Applied Biological Research 27(1): 12-25; (2025)
DOI: 10.48165/abr.2025.27.01.2

CHARACTERIZATION AND ANTIOXIDANT, ANTIDIABETIC AND IMMUNOSTIMULATING EFFICACY OF POLYSACCHARIDE EXTRACT FROM *Syzygium cumini* (L.) SEEDS

Jegadeshwari Bhakaran[1,2] and Sundara Dhakshinamurthy Saraswathy[1*]

[1,*]*Department of Biomedical Science,* [2]*Department of Marine Science, Bharathidasan University, Tiruchirappalli - 620 024, Tamil Nadu (India)*
*****e-mail: *sdswathy@bdu.ac.in*

(Received 14 October, 2024; accepted 3 February, 2025)

ABSTRACT

***Syzygium cumini* L., belonging to the family *Myrtaceae*, is an edible fruit, well known for its nutritional values and possess several beneficial effects on human beings. The present study was aimed to isolate the polysaccharide from *S. cumini* seeds and assess its biological activities. The *S. cumini* polysaccharide (SCP), extracted by hot water extraction process followed by ethanol precipitation, was subjected to physiochemical characterization and *in vitro* antioxidant, hypoglycaemic and candidacidal assays. The UV-visible spectrum analysis confirmed the extract to be protein-free polysaccharide, while Fourier transform infrared spectroscopy (FTIR) revealed polysaccharide's functional groups. The scanning electron microscopy (SEM) images depicted smooth and non-porous surface morphology, while thin layer chromatography (TLC) indicated the presence of monosaccharide constituents of crude polysaccharide hydrolysates. SCP showed higher total antioxidant capacity and greater radical scavenging activity in DPPH, ABTS and FRAP assays. Also, the extracted SCP effectively inhibited α-amylase and α-glucosidase, a carbohydrate metabolizing enzymes. Besides, SCP enhanced the intracellular killing activity of stimulated neutrophils and neutrophils candidacidal activity in a dose-dependent manner. The study showed that the isolated polysaccharides from *S. cumini* seeds possess imperative antioxidant, antidiabetic and immunostimulatory effects.**

Keywords: Antioxidant activity, α-amylase, candidacidal effect, α-glucosidase, polysaccharides, *Syzygium cumini*

INTRODUCTION

Polysaccharides are natural biopolymers, which play major role in regulating and supporting the developmental and metabolic processes in plants (Delattre *et al*., 2011; Liu *et al*., 2020). They exist both in simple and complex glycol-conjugated forms and display structural and functional significance. Their complex structure includes diverse functional groups mainly hydroxyl, carboxylate, amino, ester, and sulphate groups, which exhibit excellent biological actions and are often used in food and nutraceutical industry (Yadav and Karthikeyan, 2019). The bioactivity of polysaccharides is related to their physicochemical and structural properties like molecular weight, monosaccharide composition, and glycosidic bond (Thakur, 2017). Polysaccharides with active ingredients, different structural features and biological activity have been purified and characterized from a number of important medicinal plants (Xie *et al*., 2016). A variety of polysaccharides, extracted and isolated from diverse natural sources, have attracted considerable attention as they reportedly exhibit various biological activities like antitumor (Dong *et al*., 2019), antiviral (He *et al*., 2020), hypoglycemic (Wu

et al., 2019), anti-inflammatory (Lee *et al*., 2020), antioxidative (Huang *et al*., 2019), immuno-stimulatory (Tabarsa *et al*., 2020), regulation of gut microbiota (Shao *et al*., 2019), etc.

Syzygium cumini L., locally known as jamun, is an evergreen tree belonging to Myrtaceae family and is native to India, widely found in tropical and subtropical regions of the world. Various parts of this plant like stem bark, leaves, fruits, and seeds have frequently been exploited in folk medicine (Ayyanar and Subash-Babu, 2012). The fruits are widely consumed as a nutritious drupe and also are used as astringent, antiscorbutic, diuretic, and in treating chronic diarrhoea and enlargement of spleen (Kumar and Singh, 2021). Besides antidiabetic properties, jamun fruits possess strong antioxidant and anti-genotoxic potential (Rizvi *et al*., 2022; Qamar *et al*., 2023). Its seed are widely used for different medical purposes due to the bioactivities like antidiabetic, antioxidant, anti-inflammatory, antipyretic, anticancer, antibacterial, gastroprotective, and radioprotective effects (Kumar *et al*., 2022). Due to their various biological effects and low toxicity, polysaccharides from genus *Syzygium* have received much attention recently (Aung *et al*., 2020; de Araújo *et al*., 2024). The polysaccharide structure and bioactivity reportedly are diverse among various genera of *Syzygium* (Tamiello *et al*., 2018). There are scanty studies on the identification of bioactive compounds from *S. cumini* seeds which are considered as a food waste material (Al-Dhabi and Ponmurugan, 2020). The present study was aimed to isolate water soluble polysaccharides from *S. cumini* seeds by hot water extraction process and characterize it for physicochemical constituents as well as assess its biological competence by *in vitro* antioxidant, antidiabetic and immunostimulatory studies.

MATERIALS AND METHODS

All the chemicals used were purchased from Sigma, Aldrich, SRL, Fine Chemicals and Merck (India) and the solvents used were of analytical grade.

Collection of seeds and extraction of polysaccharide

The *Syzygium cumini* fruits were purchased from the Regional Gandhi Market, Tiruchirappalli (India). The seeds were removed from the pulp and shade-dried for 10-15 days. The seed kernels were separated and ground well using a blender. Then 50 g powdered seed sample was subjected to defatting using petroleum ether for 12 h in a Soxhlet apparatus. The excessive solvent was evaporated completely by placing the paste in an oven at 45°C for 48 h. The crude polysaccharide from the seeds was extracted by hot water extraction process with slight modifications (Hu *et al.*, 2018). The dried defatted seed powder was suspended in 500 mL distilled water and subjected to extraction process at 60°C for 3 h. The mixture was then cooled, centrifuged at 10,000 rpm for 20 min and supernatant collected. This process was repeated twice and the collected supernatant was concentrated using a rotary evaporator. From the concentrated supernatant, the polysaccharides were precipitated by mixing it with fourfold volume of 95% ethanol and left overnight at 4°C. The precipitated polysaccharides were pelleted out by centrifugation at 8000 rpm for 15 min and the resultant pellet was suspended in distilled water. The polysaccharides were then subjected to deproteinization process (Sevag *et al*., 1938). Aqueous polysaccharide (15 mL) was transferred to a flask and then deproteinized by adding 3-fold volume of chloroform and n-butanol mixture (4:1), then mixed vigorously in a shaker for 20 min and centrifuged at 4500 rpm for 15 min. The upper layer was collected and concentrated using a rotary evaporator. The concentrated solution was again precipitated by adding fourfold volumes of 95% ethanol and centrifuged at 8000 rpm for 15 min. The pellet was then dissolved in water and lyophilized. The crude polysaccharide was used for further analysis.

Physiochemical characterization

UV-Visible absorption spectrum: The *S. cumini* polysaccharide (SCP) solution (1%) was prepared with Millipore water after deproteinization and the absorption spectrum was recorded from 250 to 800 nm

by a Shimadzu UV-Vis 1900 spectrophotometer (Deng *et al*., 2020).

FTIR analysis: FTIR spectra of polysaccharides were obtained by FTIR-6600 spectrophotometry (JASCO, Japan). The sample (10 mg) was incorporated into spectroscopic grade potassium bromide powder and then pressed into 1 mm pellets. The measurement was made in the 4000-400 cm^{-1} range with a resolution of 4 cm^{-1} at room temperature (Eljoudi *et al*., 2022).

Scanning electron microscopy (SEM): Dried polysaccharide samples were sputter-coated with a platinum layer and morphology was analysed by SEM imaging [TESCAN, VEGA 3]. The images were captured at a voltage of 10 kV, with magnification at 2000-40000X under high vacuum condition (Patel *et al*., 2019).

Thin-layer chromatography (TLC): The polysaccharide extract was hydrolysed using trifluoroacetic acid (2 M) for 5 h at 90˚C. The hydrolysates were dotted on to 60 F_{254} silica gel plate and separated using the mobile phase solvent mixture butanol: ethanol: water (5.0:2.5:2.5). The visualization of spots was carried out with diphenylamine reagent and dried in a hot air oven for 30-40 min at 115˚C or until the blue colour spots appeared (Zhang *et al*., 2009).

Antioxidant activity

Total antioxidant capacity (TAC): Different volumes of SCP (20-100 µg mL^{-1}) were mixed with 3 mL ammonium molybdate reagent and the tubes were kept at 95˚C for 90 min in a water bath. After cooling the absorbance was measured at 765 nm with a double beam spectrophotometer (Shimadzu UV-1800) [Li *et al.*, 2020]. The calibration curve was plotted using ascorbic acid standard (20-100 µg mL^{-1}). TAC of polysaccharide extract was expressed as ascorbic acid equivalents (AAE g^{-1}).

DPPH assay: For this, 3 mL (0.1 mM) 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent was added to the different concentrations of SCP (10-100 µg mL^{-1}) and absorbance measured at 517 nm using a Shimadzu UV-1800 spectrophotometer (Tian *et al*., 2011). The DPPH scavenging activity was expressed in percentage values using the formula:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \; x\ 100$$

ABTS assay: ABTS **[**2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt], radical cation solution was prepared by mixing equal volumes of ABTS (7 mM) and potassium persulphate solution (2.45 mM), followed by incubation in dark at room temperature for 14-16 h, before use. For the assay, the prepared ABTS radical cation solution was diluted with phosphate buffered saline (pH 7.4) to an initial absorbance of 0.70 $\pm$ 0.02 at 734 nm using UV-visible spectrophotometer (Shimadzu UV-1800). ABTS radical scavenging activity was assessed by mixing different concentrations of polysaccharide extract (20-100 µg mL^{-1}) separately with 3 mL diluted ABTS radical cation solution and incubating it 10 min at 30˚C and then measuring the absorbance at 734 nm (Shen *et a*., 2019). ABTS radical scavenging activity was expressed in percentage values using the formula:

$$\text{ABTS scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \; x\ 100$$

FRAP assay: The antioxidant activity of polysaccharide extract was measured by ferric-reducing antioxidant power (FRAP) as per Sun *et al*. (2013). Different concentrations of polysaccharide extract (20-100 µg mL^{-1}) were separately mixed with 2.5 mL potassium ferricyanide (1%) and 2.5 mL phosphate buffer (0.2 M, pH 6.6). The mixture was incubated at 50˚C for 20 min, then 2.5 mL trichloroacetic acid (10%) was added and centrifuged at 3000 rpm for 10 min. Subsequently, 2.5 mL distilled water and 0.5 mL ferric chloride (0.1%) was added to 2.5 mL supernatant and the solution incubated at ambient temperature for 30 min for colour development. The absorbance was measured at 700 nm using UV-visible spectrophotometer (Shimadzu UV-1800). The FRAP activity of test sample was compared with those of standards *viz*., ascorbic acid and butylated hydroxytoluene (BHT).

Antidiabetic activity

α-amylase inhibition assay: The extracted polysaccharide samples at varying concentrations (100-500 µg mL^{-1}) were separately mixed with α-amylase solution (0.5 mg mL^{-1}) containing sodium phosphate buffer (0.02 M, pH 6.9) and left as such at room temperature for 10 min. Then, 0.25 mL of 1% starch prepared in sodium phosphate buffer (0.02 M, pH 6.9) was added and the tubes kept at room temperature for another 10 min. The reaction was terminated by adding dinitrosalicylic acid reagent and kept in water bath at 100˚C for 5 min. After cooling the absorbance was measured at 540 nm with a double beam spectrophotometer (Shimadzu UV-1800) [Deng *et al*., 2020]. The inhibition of α-amylase activity by SCP extract was expressed in percentage values using the following formula:

$$\text{Inhibition of α-amylase activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \; x\; 100$$

α-glucosidase inhibition assay: The polysaccharide extract at various concentrations (10-50 µg mL^{-1}) was mixed with 320 µL phosphate buffer (100 mM, pH 6.8) and 50 µL 4-nitrophenyl-β-D-glucopyranoside in phosphate buffer (10 mM, pH 6.8). The reaction mixture was incubated at 30˚C for 5 min, followed by the addition of 20 µL α-glucosidase solution (0.5 mg mL^{-1}), which was kept at room temperature for another 5 min. To stop the reaction, 3 mL 50 mM sodium hydroxide was added and absorbance measured spectrophotometerically (Shimadzu UV 1800) at 405 nm (Wang *et al*., 2019). The inhibitory action was expressed in percentage and calculated using the formula:

$$\text{Inhibition of α-glucosidase activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \; x\; 100$$

Immunostimulatory effects

Nitroblue tetrazolium (NBT) assay: Leucocyte suspension (0.5 mL) was mixed with 100 µL polysaccharide solution using its different concentrations (125, 250, 500 and 1000 µg mL^{-1}) and 100 µL lipopolysaccharide [LPS] (10 µg mL^{-1}) was used as a standard. Then to all tubes, 200 µL of 0.15% NBT solution was added and incubated at 37˚C for 20 min. The tubes were centrifuged at 1700 rpm for 3 min and supernatant discarded. The cells were then treated with a phosphate buffered saline (pH 7.2) and a thin film was made on clean glass slide. The slides were dried, fixed by heating, stained with Giemsa stain and examined under microscope (Labomed) at 40X magnification (Bhanwase and Alagawadi, 2016). The percentage of the NBT positive cells containing blue lumps or granules was determined by light microscopy after counting total 200 neutrophils under a 40X objective.

$$\text{NBT positive cells (\%)} = \frac{\text{Number of blue coloured cells}}{\text{Total nmber of cells examined}} \; x\; 100$$

In vitro candida phagocytosis test

Candida albicans was cultured in nutrient broth at 35˚C for 24 h and centrifuged at 4000 rpm to collect the cells at bottom. The collected cell pellet was washed with sterile Hank's balanced salt solution (HBSS) and centrifuged again. This washing step was repeated 3-4 times and the final cell pellet mixed with a mixture of sterile HBSS and human serum (4:1). The concentration of cell suspension used in the experiment was 1 x 10^8 (Heroor *et al*., 2011). The slide having polymorphonuclear neutrophils was flooded with a predetermined concentration of SCP, and incubated at 37˚C for 15 min. Then it covered with *C. albicans* suspension and re-incubated at 37˚C for 1 h. The slide was drained, fixed with methanol, and stained (Patil *et al*., 2010). Then 200 neutrophils were examined and the mean particle number (MPN) calculated.

Statistical analysis

All experiments and assays were conducted in triplicates (n = 3) in a completely randomized design and the values expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism software. The results were considered statistically significant if $*p \leq 0.05$.

RESULTS AND DISCUSSION

UV-visible spectrum analysis

The purity of SCP was confirmed by recording UV-vis absorption spectra at 250-800 nm range (Fig. 1).

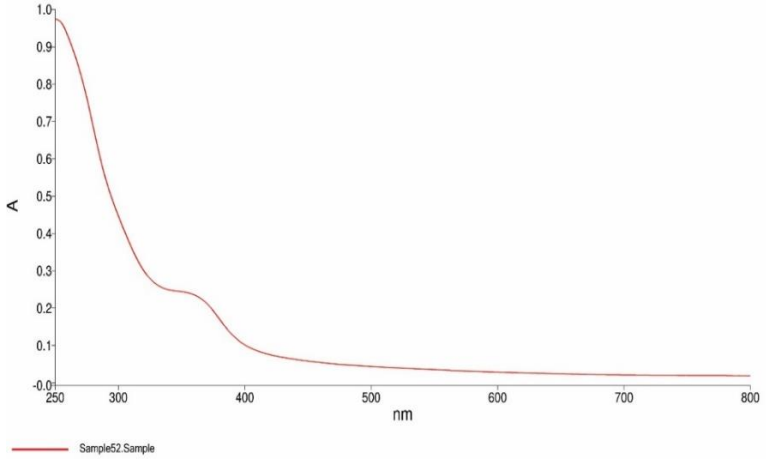


Fig. 1: UV-visible spectrum of polysaccharides extracted from *S. cumini* seeds

The detected absorption peak observed around 350-400 nm indicated the presence of polyhydroxyl groups that was similar to the previous reports of polysaccharides extracted from plants (Sun *et al*., 2018). The extracted polysaccharide was not having any protein constituents as no peaks were seen between 260-280 nm, so the extracted polysaccharide was protein-free and constituted only carbohydrates in the final extracted product.

FTIR analysis

The FTIR spectra of SCP illustrated two strong singles (Fig. 2) between 2100-3800 cm^{-1} and 1000-1700 cm^{-1}. A minor stretching peak was detected around 2100 cm^{-1}. The major peak with broad signal at 3336.25 cm^{-1} was attributed to the O-H stretching regions rising from the hydrogen bonding of gluco-pyranose hydroxyl groups of carbohydrates (Ahmadi *et al*., 2019). The narrow signal detected at 1635.34 cm^{-1} was assumed to be C-O stretching situations of uronic acids or acyl amino group (Wang *et al*., 2017). The minor stretching peaks at 2149.28, 2363.34 and 2340.19 cm^{-1} were attributed to C-H_2 groups and C-H bond vibrations of aliphatic alkanes (Bagchi and Kumar, 2016). Thus, the extracted SCP was confirmed with the sugar monomeric molecular structures.

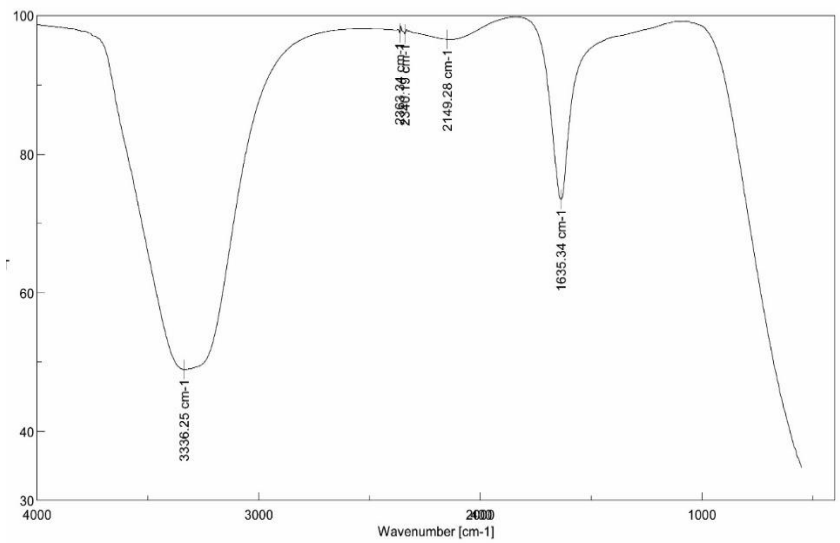


Fig. 2: FTIR spectrum of *S. cumini* polysaccharide extract

SEM analysis

The ultra-structural images of freeze-dried sample of SCP at 2000 - 40000 X magnification is given in Fig. 3. Under low magnification the SCP depicted a regular strip-like structure with flat and smooth surface. It represented slight clefts under high magnification. The non-porous structure with diverse morphology observed in SEM was in agreement with the previously reported ultra-structural studies of polysaccharides extracted from purple-heart radish (Lin *et al*., 2022). From this analysis, we further outlined the structural features of extracted SCP.

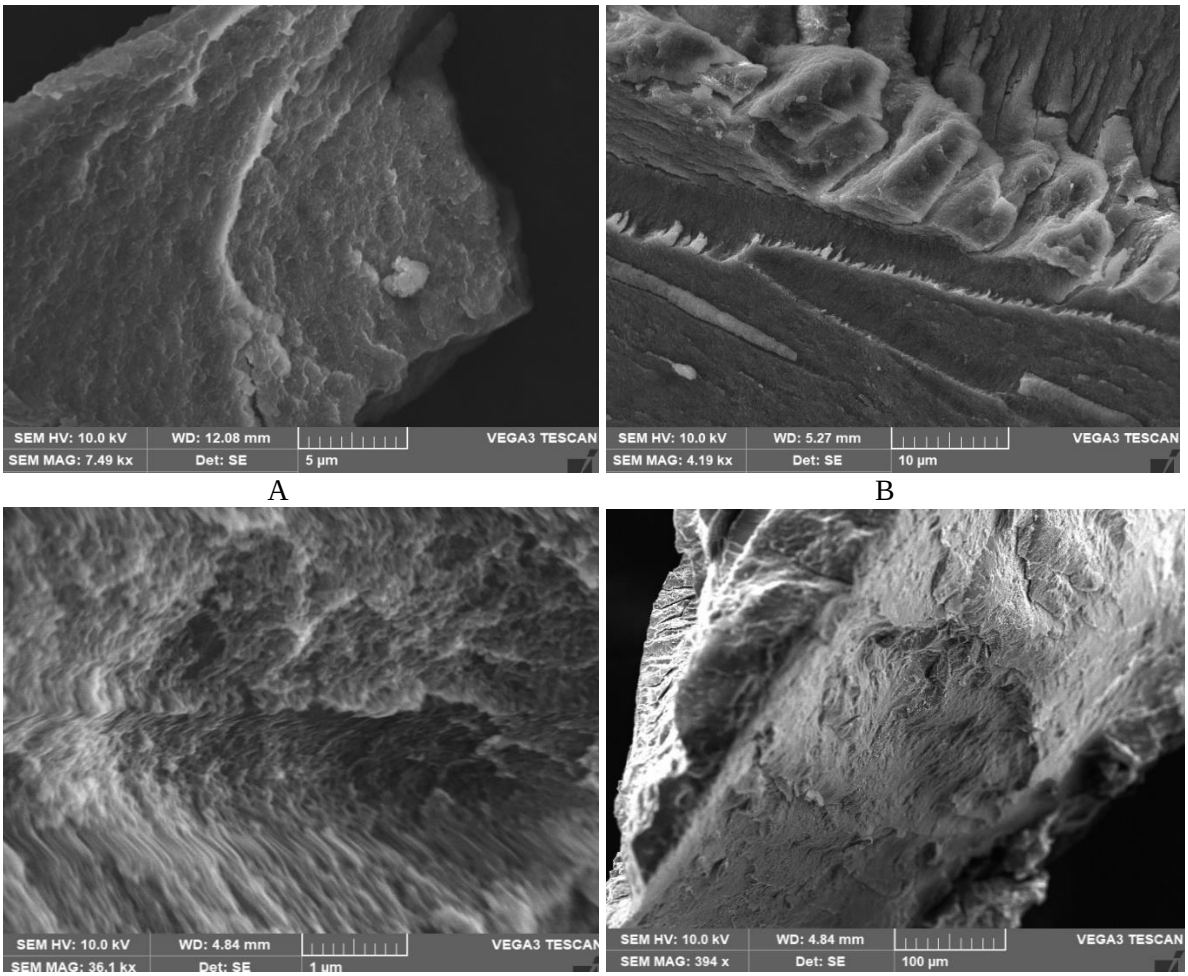


Fig. 3: Surface structure of *S. cumini* polysaccharide extract on scanning electron microscopy; A - Smooth and flat morphology; B - Strip-like structure; C - Non-porous structure; D - Smooth and rigid surface

TLC analysis

TLC is the most commonly employed method for analysing the monosaccharides composition in polysaccharides. The monosaccharide composition of SCP extract was screened by TLC (Fig. 4). The result of TLC analysis with *S. cumini* polysaccharide were in line with the earlier reports of polysaccharide extract from *Sorghum bicolor* seeds (Slima *et al*., 2018). TLC analysis of acid-hydrolysed SCP sample revealed the presence of monosaccharaides like dextrose, fructose, and arabinose.

In vitro antioxidant assays

The TAC of SCP varied from 96.0 to 138.77 AAE g^{-1} (Fig. 5A). The antioxidant activity increased with increase in the concentration of SCP. The free radicals are the molecules that are highly reactive and unstable and can cause damage to cells. However, the antioxidant compounds can effectively inhibit this cell damage. The result of DPPH free radical-scavenging ability of extracted polysaccharides is given in Fig. 5B in which the radical scavenging activity was found to be 18.61-60.04% with increase in concentration of SCP (20 to 100 µg mL^{-1}). Highest concentration tested depicted 60.04 ± 1.11% DPPH radical scavenging activity of SCP as compared to the standard ascorbic acid (83.48 ± 0.12%). DPPH is a very stable nitrogen-centered free radical, which accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Rjeibi *et al*., 2019; Chen and Huang, 2019). DPPH assay is based on the reduction of alcoholic DPPH solution in presence of

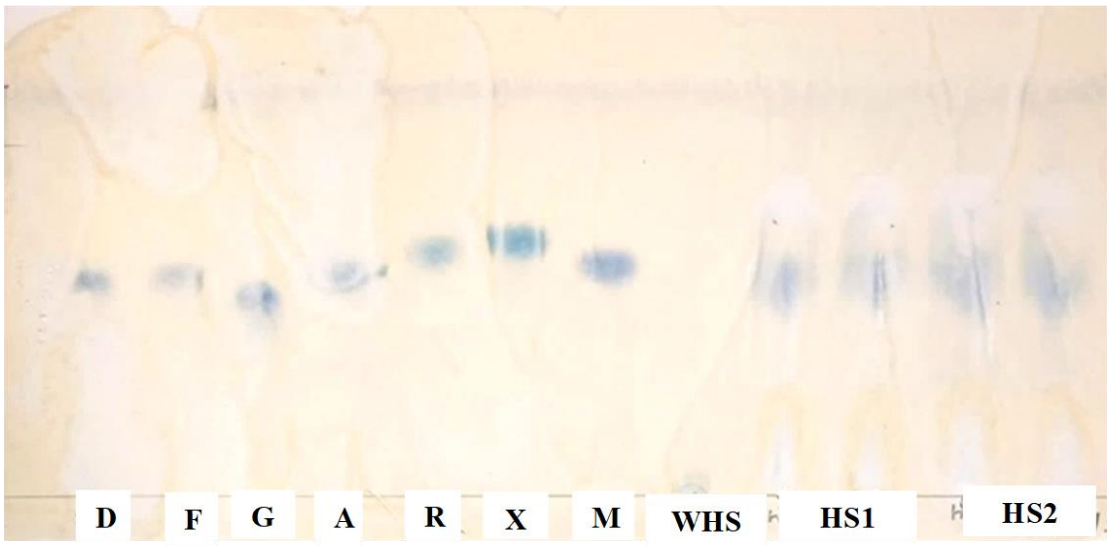


Fig. 4: Separation of monosaccharide components from *S. cumini* polysaccharide extract by thin layer chromatography (TLC); D - Dextrose; F - Fructose; G - Galactose; A - Arabinose; R - Ribose; X - Xylose; M - Mannose; WHS - SCP sample; HS1 - SCP sample after hydrolysis (2 h); HS2 - SCP sample after hydrolysis (5 h).

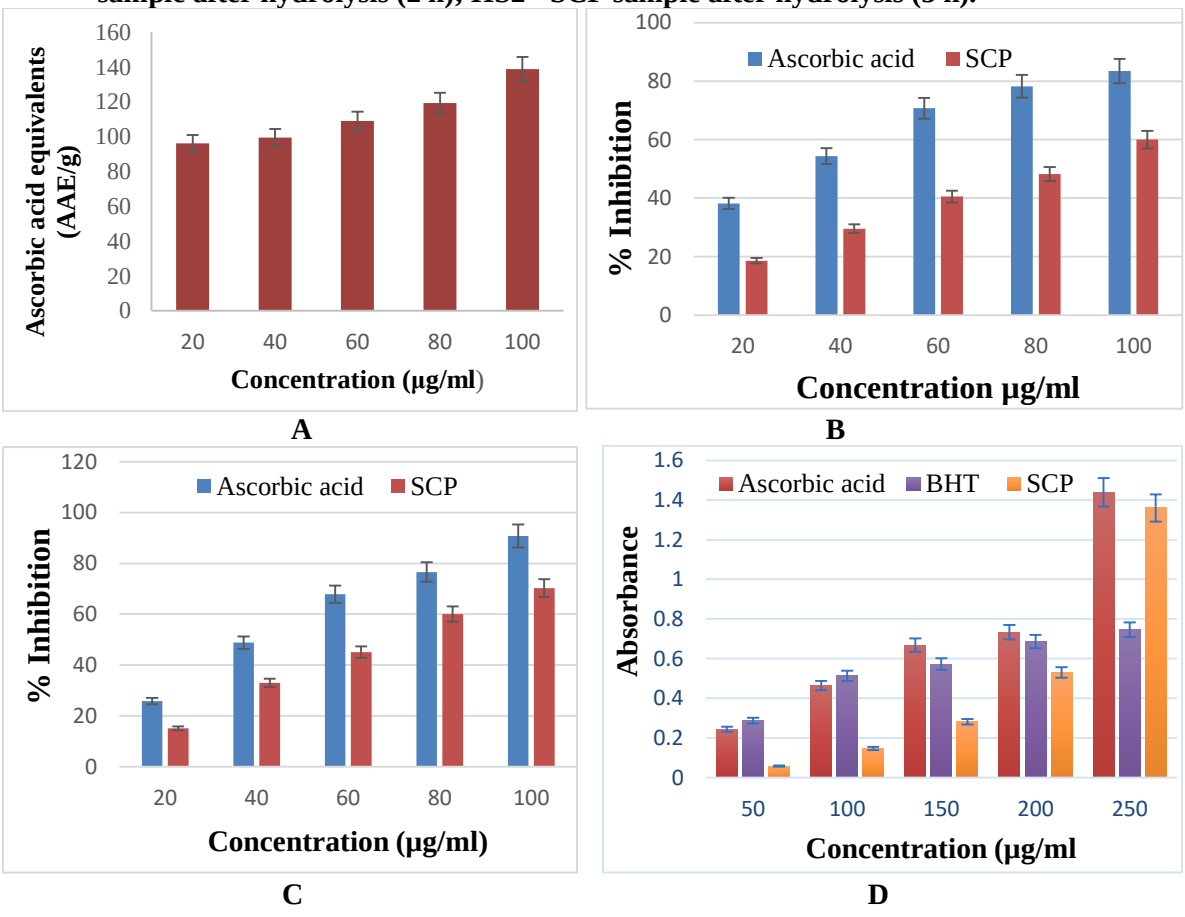


Fig. 5: Antioxidant activity of polysaccharide extract of *S. cumini* seeds (SCP). A) Total antioxidant capacity; B) DPPH radical scavenging activity; C) ABTS radical scavenging activity, and D) FRAP activity. The values are expressed as mean ± SD. $P \leq 0.05$ is regarded as significant (n = 3).

hydrogen-donating antioxidant due to the formation of non-radical form DPPH-H by reaction (Ye *et al*., 2011; Thambiraj *et al*., 2015). The increase in DPPH radical scavenging activity of SCP could be attributed to the antioxidant potential of crude polysaccharide extract and its neutralizing action against free radicals. The ABTS assay is used to evaluate the free radical scavenging ability of hydrophilic antioxidant compounds (Shi *et al*., 2016; Ma *et al*., 2020). SCP demonstrated similar concentration-dependent ABTS radical scavenging activity (57.89 ± 0.28%) [Fig. 5C] comparable with standard (99.35 ± 0.17%) at a concentration of 100 µg mL^{-1}. The result revealed that SCP possess excellent radical scavenging ability in hydrophilic system and could be used as one of the natural antioxidant ingredients in food and healthcare products. The results of FRAP illustrated in Fig. 5D revealed that SCP has significantly greater reducing effect (1.36) which was comparable to ascorbic acid (1.43), and BHT (0.740) at 100 µg mL^{-1}. The reducing power of polysaccharide extract, ascorbic acid, and BHT at 100 µg mL^{-1} were 1.36, 1.43, and 0.74, respectively. The polysaccharides can act as electron donors and can react with free radicals to convert them to more stable products, thereby terminate chain of free radical reactions (Zhang *et al*., 2016; Mohammed *et al*., 2020).

In vitro antidiabetic effects

Carbohydrates, such as starch and glycogen, are essential sources of nourishment that provide body with necessary energy for normal daily activities. Once hydrolysed and absorbed, these complex carbohydrates can lead to postprandial hyperglycemia (Trinh *et al*., 2016). The results of carbohydrate metabolizing enzyme inhibitory effects of SCP are shown in Fig. 6A and B. Though SCP exhibited a

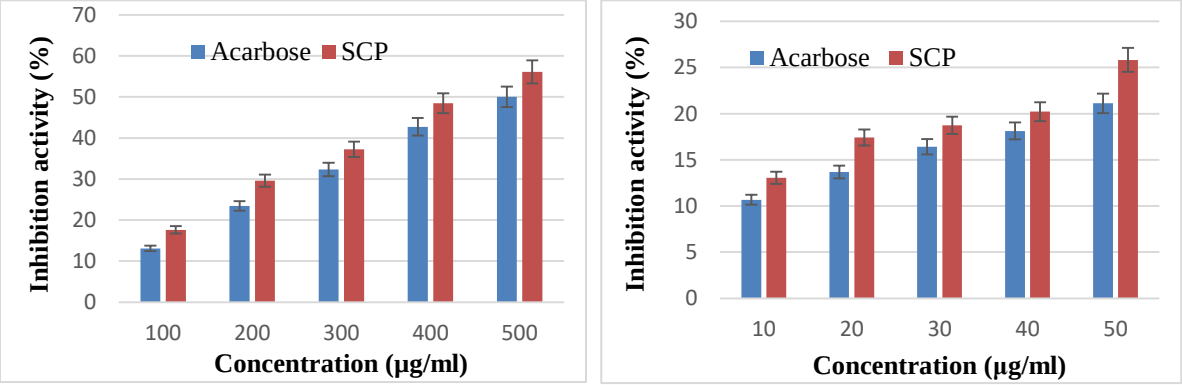


Fig. 6: *In vitro* A) α-amylase B) α-glucosidase (carbohydrate metabolizing enzymes) inhibitory effects of *S. cumini* seed polysaccharide. Values are expressed as mean ± SD. P ≤ 0.05 is regarded as significant (n = 3).

dose-dependent inhibitory effect on α-amylase and α-glucosidase activities, the SCP exhibited strong inhibitory effect on α-glucosidase enzyme than the standard acarbose standard.

The α-amylase inhibitory action of SCP was 56.12% at a concentration of 500 µg mL^{-1}. The enzyme α-amylase plays a crucial role in this process by breaking down the polysaccharides into smaller oligosaccharides (Xu *et al*., 2018). The brush border of small intestine contains membrane-bound α-glucosidase enzyme which plays a crucial role in carbohydrate digestion (Wang *et al*., 2018). The present study revealed that SCP was effective in controlling the human pancreatic α-amylase and α-glucosidase activities and plays an vital role in type 2 diabetes mellitus treatment. Earlier studies have also indicated anti-diabetic effect of polysaccharides extracted from various natural sources (Zhang *et al*., 2016; Lv *et al*., 2021).

Immunostimulatory activity

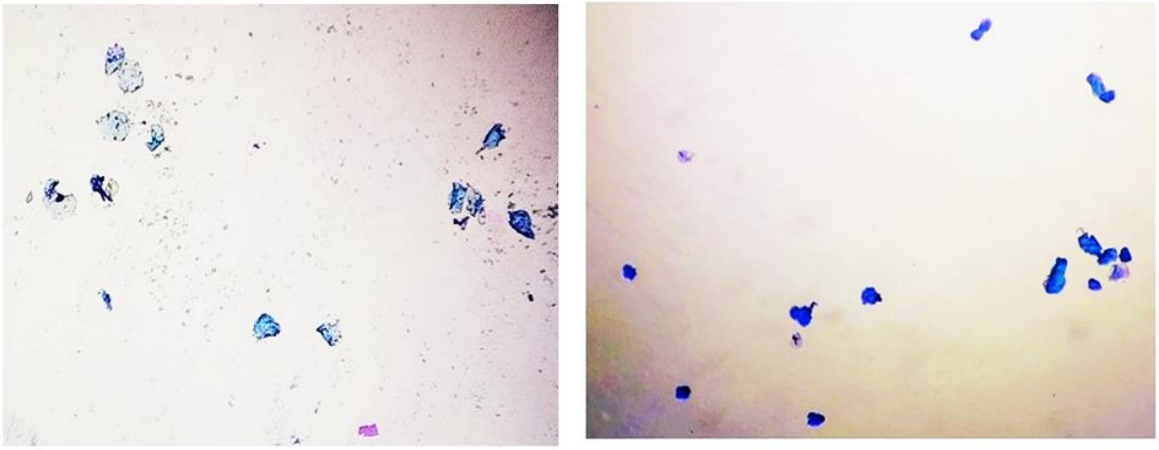
NBT test is used to assess the immunomodulatory potential of newly derived compounds from various sources and studied by stimulating the phagocytic activity of leucocytes (Yadav *et al*., 2016; Shantilal and Vaghela, 2020). The extracted water-soluble SCP stimulated leucocyte phagocytic activity in a dose-dependent manner (Table 1). The neutrophils with reduced NBT were portrayed in Fig. 7. The

Table 1: NBT test of *S. cumini* polysaccharide on human neutrophils

Normal control	LPS (Positive control)	Concentration (µg mL^{-1})	Mean percentage of reduced neutrophils by SCP
22.01 ± 0.31	66.54 ± 0.25	125	11.17
		250	26.03
		500	34.95
		1000	43.34
		Mean ± SE	28.87 ± 0.25

study revealed that SCP was able to provoke phagocytic response (43%) at a concentration of 1000 µg mL^{-1} as compared to the positive control lipopolysaccharides (66%).

On treatment with polysaccharide extract, neutrophils demonstrated enhanced response (Fig. 7B), thereby suggesting its immuno-stimulatory effect as compared to the control group (Fig. 7A) which showed only baseline activity. The present study explored the immunostimulating effect



A **B**

Fig. 7: NBT qualitative test of *S. cumini* polysaccharide on human neutrophils at 40X magnification; A) Control neutrophils B) Neutrophils incubated with polysaccharide extract

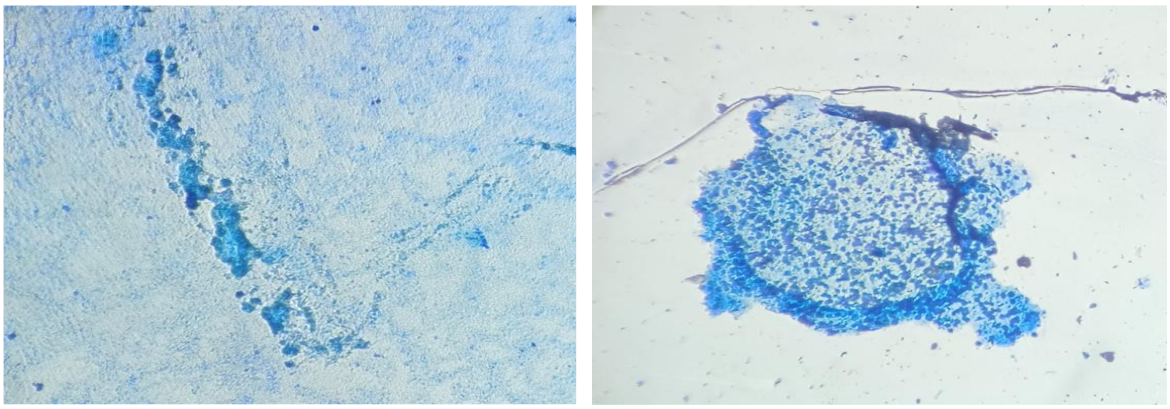
of SCP on leucocyte phagocytic activity because the percentage of NBT positive cells showed increase with increase in SCP concentrations. The enhanced activity may be attributed to the fact that the extracted polysaccharide could be used in various natural food formulations as an immune booster.

Phagocytosis of killed C. albicans

The phagocytosis of killed *C. albicans* assay is a method that measures phagocytosis by phagocytes over a short period. The most probable number of *C. albicans* has been used to evaluate phagocytosis of killed *C. albicans* (Rawat *et al*., 2018; Sunitha and Nagulu, 2019). In present study an increase in phagocytosis of killed *C. albicans* was noticed on treatment with SCP (Table 2). Fig. 8 illustrates the phagocytosis of killed *C. albicans* for SCP on human neutrophils that was assessed by the uptake of particles by phagocytes after a short period. The mean number of neutrophils attracted per field was noted against positive control (casein). The average value of

Table 2: Phagocytosis of killed *C.albicans* by human neutrophils with polysaccharide extract of *S. cumini*

Normal control	Pooled serum (Positive control)	SCP concentration (µg mL^{-1})	Mean particle number of killed *C. albicans* by human neutrophils with SCP
25 ± 0.0	45 ± 0.0	125	23
		250	29
		500	32
		1000	38
		Mean ± SE	30.5 ± 3.12



A **B**

Fig. 8: Photograph showing neutrophil during the process of phagocytosis of killed *C. albicans* at 40X magnification. A) Normal control B) SCP treated neutrophils

mean particle number (MPN) of killed *C. albicans* on incubation with SCP was scored as 30. Overall, the number of particles phagocytosed by neutrophils was found to increase with an increase in concentration of the polysaccharide extract. In untreated control neutrophils (Fig. 8A) the phagocytosis of killed *C. albicans* was found to occur at lesser rate. In contrast, neutrophils that were treated with the *S. cumini* polysaccharide extract, showed enhanced phagocytic response (Fig. 8B) and stimulated their ability to engulf and internalize killed *C. albicans*. An increase in MPN of killed *C. albicans* values on SCP treatment evidenced enhancement of immune reaction on neutrophils by promoting increased phagocytic activity, hence SCP could perform as an immuno-stimulatory agent. Further, the immune-stimulation of SCP was improved as the concentration was increased, and it demonstrated the potent *in vitro* immune-modulatory activity.

Conclusion: The extracted water-soluble seed SCP showed exceptional antioxidant activity, potential inhibitory effect on carbohydrate metabolizing enzymes and distinguished immunomodulatory property, and might have a promising role in food and pharmaceutical industries as a natural ingredient. However, further purification and structural elucidation studies are needed to identify the major polysaccharide constituents and to evaluate its biological efficacy.

Acknowledgment: The authors would like to thank University Science Instrumentation Centre (USIC), Bharathidasan University, Trichy for permission to carryout FTIR and SEM analysis.

Funding: This study was supported by Rashtriya Uchchatar Shiksha Abhiyan (RUSA) 2.0, Biological Sciences of Bharathidasan University, Tiruchirappalli (Ref. No. 2-20/BDU/RUSA 2.0/TRP/BS dated 22/04/2021).

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

REFERENCES

Ahmadi, S., Sheikh, Z.M., Soleimanian, Z.S., Alihosseini, F. and Yadav, H. 2019. Effects of different drying methods on the physicochemical properties and antioxidant activities of isolated acorn polysaccharides. *Lebensm Wiss Technology*, **100**: 1-9.

Al-Dhabi, N.A. and Ponmurugan, K. 2020. Microwave-assisted extraction and characterization of polysaccharide from waste jamun fruit seeds. *International Journal of Biological Macromolecules*, **152**: 1157-1163.

Aung, E., Kristanti, A., Aminah, N., Takaya, Y. and Ramadhan, R. 2020. Plant description, phytochemical constituents and bioactivities of *Syzygium* genus: A review. *Open Chemistry*, **18**: 1256-1281.

Ayyanar, M. and Subash-Babu, P. 2012. *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asian Pacific Journal of Tropical Biomedicine*, **2**: 240-246.

Bagchi, S. and Kumar, K.J. 2016. Studies on water soluble polysaccharides from *Pithecellobium dulce* (Roxb.) Benth. seeds. *Carbohydrate Polymers*, **138**: 215-221.

Bhanwase, A.S. and Alagawadi, K.R. 2016. Antioxidant and immunomodulatory activity of hydroalcoholic extract and its fractions of leaves of *Ficus benghalensis* Linn. *Pharmacognosy Research*, **8**: 50-55.

Chen, F. and Huang, G. 2019. Extraction, derivatization and antioxidant activity of bitter gourd polysaccharide. *International Journal of Biological Macromolecules*, **141**: 14-20.

de Araújo, A.N.V., de Souza, E.L., dos Santos N.D., Alves, J.M., Sampaio, K.B., da Silva, S.R.F. *et al.*, 2024. Revisiting the nutritional and functional value and health-promoting potential of *Syzygium* species. *Journal of Functional Foods*, **118**: 106265. [*https://doi.org/10.1016/j.jff. 2024.106265*].

Delattre, C., Fenoradosoa, T.A. and Michaudll, P. 2011. Galactans: an overview of their most important sourcing and applications as natural polysaccharides. *Brazilian Archives of Biology and Technology*, **54**: 1075-1092.

Deng, Y., Huang, L., Zhang, C., Xie, P., Cheng, J., Wang, X. *et al.*, 2020. Novel polysaccharide from *Chaenomeles speciosa* seeds: Structural characterization, α-amylase and α-glucosidase inhibitory activity evaluation. *International Journal of Biological Macromolecules*, **153**: 755-766.

Dong, X.D., Yu, J., Feng, Y.Y., Ji, H.Y., Yu, S.S. and Liu, A.J. 2019. Alcohol-soluble polysaccharide from *Castanea mollissima* Blume: Preparation, characteristics and antitumor activity. *Journal of Functional Foods*, **63**: 103563. [*https://doi.org/10.1016/j.jff.2019.103563*].

Eljoudi, S., Feki, A., Bkhairia, I., Barkia, A., Ben Amara, I., Nasri, M. *et al.*, 2022. New polysaccharides extracted from *Malcolmia triloba*: Structure characterization, biological properties and application to beef meat preservation. *Journal of Food Composition and Analysis*, **107**: 104380. [*https://doi.org/10.1016/j.jfca.2021.104380*].

He, X., Fang, J., Guo, Q., Wang, M., Li, Y., Meng, Y. *et al.*, 2020. Advances in antiviral polysaccharides derived from edible and medicinal plants and mushrooms. *Carbohydrate Polymers*, **229**: 115548. [*https://doi.org/10.1016/j.carbpol.2019.115548*].

Heroor, S., Beknal, A.K. and Mahurkar, N. 2011. Immunomodulatory activity of methanolic extracts of *Ficus glomerata* Roxb. leaf, fruit and bark in cyclophosphamide induced mice. *International Journal of Modern Botany*, **1**: 4-7.

Hu, H.B., Liang, H.P., Li, H.M., Yuan, R.N., Sun, J., Zhang, L.L., *et al.*, 2018. Isolation, purification, characterization and antioxidant activity of polysaccharides from the stem barks of *Acanthopanax leucorrhizus*. *Carbohydrate Polymers*, **196**: 359-367.

Huang, L., Huang, M., Shen, M., Wen, P., Wu, T., Hong, Y. *et al.*, 2019. Sulfated modification enhanced the antioxidant activity of *Mesona chinensis* Benth polysaccharide and its protective effect on cellular oxidative stress. *International Journal of Biological Macromolecules*, **136**: 1000-1006.

Kumar, M., Zhang, B., Nishad, J., Verma, A., Sheri, V. and Dhumal S. 2022. Jamun (*Syzygium cumini* (L.) Skeels) seed: A review on nutritional profile, functional food properties, health-promoting applications, and safety aspects. *Processes*, **10**: 2169. [*https://doi.org/10.3390/pr101 12169*].

Kumar, S. and Singh, B. 2021. *Syzygium cumini* (Jamun) its medicinal uses. *International Journal of Unani and Integrative Medicine*, **5**: 89-97.

Lee, Y.K., Jung, S.K. and Chang, Y.H. 2020. Rheological properties of a neutral polysaccharide extracted from maca (*Lepidium meyenii* Walp.) roots with prebiotic and anti-inflammatory activities. *International Journal of Biological Macromolecules*, **152**: 757-765.

Li, W., Wang, Y., Wei, H., Zhang, Y., Guo, Z., Qiu, Y., *et al.*, 2020. Structural characterization of Lanzhou lily (*Lilium Davidii* var. *unicolor*) polysaccharides and determination of their associated antioxidant activity. *Journal of the Science of Food and Agriculture*, **100**: 5603-5616.

Lin, Y., Pi, J., Jin, P., Liu, Y., Mai, X., Li, P. and Fan, H. 2022. Enzyme and microwave co-assisted extraction, structural characterization and antioxidant activity of polysaccharides from purple-heart radish. *Food Chemistry*, **372**: 131274. [*https://doi.org/10.1016/j.foodchem.2021.131274*].

Liu, Z.H., Niu, F.J., Xie, Y.X., Xie, S.M., Liu, Y.N., Yang, Y.Y., *et al.*, 2020. A review: Natural poly saccharides from medicinal plants and microorganisms and their anti-herpetic mechanism. *Biomedicine & Pharmacotherapy*, **129**: 110469. [*https://doi.org/10.1016/j.biopha.2020.110469*].

Lv, Q.Q., Cao, J.J., Liu, R. and Chen, H.Q. 2021. Structural characterization, α-amylase and α-glucosidase inhibitory activities of polysaccharides from wheat bran. *Food Chemistry*, **341**: 128218. [*https://doi.org/10.1016/j.foodchem.2020.128218*].

Ma, J.S., Liu, H., Han, C.R., Zeng, S.J., Xu, X.J., Lu, D.J. *et al.*, 2020. Extraction, characterization and antioxidant activity of polysaccharide from *Pouteria campechiana* seed. *Carbohydrate Polymers*, **229**:115409. [*doi: 10.1016/j.carbpol.2019.115409*].

Mohammed, J.K., Mahdi, A.A., Ahmed, M.I., Ma, M. and Wang, H. 2020. Preparation, deproteinization, characterization, and antioxidant activity of polysaccharide from *Medemia argun* fruit. *International Journal of Biological Macromolecules*, **155**: 919-926.

Patel, M.K., Tanna, B., Gupta, H., Mishra, A. and Jha, B. 2019. Physicochemical, scavenging and anti-proliferative analyses of polysaccharides extracted from psyllium (*Plantago ovata* Forssk) husk and seeds. *International Journal of Biological Macromolecules*, **133**: 190–201.

Patil, J.K., Jalalpure, S.S., Hamid, S. and Ahirrao, R.A. 2010. *In-vitro* immunomodulatory activity of extracts of *Bauhinia vareigata* Linn stem bark on human neutrophils. *Iranian Journal of Pharmacology and Therapeutics*, **9**: 41-46.

Qamar, M., Akhtar, S., Ismail, T., Wahid, M., Abbas, M.W., Mubarak, M.S., *et al.*, 2022. Phytochemical profile, biological properties, and food applications of the medicinal plant *Syzygium cumini*. *Foods*, **11**: 378. [*https://doi.org/10.3390/foods11030378*].

Rawat, P., Bachheti, R.K., Kumar, N. and Rai, N. 2018. Phytochemical analysis and evaluation of *in vitro* immunomodulatory activity of *Rhododendron arboreum* leaves. *Asian Journal of Pharmaceutical and Clinical Research*, **11**: 123-128.

Rizvi, M.K., Rabail, R., Munir, S., Inam, R.M., Qayyum, M.M.N., Kieliszek, M., *et al.*, 2022. Astounding health benefits of jamun (*Syzygium cumini*) toward metabolic dyndrome. *Molecules*. **27**: 7184. [*https://doi.org/10.3390/molecules27217184*].

Rjeibi, I., Feriani, A., Hentati, F., Hfaiedh, N., Michaud, P. and Pierre G. 2019. Structural characterization of water-soluble polysaccharides from *Nitraria retusa* fruits and their antioxidant and hypolipidemic activities. *International Journal of Biological Macromolecules*, **129**: 422-432.

Sevag, M.G., Lackman, D.B. and Smolens, J. 1938. The isolation of the components of Streptococcal nucleoproteins in serologically active form. *Journal of Biological Chemistry*, **124**: 425-436.

Shantilal, S. and Vaghela, J.S. 2020. Evaluation of immunomodulatory activity of ethanolic extracts of *Moringa concanensis* nimmo in mice. *Journal of Advanced Scientific Research*, **11**: 187-194.

Shao, S., Wang, D., Zheng, W., Li, X., Zhang, H., Zhao, D. *et al.*, 2019. A unique polysaccharide from *Hericium erinaceus* mycelium ameliorates acetic acid-induced ulcerative colitis rats by modulating the composition of the gut microbiota, short chain fatty acids levels and GPR41/43 respectors. *International Immunopharmacology*, **71**: 411-422.

Shen, D.D., Li, X., Qin, Y.L., Li, M.T., Han, Q.H., Zhou, J., *et al.*, 2019. Physicochemical properties, phenolic profiles, antioxidant capacities, and inhibitory effects on digestive enzymes of okra (*Abelmoschus esculentus*) fruit at different maturation stages. *Journal of Food Science and Technology*, **56**:1275-1286.

Shi, J.J., Zhang, J.G., Sun, Y.H., Qu, J., Li, L., Prasad, C. *et al.*, 2016. Physicochemical properties and antioxidant activities of polysaccharides sequentially extracted from peony seed dreg. *International Journal of Biological Macromolecules*, **91**: 23-30.

Shi, L. 2016. Bioactivities, isolation and purification methods of polysaccharides from natural products: A review. *International Journal of Biological Macromolecules*, **92**: 37-48.

Slima, S.B., Ktari, N., Trabelsi, I., Moussa, H., Makni, I. and Salah, R.B. 2018. Purification, characterization, and antioxidant properties of a novel polysaccharide extracted from *Sorghum bicolor* (L.) seeds in sausage. *International Journal of Biological Macromolecules*, **106**:168-178.

Sun, J., Zhou, B., Tang, C., Gou, Y., Chen, H., Wang, Y., *et al.*, 2018. Characterization, antioxidant activity, and hepatoprotective effect of purple sweet potato polysaccharides. *International Journal of Biological Macromolecules*, **115**: 69-76.

Sun, Y., Yang, X., Lu, X., Wang, D. and Zhao, Y. 2013. Protective effects of Keemun black tea polysaccharides on acute carbon tetrachloride-caused oxidative hepatotoxicity in mice. *Food and Chemical Toxicology*, **58**: 184-192.

Sunitha, K. and Nagulu, M. 2019. *In vitro* screening of immunomodulatory activity of methanolic leaves extract of *Tecoma stans*. *International Research Journal of Pharmcy and Medical Sciences*, **2**: 52-54.

Tabarsa, M., You, S., Yelithao, K., Palanisamy, S., Prabhu, N.M. and Nan, M. 2020 Isolation, structural elucidation and immuno-stimulatory properties of polysaccharides from *Cuminum cyminum*. *Carbohydrate Polymers*, **230**: 115636. [*https://doi.org/10.1016/j.carbpol.2019.115636*].

Tamiello, C.S., Adami, E.R., de Oliveira, N.M.T., Acco, A., Iacomini, M. and Cordeiro, L.M.C. 2018. Structural features of polysaccharides from edible jambo (*Syzygium jambos*) fruits and antitumor activity of extracted pectins. *International Journal of Biological Macromolecules*, **118**: 1414-1421.

Tian, L., Zhao, Y., Guo, C. and Yang, X. 2011. A comparative study on the antioxidant activities of an acidic polysaccharide and various solvent extracts derived from herbal *Houttuynia cordata*. *Carbohydrate Polymers*, **83**: 537-544.

Thakur, V.K. 2017. *Biopolymer Grafting: Synthesis and Properties*. Elsevier, New York, USA.

Thambiraj, S.R., Phillips, M., Koyyalamudi, S.R. and Reddy, N. 2015. Antioxidant activities and characterisation of polysaccharides isolated from the seeds of *Lupinus angustifolius*. *Industrial Crops and Products*, **74**: 950-956.

Trinh, B.T., Staerk, D. and Jäger, A.K. 2016. Screening for potential α-glucosidase and α-amylase inhibitory constituents from selected Vietnamese plants used to treat type 2 diabetes. *Journal of Ethnopharmacology*, **186**: 189-195.

Wang, L., Liu, F., Wang, A., Yu, Z., Xu, Y. and Yang, Y. 2017. Purification characterization and bioactivity determination of a novel polysaccharide from pumpkin (*Cucurbita moschata*) seeds. *Food Hydrocolloids*, **66**: 357-364.

Wang, L., Chen, C., Zhang, B., Huang, Q., Fu, X. and Li, C. 2018. Structural characterization of a novel acidic polysaccharide from *Rosa roxburghii* Tratt fruit and its α-glucosidase inhibitory activity. *Food and Function*, **9:** 3974-3985.

Wang, Z., Zhao, X., Liu, X., Lu, W., Jia, S., Hong, T., *et al.*, 2019. Anti-diabetic activity evaluation of a polysaccharide extracted from *Gynostemma pentaphyllum*. *International Journal of Biological Macromolecules*, **126**: 209-214.

Wu, G.J., Liu, D., Wan, Y.J., Huang, X.J. and Nie, S.P. 2019. Comparison of hypoglycemic effects of polysaccharides from four legume species. *Food Hydrocolloids*, **90**: 299-304.

Xie, J.H.., Jin, M.L., Morris, G.A., Zha, X.Q., Chen, H.Q., Yi, Y., *et al.*, 2016. Advances on bioactive polysaccharides from medicinal plants. *Critical Reviews in Food Science and Nutrition*, **56**: 60-84.

Xu, Y., Gao, Y., Liu, F., Niu, X., Wang, L., Li, X., *et al.*, 2018. Sulfated modification of the polysaccharides from blackcurrant and their antioxidant and α-amylase inhibitory activities. *International Journal of Biological Macromolecules*, **109**:1344-1354.

Yadav, H. and Karthikeyan, C. 2019. Natural polysaccharides: Structural features and properties. pp. 1-17. **In**: *Polysaccharide Carriers for Drug Delivery* (eds. S. Maiti and S. Jana). Woodhead Publishing, New York, USA.

Yadav, S., Bhargav, M., Ramya, R., Laureen, and Habeeb, N. 2016. *In vitro* antioxidant and immunomodulatory activity of *Citrullus lanatus* seed. *International Journal of Engineering Sciences & Research Technology*, **5**: 679-685.

Ye, C.L., Hu, W.L. and Dai, D.H. 2011. Extraction of polysaccharides and the antioxidant activity from the seeds of *Plantago asiatica* L. *International Journal of Biological Macromolecules*, **49**: 466-470.

Zhang, Z., Xiao, Z. and Linhardt, R.J. 2009. Thin layer chromatography for the separation and analysis of acidic carbohydrates. *Journal of Liquid Chromatography and Related Technologies*, **32**: 1711-1732.

Zhang, Z., Kong, F., Ni, H., Mo, Z., Wan, J.B., Hua, D. *et al.*, 2016. Structural characterization, α-glucosidase inhibitory and DPPH scavenging activities of polysaccharides from guava. *Carbohydrate Polymers*, **144**: 106-114.

Zhang, X., Li, M., Zhong, L., Peng, X. and Sun, R. 2016. Microwave-assisted extraction of polysaccharides from bamboo (*Phyllostachys acuta*) leaves and their antioxidant activity. *BioResources*, **11**: 5100-5112.