viability was expressed as relative viable cells (%) to control H9c2 cells.

2.8. Statistical analysis

Analysis was performed using SPSS (Version 17.0 for windows, SPSS Inc., Chicago, IL, USA). DATA were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by Duncan's multiple range test ($p < 0.05$). All tests and measurements were carried out in triplicate

3. Result and discussion

3.1. Functional properties

3.1.1. WHC, OHC and solubility

WHC and OHC are among the most popular functional properties in food processing, which are closely related to texture through the interactions between components, including water and oil ²⁰. Functional characteristic of PLP1 are presented in table. 1.

As it can be seen, for the WHC the value determined for was of ~7.92 g water/g PLP1, while OHC value was of ~3.57 g oil/g PLP1. The WHC value was significantly lower than those of polysaccharides from *Nostoc flagelliforme* (27.82 g/g) [20], *Gigartina pistillata* (10.22 g/g) [21], higher than that from watermelon rinds (2 g/g) [22]. Similar value of WHC was observed with polysaccharide from potato (8 g/g) [23]. A pioneering study exploring the functional properties of polysacchrides, published by Elleuch et al. [24] highlighted that the high WHC of polysaccharide present a key parameter to avoid syneresis and modify the viscosity and texture of some formulated foods. From table 1, PLP1 also showed the highest OHC with the value of ~23.57 g/g, which was much higher than that polysaccharide from black cumin seeds (2.86 and 1.26 g/g at the temperature 25 °C and 75 °C) [25].

As for OHC, it is partially correlated to the chemical composition, but it is more closely linked to and polysaccharide source to the porosity of the fiber structure than to the affinity of the fiber molecule to oil [26]. Solubility defined by Song et al. [27] as a typical representative of product behavior in aqueous phase and the common standard that determine the quality of powder reconstitution. From the result presented in table 1, we found that the solubility value is arround 78.34%, wich is slightly lower than that of polysaccharide from *Vigna radiate* L.(~88.8%) ²⁷. Researches conducted by Ahmadi et al. [28] showed that the interaction between hydrocolloids and water molecules is influenced by the hydrogen bonding, and particle size.

3.1.2. FC and FS of PLP1

The foaming property is a surface property defined by its size and stability. High-performance foaming agents are widely required in the food industry [29]. Polysaccharides as an important food macromolecule, play a crucial role in the stabilization of foams [30]. The foaming properties were investigated in terms of FC and FS shown in figure 1.

It can be observed, PLP1 accounted a FC between 27.5% and ~49.7% whereas the FS varied from ~25.5% to ~41.25% ($p < 0.05$), depending on the concentration. Our results were in line with earlier reports of polysaccharides extracted from *Trigonella foenum-graecum* [31]. According to Shen et al. [20], the foam capacity and stability of *N. flagelliforme* is related to the molecular weight, to the concentration and source of polysaccharides. The important FC and FS of PLP1 may be associated with their ability to increase the viscosity of the aqueous phase and to create a network that stabilizes the interfacial film. The flavor, texture, and shelf life of food can be greatly enhanced by using the polysaccharides as gelling or thickening agents for stabilizing dispersions.

3.1.3. EC and ES of PLP1

Knowledge of the emulsions properties of polysaccharide is an important parameter in determining its suitability in food formulations. Figure 2 illustrates the emulsion properties that include the emulsion capacity (EC) and emulsion stability (ES) of PLP1.

Interestingly, PLP1 exhibited, after 1 h, a noticeable ability to emulsify corn oil and olive oil, whose index value (E1) was 96.13% and 75.63%, respectively. Tan and Gan [32] reported that emulsiant activity of polysaccharides from *Momordica charantia*, where the emulsion prepared using palm oil at the same concentration, was 66.4%. However, at a concentration of 0.5% the EC of polysaccharide extracted from *Trigonella foenum-graecum* seeds, reported by Ktari et al. [31], was 79.08% and 62.66%. Consistent with the previous reported observations³³, the emulsifying ability of polysaccharides depends on the amount of protein linked to their

structure. From previous study of Hajji et al. [3], we found that the protein content for PLP1 is less than 0.1%, which could explain the low EC compared to that of exopolysaccharide from *Lactobacillus sp.* Ca6 [34], which is around 100%. In addition, it should be highlighted that higher concentration would result in a higher EC because of the presence of high amounts of polysaccharide that can completely saturate the oil–water interface, thus resulting in higher surface coverage. The ES of PLP1 decreased after 168 h and reached about 62.47% and 52.13% for corn oil and olive oil, respectively. Ben Slima et al [35] stated that emulsion stability had a positive effect on its physical stability, such as flocculation, gravitational separation and coalescence. ES was closely related to the size of the droplets in an emulsion based on food product.

The size of the droplets is therefore useful to accurately specify droplets size which are present within an emulsion. Thus, microscopic evaluation was performed on PLP1 corn oil and olive oil emulsions (Fig. 2B). As shown by emulsion's light micrographs, smaller and more dense droplets were observed in the emulsions prepared with corn oil, resulting in more stable emulsions, as reported by Maalej et al. [36]. Among the characteristics required for a polysaccharide to be most effective in stabilizing emulsion droplets, is the high-molecular weight of biopolymer [37]. According to our previous study³, minor proteinaceous fraction combined with its high-molecular weight, contribute to the ability of PLP1 to act as emulsifying and emulsion.

3.2. SEM analysis of PLP1

SEM analysis was used to illustrate the surface morphology of extracted polysaccharides. As presented in figure 3, and their various derivatives at 55 and 160 times magnification to evaluate the microstructures of PLP1.

SEM showed that PLP1 existed in closely aligned particles with a smooth surface, which confirmed that PLP1 is amorphous under vacuum freeze-drying process. The shape and structure, or the surface topology of the macro-biomolecules including polysaccharide may be affected by the method of extraction and purification or preparation of the product [38].

3.3. Surface tension of PLP1

The evaluation of the potential of PLP1 to reduce surface tension at the air-water interface, was made by determining their surface activity at different concentrations. Figure 4A illustrates that PLP1 surface-active macromolecule, since it is able to reduce interfacial tension relative to water (67.5 mN/m). In the scope of the testing concentration (1–5 g/100mL), the surface tension of PLP1 was concentration-dependent, which is corresponded with the results of polysaccharides from black cumin seeds [25].

From the results of surface tension, PLP1 had the greatest surface activity (43.25 mN/m) at the highest concentration (5 g/100 mL) which revealed a rapid diffusion into the air-water interface. It was likely that the surface tension of polysaccharide was strongly related with the amphiphilic character, means that polysaccharide can be adsorbed and oriented at liquid–liquid interfaces to reduce efficiently the interfacial tension.

3.4. XRD analysis of PLP1

XRD is an effective analytical tool employed to evaluate the crystalline characteristics and unraveling the structure of the polysaccharides, which can predict their various properties like solubility, swelling, flexibility, and other physical properties [39].

By studying the data obtained from the figure 4B, PLP1 showed a peaks at approximately 16°, and 37° 2θ degrees.

Consequently, it can be stated that the polysaccharide purified from *P. laevigata* (PLP1) exhibits both crystalline and amorphous portions. This finding is congruent with the

crystalline/amorphous contrast obtained by XRD of purified polysaccharides from *Musa sapientum* L. and *Vigna radiate* L. [27,40], but it is far different from the pattern of okra polysaccharide ⁴¹.

3.5. Bioactivities of PLP1

3.5.1. Effect of PLP1 on cell viability

The cytoprotective effects of PLP1 against hydrogen peroxide induced cardiomyocyte toxicity was evaluated (Figure 5).

The viability of control cells (no PLP1 or H₂O₂ added) was considered to be 100%. It was observed that at concentration of 0.5 mM H₂O₂ significantly reduced the cell viability and the total viable cells were 9.11%. The PLP1 solution at a concentration of 2 mg/mL, was found non-toxic to H9c2 cells, and the viability of cells was 97.12%. It was found that PLP1 exhibited significant (p<0.05) cytoprotective potential in a dose-dependent manner. In MTT assay, PLP1 increased the viability of H₂O₂-treated cells, thus attenuated the cytotoxic effect of H₂O₂ and provided protection against oxidative stress-induced cytotoxicity

3.5.2. Anti-protease activity of PLP1

As proteases are important in tumor growth and spread, the effect of PLP1 was tested on two selected proteases (Trypsin and Pepsin). The effect of PLP1 on acidic and alcalin proteolytic activity is shown in Fig. 6B.

The results suggested that PLP1 had a strong inhibitory effect on proteolytic activity of pepsin and trypsin. The acidic proteolytic enzyme retained about 21.5 % of their initial activities, after incubation with 2 mg of PLP1. At the same concentration trypsin was lost the totality of its activity. Furthermore, enzyme activity was decreased in concentration depending-manner. The obtained results were in agreement with several works reported in the literature with regards to the anti-protease effects of polysaccharides [42,43]. In a recent study, Wang et al. [43] reported that there may be multiple ways in which polysaccharides

affect protease activity and protein digestibility. The decrease in proteolytic activity may have been due to the ability of polysaccharide to reduce protein digestibility to some extent, possibly because of their monosaccharide compositions.

It was reported that there was a relationship between polysaccharide viscosity, protease activity⁴³ and reduction of protein digestibility [44]. Therefore, there may be multiple mechanisms by which polysaccharides affect protease activity.

3.5.3. Anti-lipase activity of PLP1

The plant kingdom have large scope to be used as an effective oral antiobesity or weight loss agents that have insignifiants side effects and that are costeffective alternative to synthetic drugs [45]. The inhibitory activity towards pancreatic lipase displayed by the PLP1 is illustrated in Fig. 6B. The analysis of pancreatic lipase activity after addition of polysaccharide, highlights that PLP1 significantly ($p < 0.5$) decreased lipase activity, with a dose–response manner. A total inhibition of lipase was obtained with concentration of 3 mg/ml (PLP1). Interestingly, PLP1 exhibited strong lipase inhibitory effects with IC_{50} values of 0.59 mg/ml. This result suggest that the new purified polysaccharide from *P. laevigata* root barks could be potential candidate specifically for the extraction of antiobesity green molecules. Our finding are in high concordance with results reported by Tsujita et al. [46] who indicated that a basic polysaccharide is able to bloked dietary fat absorption from the small intestine by inhibiting pancreatic lipase activity to get the purpose of losing weight. Hu and co-authors [47,48] suggest that based on their structure, including glycosidic bonds, monosaccharide composition, and anionic groups, polysaccharide can inhibit pancreatic lipase activity. Therefore it is ensured that the molar ratio of 1,4-linked- β -D-Glcmanp which in turn affect the activity of pancreatic lipase, as an increasing percentage of mannose decreased pancreatic lipase activity.

Tsujita et al. [49] suggest the paramount importance of extent of polymerization as a key factor in the lipase inhibition.

Conclusion

In this study, a structural characteristics, functional properties and bioactivities of a novel purified polysaccharide from *P. laevigata* root barks (PLP1) were investigated. The spectroscopic analyses by SEM and XRD, showed that PLP1 corresponded to biopolymer with crystalline and amorphous structure. Furthermore, PLP1 exhibited good functional properties, particularly for emulsion forming and stabilizing capacity, under certain conditions. Intestinal functions were characterized, and the results indicated that PLP1 could significantly improve intestinal functions, including significantly lowering protease and pancreatic lipase activities. Furthermore, the bioactivity studies demonstrated that PLP1 could slightly promote the cell viability. In addition, PLP1 exerted significant protective effects against H₂O₂-induced oxidative injury in H9c2 cells. The obtained results demonstrated the promising potential of PLP1 for different applications.

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Conflict of interest : The authors declare that they have no conflict of interest.

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LITERATURE CITED

Figures

Fig. 1. Foaming capacity (FC) and stability (FS) of PLP1

Fig. 2. Emulsification activity (A) and photomicrographs of emulsions (B) prepared with olive and corne oils. All emulsions were prepared and left at room temperature for 1, 24 and 168 h to determined E1, E24 and E168, respectively.

Fig. 3. Scanning electron microscopy (SEM) micrograph of water-soluble polysaccharide (PLP1) at different magnifications: 100 μm magnification (a), 200 μm magnification (b).

Fig. 4. Surface tension of water-soluble polysaccharide (PLP1) as a function of concentration (A). X-ray diffraction pattern of PLP1 (B).

Fig. 5. Effects of water-soluble polysaccharide (PLP1) on viability of H_2O_2 -treated H9c2 cells.

Fig. 6. Anti-protease and anti-lipase activity of water-soluble polysaccharide (PLP1) as a function of concentration

Figures:
Fig.1

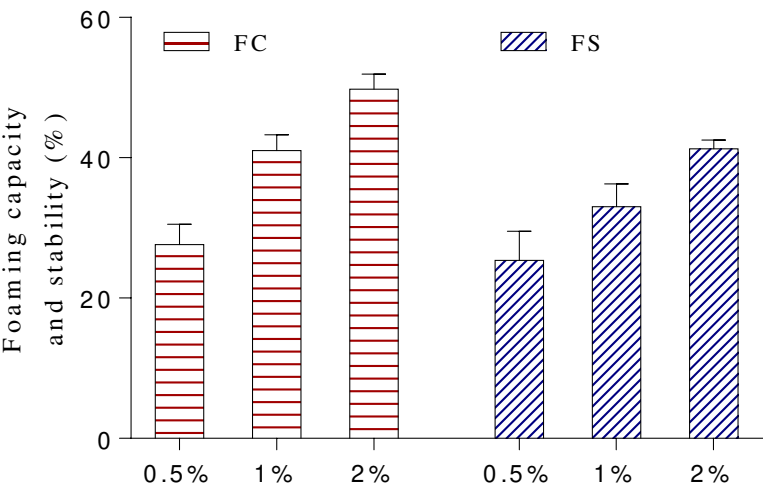


Fig.2

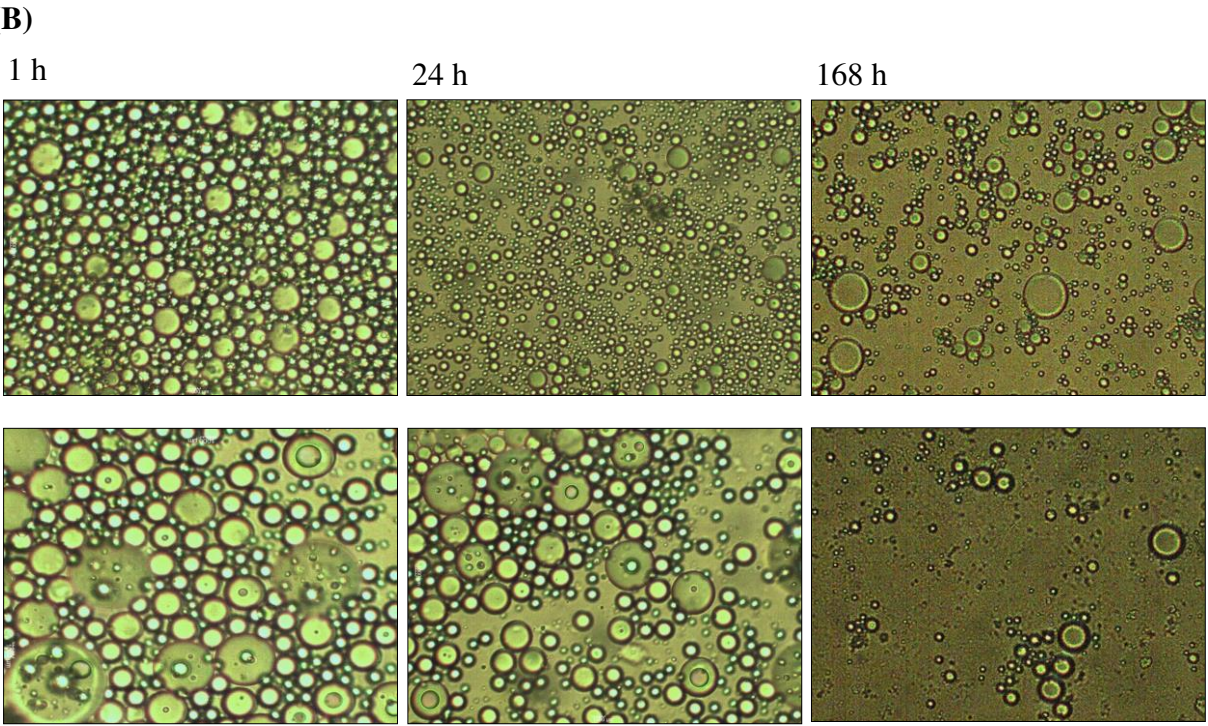
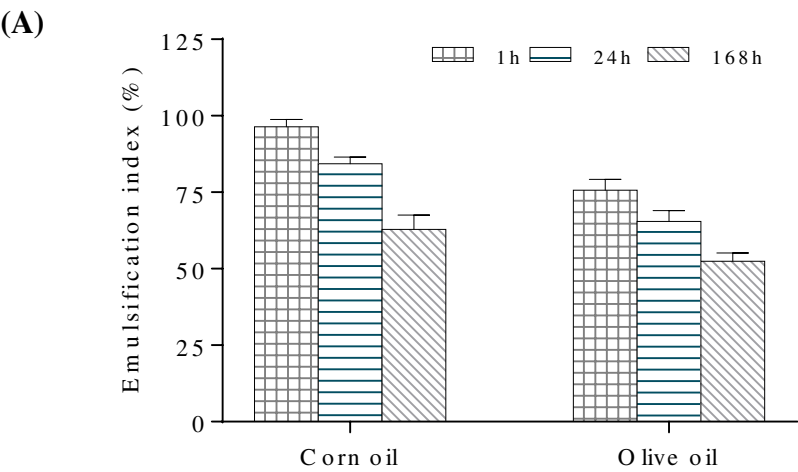
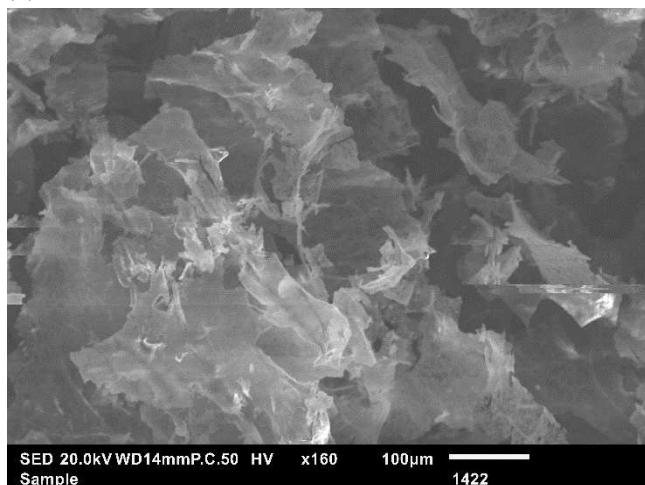


Fig.3.

(a)



(b)

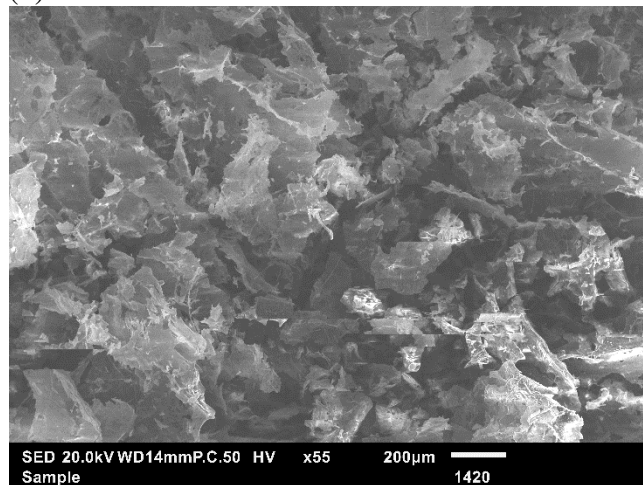
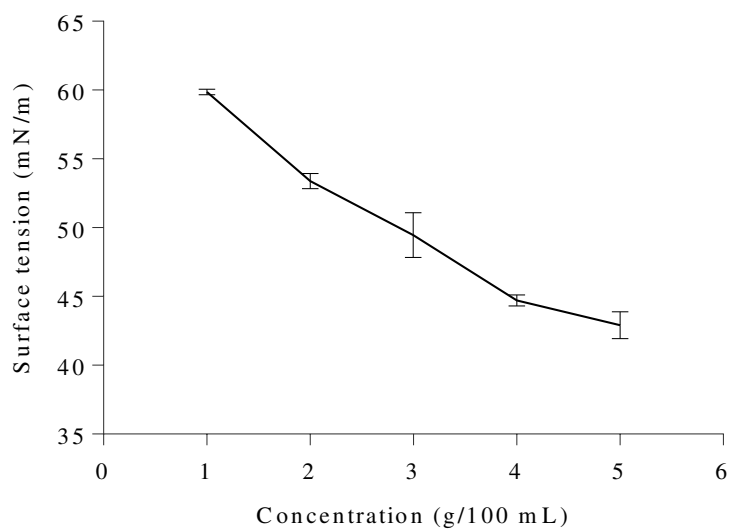


Fig. 4

(A)



(B)

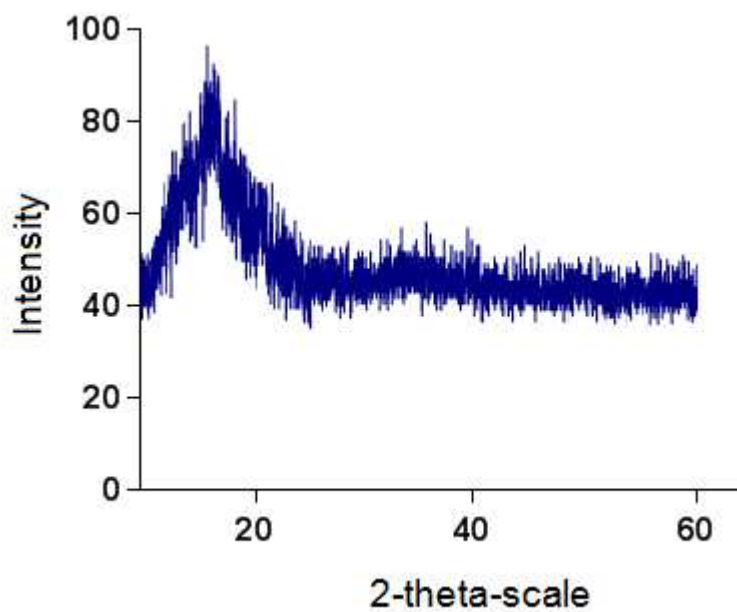


Fig.5

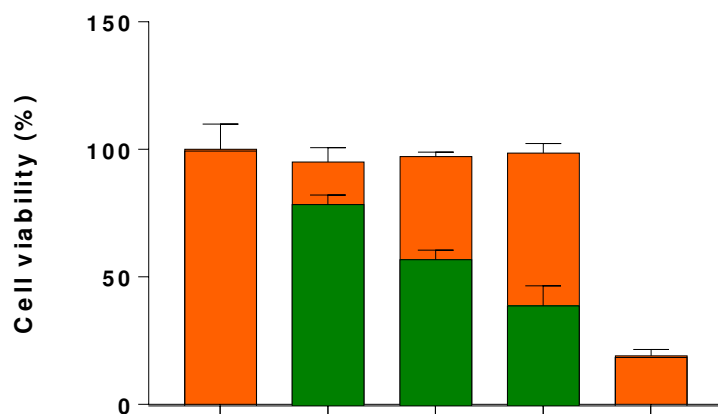
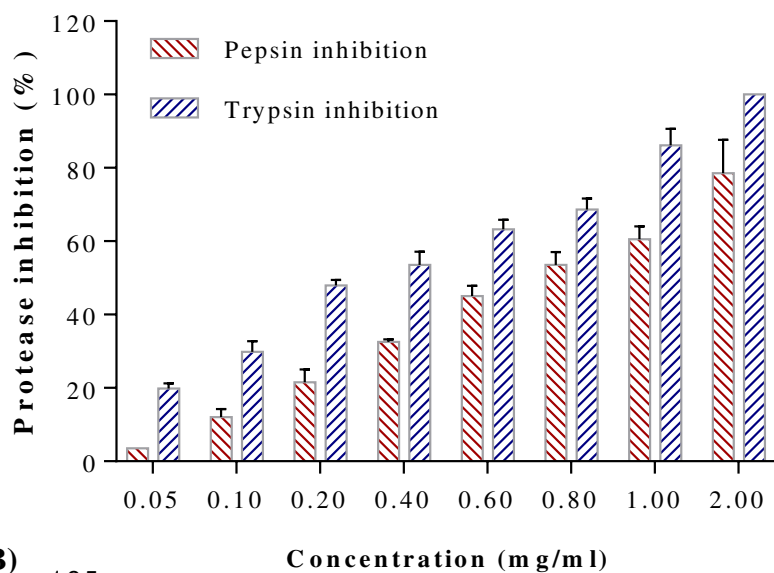


Fig.6

(A)



(B)

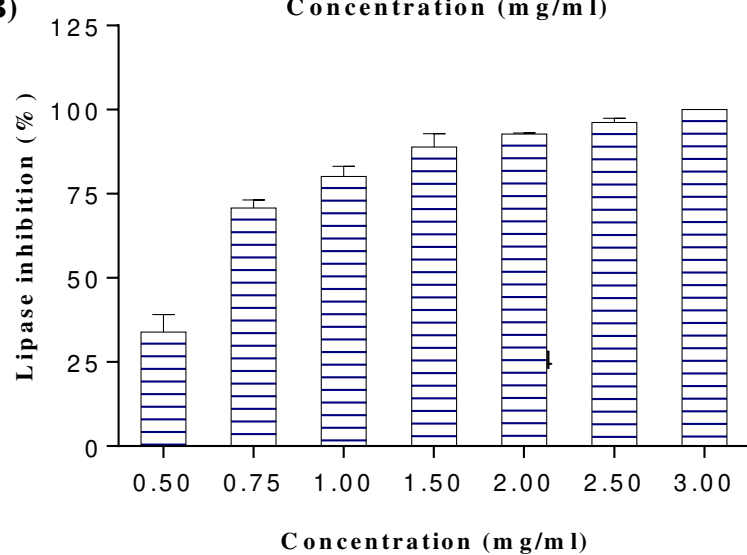


Table 1:

Water-holding capacity, oil-holding capacity and solubility of PLP1

Properties	Capacities
Water-holding capacity (g/g)	7.92±0.85
Oil-holding capacity (g/g)	23.57±0.43
Solubility (%)	78.34±2.47

Values are given as mean of three determinations ($X \pm SD$); SD: standard deviation