

# Extraction, Purification, and Characterization of Polysaccharides of *Araucaria heterophylla* L and *Prosopis chilensis* L and Utilization of Polysaccharides in Nanocarrier Synthesis

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Antony V Samrot <sup>1</sup>  
Teeshalini Kudaiyappan<sup>1</sup>  
Ummu Bisarah<sup>1</sup>  
Anita Mirarmandi<sup>1</sup>  
Etel Faradjeva<sup>1</sup>  
Amira Abubakar<sup>1</sup>  
Jenifer A Selvarani <sup>2</sup>  
Suresh Kumar Subbiah<sup>3,4</sup>

<sup>1</sup>Department of Biomedical Sciences, Faculty of Medicine and Biomedical Sciences, MAHSA University, Jenjarom, Selangor 42610, Malaysia; <sup>2</sup>Department of Biotechnology, School of Bio and Chemical Engineering, Sathyabama Institute of Science and Technology, Sholinganallur, Chennai, Tamil Nadu 600119, India; <sup>3</sup>Department of Medical Microbiology and Parasitology, Universiti Putra Malaysia, Serdang, Selangor 43400 UPM, Malaysia; <sup>4</sup>Department of Biotechnology, BIHER, Bharath University, Selaiyur, India

Correspondence: Antony V Samrot  
Department of Biomedical Sciences,  
Faculty of Medicine and Biomedical  
Sciences, MAHSA University, Jalan SP2,  
Bandar Saujana Putra, Jenjarom, Selangor  
42610, Malaysia  
Email antonysamrot@gmail.com

Suresh Kumar Subbiah  
Department of Medical Microbiology and  
Parasitology, Universiti Putra Malaysia,  
43400 UPM, Serdang, Selangor, Malaysia  
Email sureshkudsc@gmail.com

**Background:** Plant gums consist of polysaccharides which can be used in the preparation of nanocarriers and provide a wide application in pharmaceutical applications including as drug delivery agents and the matrices for drug release. The objectives of the study were to collect plant gums from *Araucaria heterophylla* L and *Prosopis chilensis* L and to extract and characterize their polysaccharides. Then to utilize these plant gum-derived polysaccharides for the formulation of nanocarriers to use for drug loading and to examine their purpose in drug delivery in vitro.

**Methods:** Plant gum was collected, polysaccharide was extracted, purified, characterized using UV-Vis, FTIR, TGA and GCMS and subjected to various bioactive studies. The purified polysaccharide was used for making curcumin-loaded nanocarriers using STMP (sodium trimetaphosphate). Bioactivities were performed on the crude, purified and drug-loaded nanocarriers. These polysaccharide-based nanocarriers were characterized using UV-Vis spectrophotometer, FTIR, SEM, and AFM. Drug release kinetics were performed for the drug-loaded nanocarriers.

**Results:** The presence of glucose, xylose and sucrose was studied from the UV-Vis and GCMS analysis. Purified polysaccharides of both the plants showed antioxidant activity and also antibacterial activity against *Bacillus* sp. Purified polysaccharides were used for nanocarrier synthesis, where the size and shape of the nanocarriers were studied using SEM analysis and AFM analysis. The size of the drug-loaded nanocarriers was found to be around 200 nm. The curcumin-loaded nanocarriers were releasing curcumin slow and steady.

**Conclusion:** The extracted pure polysaccharide of *A. heterophylla* and *P. chilensis* acted as good antioxidants and showed antibacterial activity against *Bacillus* sp. These polysaccharides were fabricated into curcumin-loaded nanocarriers whose size was below 200 nm. Both the drug-loaded nanocarriers synthesized using *A. heterophylla* and *P. chilensis* showed antibacterial activity with a steady drug release profile. Hence, these natural exudates can serve as biodegradable nanocarriers in drug delivery.

**Keywords:** *Araucaria heterophylla* L, *Prosopis chilensis* L, gum polysaccharide

## Introduction

Nanotechnology is becoming an increasingly important subject that highlights the variation of matter which ranges between 1 and 100 nm. The system is becoming the most interesting field of science due to their size and unique properties.<sup>1,2</sup> Their surface area interaction of these nanoparticles leads to unique properties<sup>3,4</sup> and it

can be applied in electronics, biomedicine, imaging, environmental remediation etc.<sup>5–10</sup> In nanobiotechnology, the recent decade has recorded extensive research on formulation of nanocarriers since they promote easy transport and availability of drug molecules to the targeted site. Nanocarriers are being formulated from both inorganic and organic resources.<sup>11</sup> They include metal nanoparticles like gold, silica, silver and magnetic iron oxide nanoparticles.<sup>5,12,13</sup> But the use of these metal nanoparticles as drug carriers can pose a threat of metal poisoning within the system. Some of the organically derived nanocarriers like polymeric micelles, liposomes, chitosan-based nanocarriers and polymeric nanogels are good for drug delivery.<sup>14–16</sup> Likewise, plant gum can be used as readily accessible and non-noxious material for nanocarrier synthesis. Making nanocarriers as colloidal drug carriers helps in delivering drugs to targeted sites and also offers slow and constant delivery of drugs. This even improves the pharmacological activity of the drug inside the system.<sup>17</sup> Most drugs are cleared in the reticuloendothelial system (RES), where the nanocarriers of biopolymers helps for slower delivery.<sup>18</sup> Biopolymers are polymers derived from living organisms,<sup>19</sup> including DNA, proteins and polysaccharides.<sup>20</sup> Gum exudates are generally high molecular mass constituents which are produced by plants as a response to biotic and abiotic stress. These gums are hydrophilic in nature and consists of polysaccharides, fats and some proteins.<sup>21,22</sup> These polysaccharides are composed of branched structures of monosaccharides with glycosidic linkages. These polysaccharides are highly biocompatible and biodegradable and can be used in pharmaceutical actions for drug carrier and drug release.<sup>8,23</sup>

*Araucaria* genus contains about 14 species and most species release gum through the bark of the plant.<sup>24</sup> These gums are brittle and irregular in shape. They are reported to have antioxidant, anticytotoxic, antibacterial and anti-ulcerogenic effects.<sup>8,25</sup> The genus *Prosopis* belongs to the family *Fabaceae* with 45 species, consist of spines and grow into either trees or shrubs. This gum has been reported to have various bioactivities including anticancer, antioxidant etc.<sup>26</sup> There are reports where polysaccharides derived from plant gums have been made into nanocarriers in the presence of chelators like STMP.<sup>4,8</sup> Polysaccharides make the nanocarrier biocompatible and also enhance the activity of drug loading by releasing the loaded drug slowly, whilst the polysaccharide itself has bioactivity. In this study, gum-derived polysaccharides of *Araucaria heterophylla* and *Prosopis chilensis* were purified,

characterized and exploited for various bioactive studies including antibacterial and antioxidant. They were further used for nanocarrier synthesis and loaded with curcumin. These drug-laden nanocarriers were examined for in-vitro bioactivity like antibacterial activity and the drug release was also checked.

## Materials and Methods

### Chemicals, Reagents and Instruments

Chloroform (HmbG Chemicals), Ethanol (System Chemicals), Trichloroacetic acid (Merck Chemicals), Acetone (Friendemann Schmidt Chemicals), Sulphuric acid (HmbG Chemicals), Phenol (R&M Chemicals), Sodium Tri Meta Phosphate (SRL Chemicals), Crystal violet (Friendemann Schmidt Chemicals), Mueller Hinton broth (SRL Chemicals), Nutrient agar (SRL Chemicals), Nutrient broth (SRL Chemicals), DPPH reagent (SRL Chemicals), Ascorbic acid (SRL Chemicals), Methanol (R&M Chemicals), Curcumin (SRL Chemicals), Hydrochloric acid (R&M Chemicals) etc. Centrifuge (Thermo Scientific), ELISA microplate reader (Lab Tech), Weighing balance (A&D Company Ltd), UV-Vis spectrophotometer (Genesys IOS UV-VIS- Thermoscientific), FTIR (Parkin Elmer Spectrum Two), SEM (Scanvac, ZEISS Ultra55), AFM (Bruker, Dimension icon model), Magnetic stirrer (Lab Tech).

### Collection of the Plant Gums

Plant gum from *A. heterophylla* was obtained from Melaka, Malaysia and plant gum from *P. chilensis* was obtained from Chennai, Tamil Nadu, India (Figure 1).

### Extraction and Purification of the Polysaccharides

The gum was crushed using a pestle and mortar. Water extract was obtained by mixing the gum with distilled water, centrifuged and the supernatant was dried. In the purification process, gum was soaked in 100% ethanol to de-fat, followed with drying at 60°C. Then the gum was allowed to dissolve in 50 mL water and continuously stirred in a magnetic stirrer. The solution was boiled to increase its viscosity and filtrated using filter paper. Equal volume of 10% TCA (Trichloroacetic acid) was added to precipitate the proteins<sup>27</sup> and proteins were removed as pellets after subjecting to centrifugation at 7000 rpm. The collected supernatant was added with half the volume with acetone on continuous stirring to form a white mass. The obtained

white mass was dialyzed against milli Q water for 3 days, then subjected to lyophilization and stored at 4°C.<sup>28</sup>

### Characterization of the Polysaccharides

The polysaccharides were characterized using UV-Vis spectroscopy analysis which was measured at 200–800 nm.<sup>29</sup> The polysaccharides were then subjected for FT-IR analysis at the frequency range of 450–4000 cm<sup>-1</sup>. Phenol sulphuric assay and GC-MS were performed to find any monosaccharides present. Phenol sulfuric acid assay was done by adding 1 mg/

mL of purified samples with 5% aqueous phenol and 2 mL of concentrated sulphuric acid, kept undisturbed for about 10 minutes, followed by vortex, left at room temperature and the solution was now measured between 200 nm and 800 nm.<sup>30</sup>

TGA (thermogravimetric analysis) was performed for purified polysaccharides.

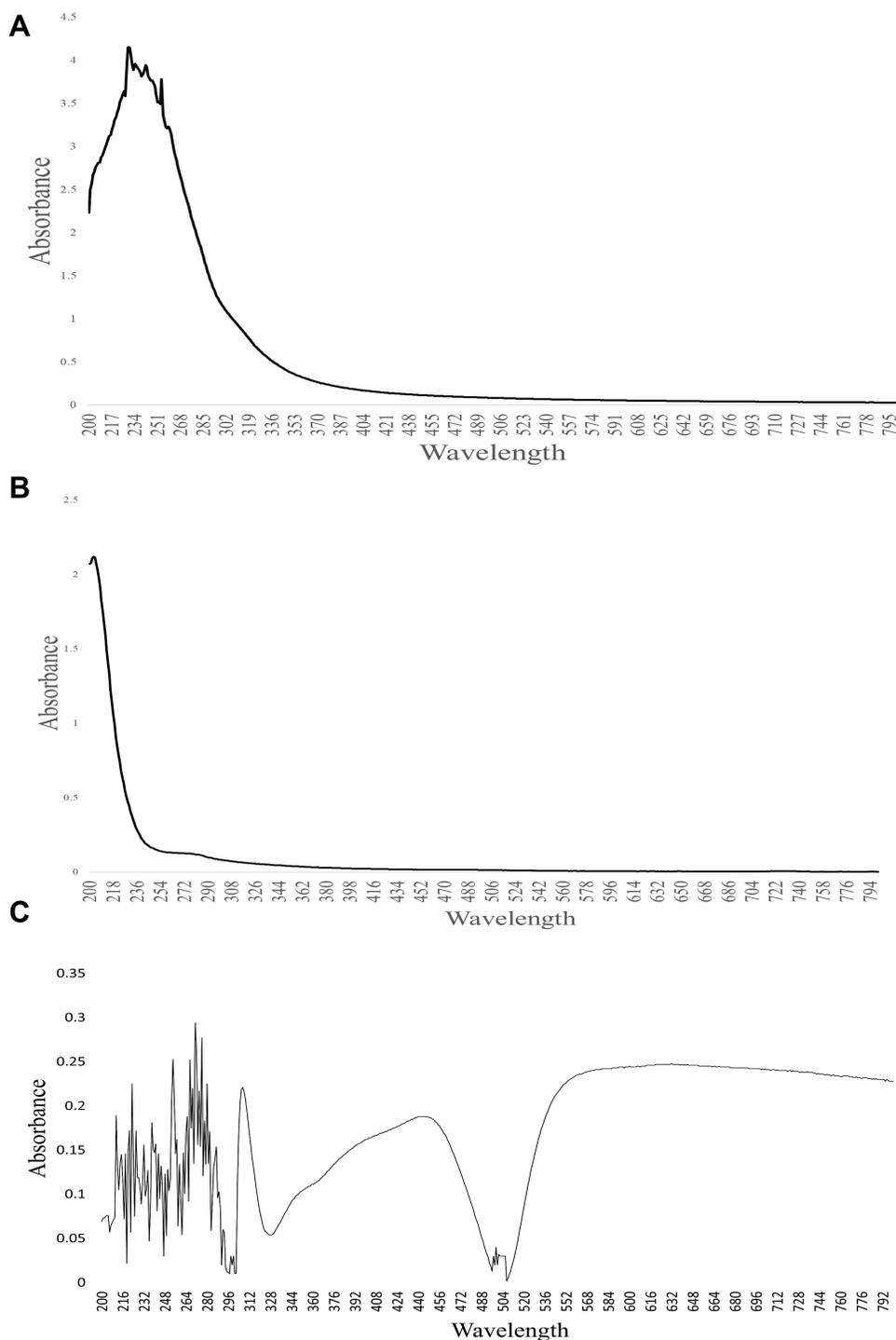
Pure gum exudate consists of complex hydrophilic polysaccharides, therefore, the purified polysaccharides were derivatized to their simple sugar moieties before detecting them in GC analysis as described earlier.<sup>31</sup> 1



**Figure 1** Collected plant gum. (A) Plant gum of *A. heterophylla* and (B) Powdered gum of *A. heterophylla*; (C) Plant gum of *P. chilensis*; and (D) Powdered gum of *P. chilensis*.

$\mu\text{L}$  of the derivatized sample was loaded and the analysis was performed using JEOL GC MATE II HP 5 MS (5% phenyl-methylpolysiloxane) fitted with a Quadrupole double focusing Mass analyzer. The mobile phase used here was helium gas with a flow

rate of 1mL/min. The column temperature was raised from 50 °C to 250 °C at 10 °C/min and the detection temperature was maintained as 250 °C in a scan range of 50–600 amu. Ionization was achieved by electron impact mode at 70 eV.



**Figure 2** UV-Vis spectroscopy analysis: (A) Crude of *A. heterophylla*, (B) Purified *A. heterophylla*, and (C) Phenol sulphuric acid assay of *A. heterophylla*.

## Bioactivities of Polysaccharides

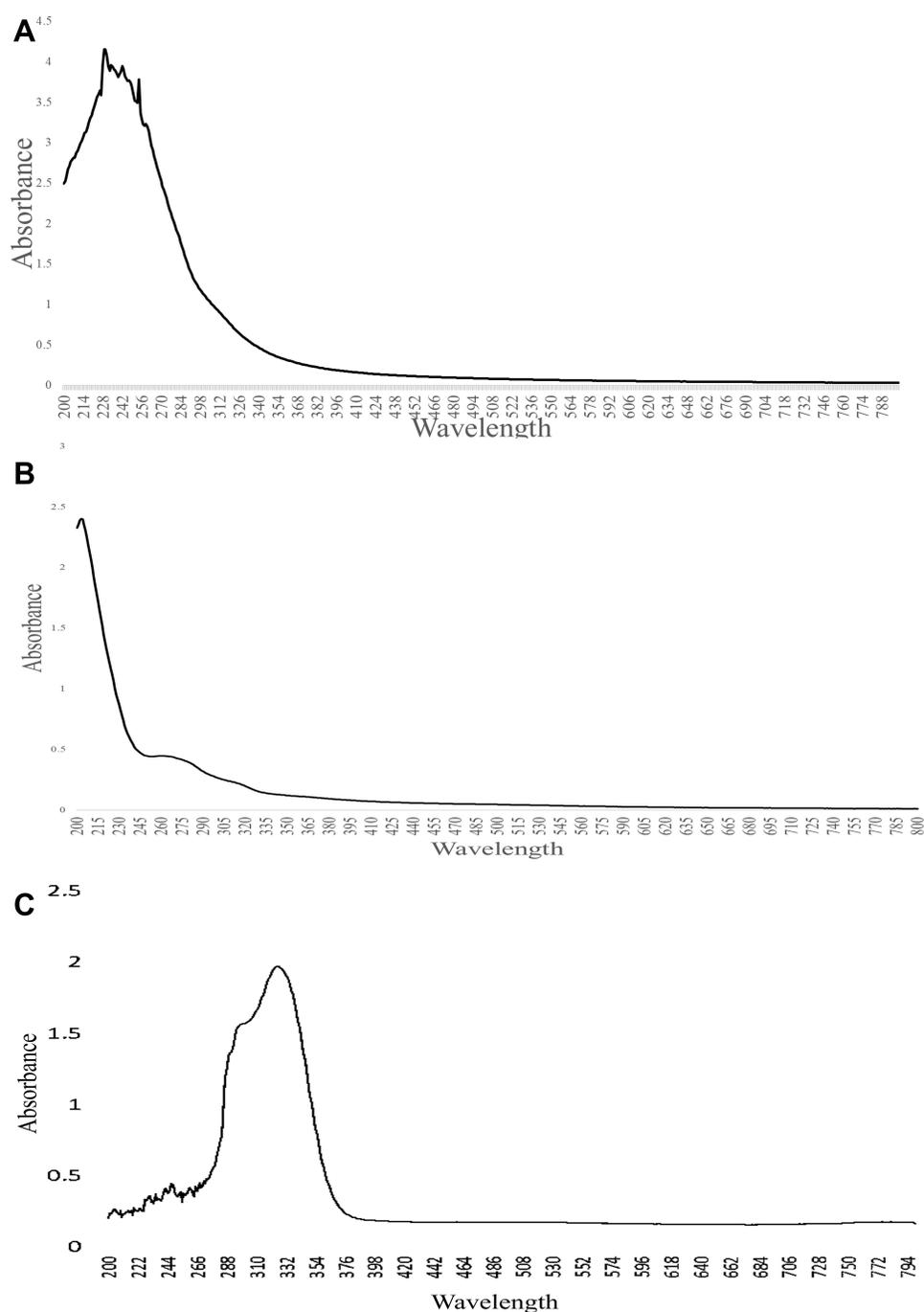
### Antibacterial Activity

Purified dried polysaccharide was weighed and mixed with sterile distilled water and used for the antibacterial activity studies against *Klebsiella* sp, *Bacillus* sp, *Staphylococcus aureus*, and *Escherichia coli*<sup>32</sup> which were swabbed all over the nutrient agar plate. The sample which showed activity was subjected for MIC (Minimal inhibition

concentration) and biofilm inhibition assay in 96-well plates (micro dilution method).<sup>33</sup> Swarming motility was performed as described before.<sup>10</sup>

### Antioxidant Assay (DPPH)

DPPH solution (0.1 mg/mL) in methanol was prepared. Then, 3 mL of purified polysaccharide (different concentration) was added with 1 mL of DPPH solution. After



**Figure 3** UV-Vis spectroscopy analysis. (A) Crude of *P. chilensis*, (B) Purified *P. chilensis*, and (C) Phenol sulphuric acid assay of *P. chilensis*.

vigorously shaking, it was left in the dark for 1 hour. Absorbance was recorded at 517 nm. Ascorbic acid was used as standard. % DPPH radical scavenging activity was determined as mentioned by Shen et al.<sup>34</sup>

### Preparation of Nanocarriers and Drug-Loading Nanocarriers

Purified gum was carboxymethylated with 0.1 M NaOH and 2.36 g of chloroacetic acid as described earlier.<sup>4</sup> The obtained 0.5 g of polysaccharide was added to 10 mL of distilled water and stirred for 2 hours using a magnetic stirrer. 100 mL of 0.01% of STMP (Tri Sodium Tri Meta Phosphate) was added dropwise into the solution under continuous stirring. The mixture was left stirring overnight. The solution was then centrifuged at 12,000 rpm for 15 minutes. The supernatant was removed and the pellet was lyophilized. 15 mg curcumin/10 mL of ethanol: water (1:1) was added to the nanocarrier formed. The mixture was stirred for 3 hours. The solution was adjusted to pH 5 using 0.01 M hydrochloric acid and magnetic stirred at room temperature overnight. The solution was then centrifuged and the pellet was lyophilized.<sup>4</sup>

### Characterization of Synthesized Nanocarriers

Both the loaded and unloaded nanocarriers were subjected for UV-Vis, FTIR, Atomic force microscopy (AFM) and Scanning electron microscopy (SEM).<sup>9</sup>

### Antibacterial Activity of Loaded Nanocarriers

A total of 1 mg of nanocarrier was dispersed in 1 mL of water and ethanol separately. Antibacterial activity of drug-loaded nanocarriers was performed against both Gram-positive (*S. aureus* and *Bacillus* sp.) and Gram-negative bacteria (*E. coli* and *Klebsiella* sp). The solvent used and unloaded nanocarrier were used as controls.

### Drug Release Kinetics

A total of 1 mg of drug-loaded nanocarrier was added to 1 mL of distilled water in a dialysis tube and was dialyzed against distilled water. Absorbance was taken at every 15 minutes using UV-Vis spectrophotometer at the wavelength of 420 nm. The analysis was done for 3 hours.<sup>35</sup>

### Statistical Analysis

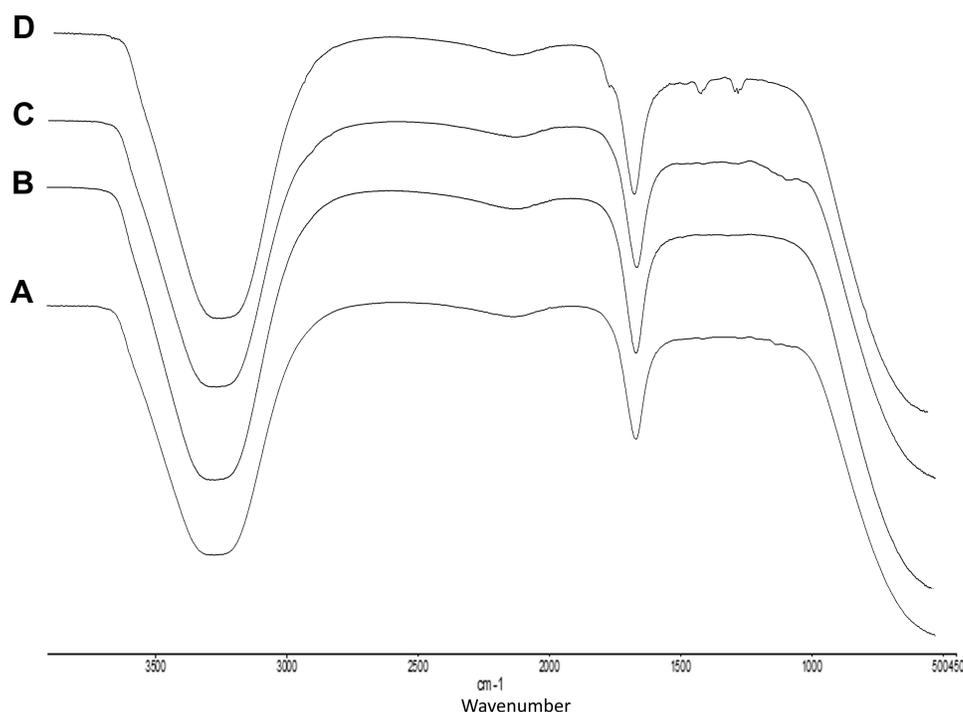
All the data obtained in this study were performed thrice and illustrated as mean  $\pm$  standard error.

## Results

### Characterization of the Polysaccharides

#### UV Vis Analysis

Figures 2 and 3 shows UV-Vis analysis of crude, purified polysaccharide and phenol sulphuric assay of *A. heterophylla* and *P. chilensis*. For *A. heterophylla*, the maximum absorbance for the analysis of crude was found between 234 and 300 nm



**Figure 4** FTIR analysis. (A) Water extract of *A. heterophylla*, and (B) Purified *A. heterophylla*, (C) Water extract of *P. chilensis*, and (D) Purified *P. chilensis*

(Figure 2A). For the purified sample, there was absorbance around 210 nm which indicated the presence of xylose (Figure 2B and C). For *P. chilensis*, the maximum absorbance for the analysis of the purified sample was found between 264 and 290 nm (Figure 3B). For the crude sample, absorbance around 210 nm was indicative of xylose and the absorbance peak around 265 nm was because of glucose (Figure 3A), whilst phenol sulfuric acid assay showed peaks from 255 nm indicating the presence of xylose (Figure 3C).<sup>36</sup>

### FTIR Analysis

Figure 4 shows FTIR analysis of *A. heterophylla* and *P. chilensis*. In the study, all polysaccharides showed a broad peak at  $3417\text{ cm}^{-1}$  for OH stretching,  $1635\text{ cm}^{-1}$  peak was for C=C stretching of alkenes.<sup>4</sup> Both the polysaccharides showed peaks at  $1384\text{ cm}^{-1}$ ,  $1260\text{ cm}^{-1}$ , and  $1751\text{ cm}^{-1}$  which correspond to esters of C=O stretching,  $\text{CH}_3\text{CH}$  stretching, and C-H stretching.<sup>37</sup>

### TGA Analysis

Thermogravimetric analysis of both the polysaccharides found that they were losing weight to around 70% between the temperatures 55 and  $500^\circ\text{C}$  (Figure 5A and B). Likewise, polysaccharides of *A. heterophylla* gum had the same kind of thermogravimetry.<sup>8</sup>

### GC-MS Analysis

The gums exudate of *Araucaria heterophylla* was previously reported by Anderson and Munro<sup>38</sup> to contain galactose, uronic acid, arabinose, rhamnose and xylose by molecular sieve and TLC chromatography. For GC-MS analysis, the polysaccharides are generally hydrolyzed to their simple sugars. Hydrolysis with TFA and acetic anhydride convert these sugars to aldonitrile acetate derivatives.<sup>39</sup> In GC-MS, the spectra were obtained for the aldonitrile acetate derivatives of aldose sugars. The peaks were identified by comparing

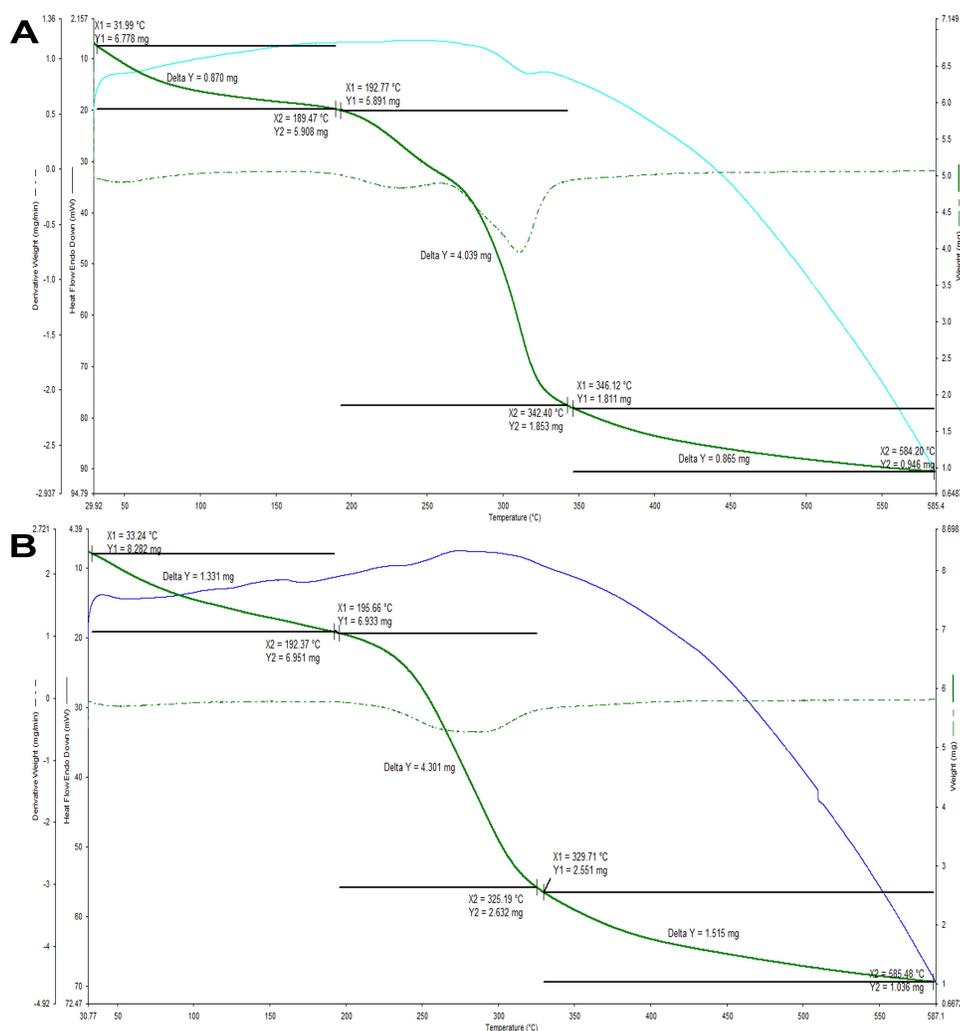


Figure 5 TGA analysis of: (A) *A. heterophylla* and (B) *P. chilensis*.

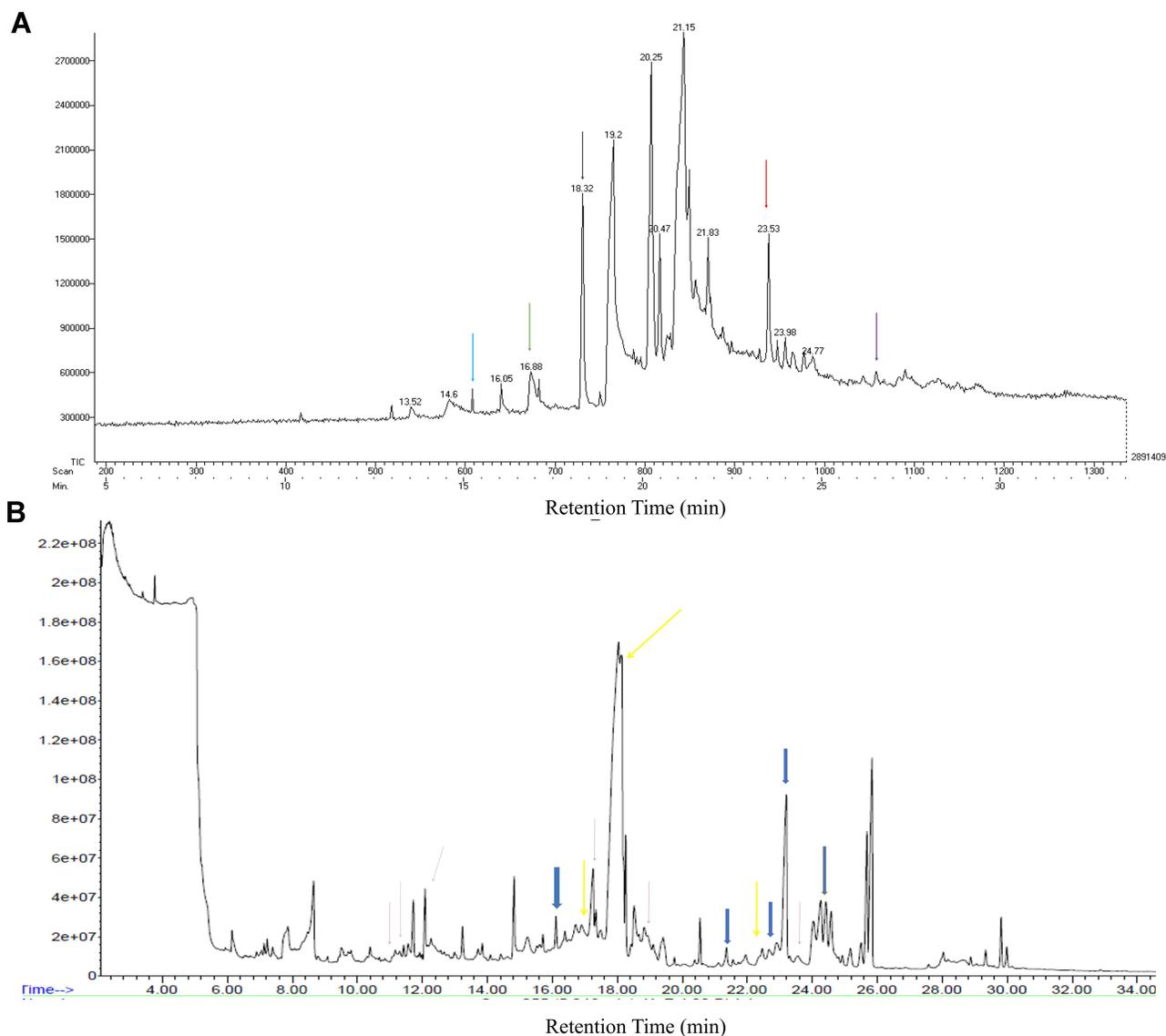
the retention time and mass spectra of previous reports. Figure 6A represents the GC-MS chromatogram of monosaccharides of *Araucaria heterophylla*. The peaks recorded at 15.4, 16.88, 18.32, 23.53, 26.5 correspond to erythrose, ribose, xylose, arabinose and rhamnose, respectively.<sup>40,41</sup> In the earlier studies of Samrot et al,<sup>8</sup> he also reported rhamnose, allose, glucosinolate, threose, idosan, galactose and arabinose as the monosaccharide residues of *Araucaria heterophylla* gum polysaccharide. Sugar derivatives seen in *P. chilensis* were: Peak at 11.169, 18.516, 23.571 were for xylose, peak at 11.726, 12.086 were for

erythrose, D-Erythronic acid, 16.112 for a derivative of ribose, 17.252, 18.045, 22.456 for arabinose, 17.339 for rhamnose, 21.353, 22.666, 23.199 for galactose, 24.413 for a derivative of mannose (Figure 6B).

## Bioactivities of the Polysaccharides

### Antibacterial Activity

There was no zone of inhibition found for water extract. For chloroform extract, the zone of inhibition can only be seen against *S. aureus*, *E. coli* and *Bacillus*. For the purified polysaccharide of *A. heterophylla* antibacterial activity was found against *Bacillus* sp (Table 1). Water extract



**Figure 6** GC-MS analysis of: **(A)** *Araucaria heterophylla*, erythrose – 15.4 (blue arrow), ribose – 16.88 (green arrow), xylose – 18.32 (black arrow), arabinose – 23.53 (red arrow), rhamnose – 26.5 (purple arrow). **(B)** *Prosopis chilensis* peak at 11.169, 18.516, 23.571 (red arrow) – derivative of xylose (xylitol), peak at 11.726, 12.086 (blue arrow) – erythrose, D-erythronic acid, peak at 16.112 (green arrow) – derivative of ribose, peak at 17.252, 18.045, 22.456 (yellow arrow) – arabinose, peak at 17.339 (black dotted arrow) – rhamnose, peak at 21.353, 22.666, 23.199 (blue big arrow) – galactose, peak at 24.413 (orange arrow) - derivative of mannose.

**Table 1** Antibacterial Activity of *A. heterophylla*

Organism	Zone of Inhibition (cm)								
	Solvent	2 mg		4 mg		6 mg		8 mg	
	H <sub>2</sub> O	H <sub>2</sub> O	Pur						
<i>S. aureus</i>	–	–	–	–	–	–	–	–	–
<i>Bacillus</i> sp	–	–	–	–	–	–	–	–	0.2
<i>E. coli</i>	–	–	–	–	–	–	–	–	–
<i>Klebsiella</i> sp	–	–	–	–	–	–	–	–	–

**Abbreviations:** H<sub>2</sub>O, water extract; Pur, purified.

**Table 2** Antibacterial Activity of *P. chilensis*

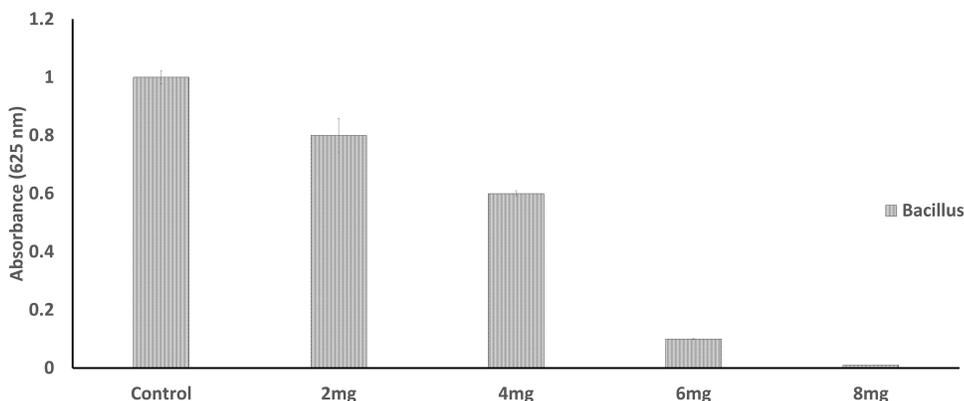
Organism	Zone of Inhibition (cm)								
	Solvent	2 mg		4 mg		6 mg		8 mg	
	H <sub>2</sub> O	H <sub>2</sub> O	Pur						
<i>S. aureus</i>	–	–	–	–	–	0.1	–	0.1	–
<i>Bacillus</i> sp	–	–	0.3	–	0.3	–	0.4	–	0.5
<i>E. coli</i>	–	–	–	–	–	–	–	–	–
<i>Klebsiella</i> sp	–	–	–	–	–	–	–	–	–

**Abbreviations:** H<sub>2</sub>O, water extract; Pur, purified.

of *P. chilensis* showed a zone of inhibition against *S. aureus* and *Bacillus* sp. For the purified polysaccharide of *P. chilensis*, antibacterial activity was found against *Bacillus* sp alone (Table 2). The presence of zone of inhibition shows that the extract was able to inhibit the bacterial growth.<sup>42</sup> Gum of plants like Acacia gum and gum-derived silver nanoparticles were reported to have antibacterial activity.<sup>9</sup> Minimal inhibitory concentration (MIC) of purified *A. heterophylla* and *P. chilensis* polysaccharide extract against *Bacillus* sp was found to be 8 mg (Figures 7 and 8). Thus, both the polysaccharides were tested further against *Bacillus* sp alone.

### Biofilm Inhibition Assay

The onset of bacterial infection is achieved by biofilm formation.<sup>43</sup> Plant polysaccharides are notably reported to show anti-biofilm action by preventing the adhesion of various bacteria.<sup>44</sup> The extracted polysaccharide was also found to be interfering in biofilm formation of *Bacillus* sp. The purified gum of *A. heterophylla* (8 mg) was inhibiting the development of biofilm to 79.49% (Figure 9) whereas it was 87.6% for *P. chilensis* (8 mg) against *Bacillus* sp. (Figure 10). The result showed that increase of polysaccharide extract concentration causes an increase of biofilm inhibition percentage. Grishin et al.<sup>45</sup> evaluated the impact

**Figure 7** Minimal inhibitory concentration (MIC) activity of *A. heterophylla*.

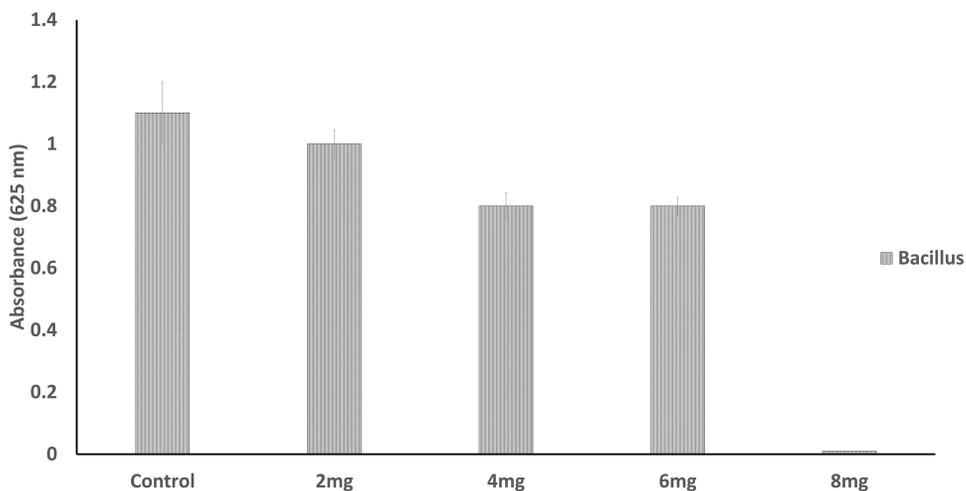


Figure 8 Minimal inhibitory concentration (MIC) activity of *P. chilensis*.

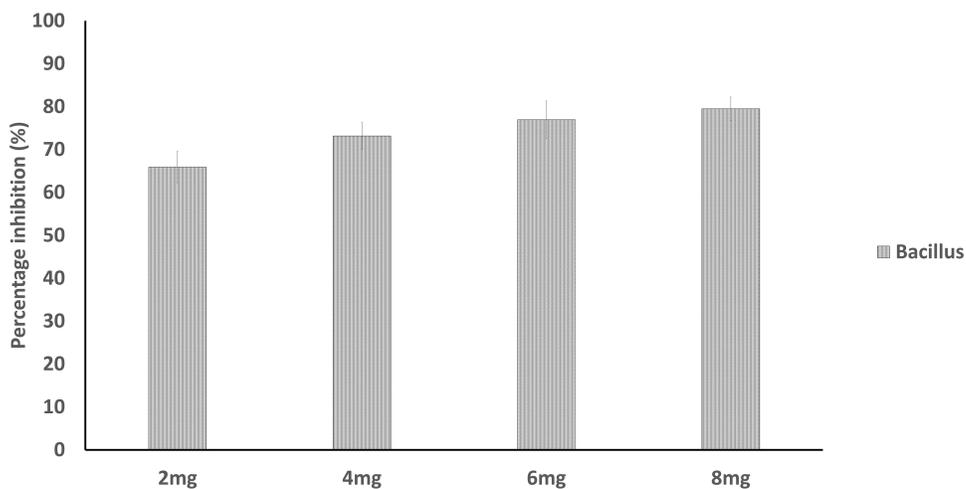


Figure 9 Biofilm inhibition assay using purified gum of *A. heterophylla*.

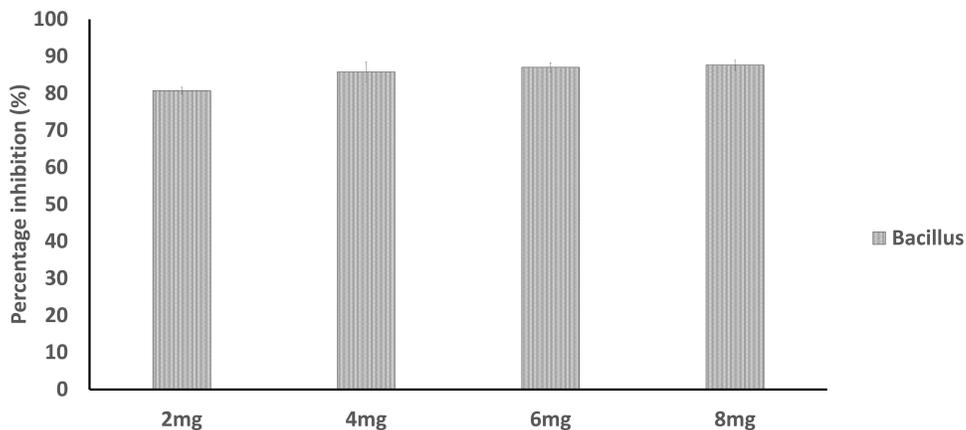


Figure 10 Biofilm inhibition assay using purified gum of *P. chilensis*.

**Table 3** Swarming Motility

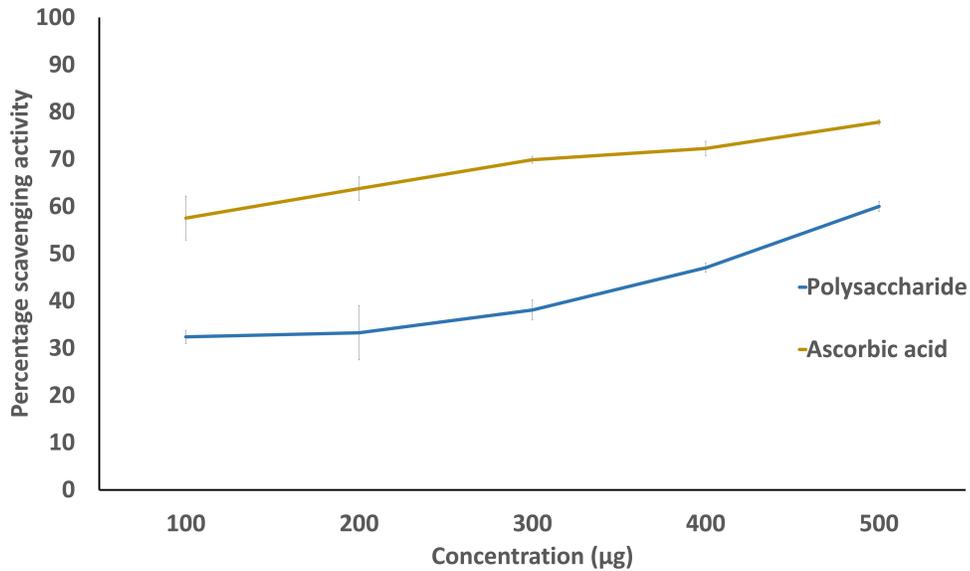
Plant Source	Organism	Biofilm(cm)	
		Control	Test
<i>A. heterophylla</i>	<i>Bacillus</i>	1.3	1.0
<i>P. chilensis</i>	<i>Bacillus</i>	1.3	1.0

of various plant polysaccharides in biofilm formation of *Pseudomonas aeruginosa*. They concluded that the polysaccharides galactan and galactomannan greatly

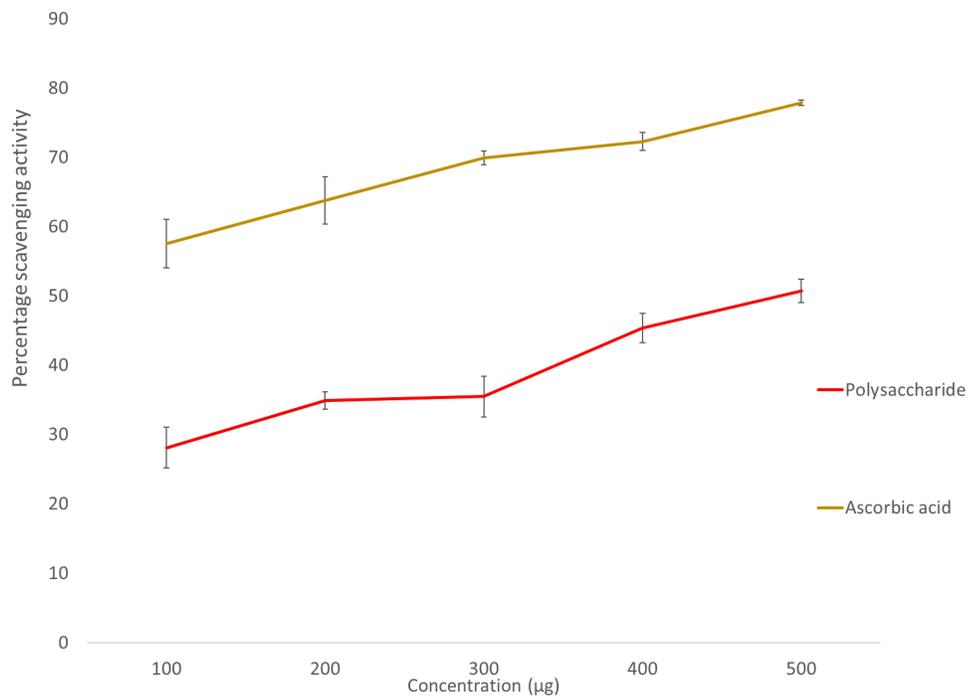
reduced the biofilm formation of clinical isolate, *P. aeruginosa* 216.

**Swarming Motility Assay**

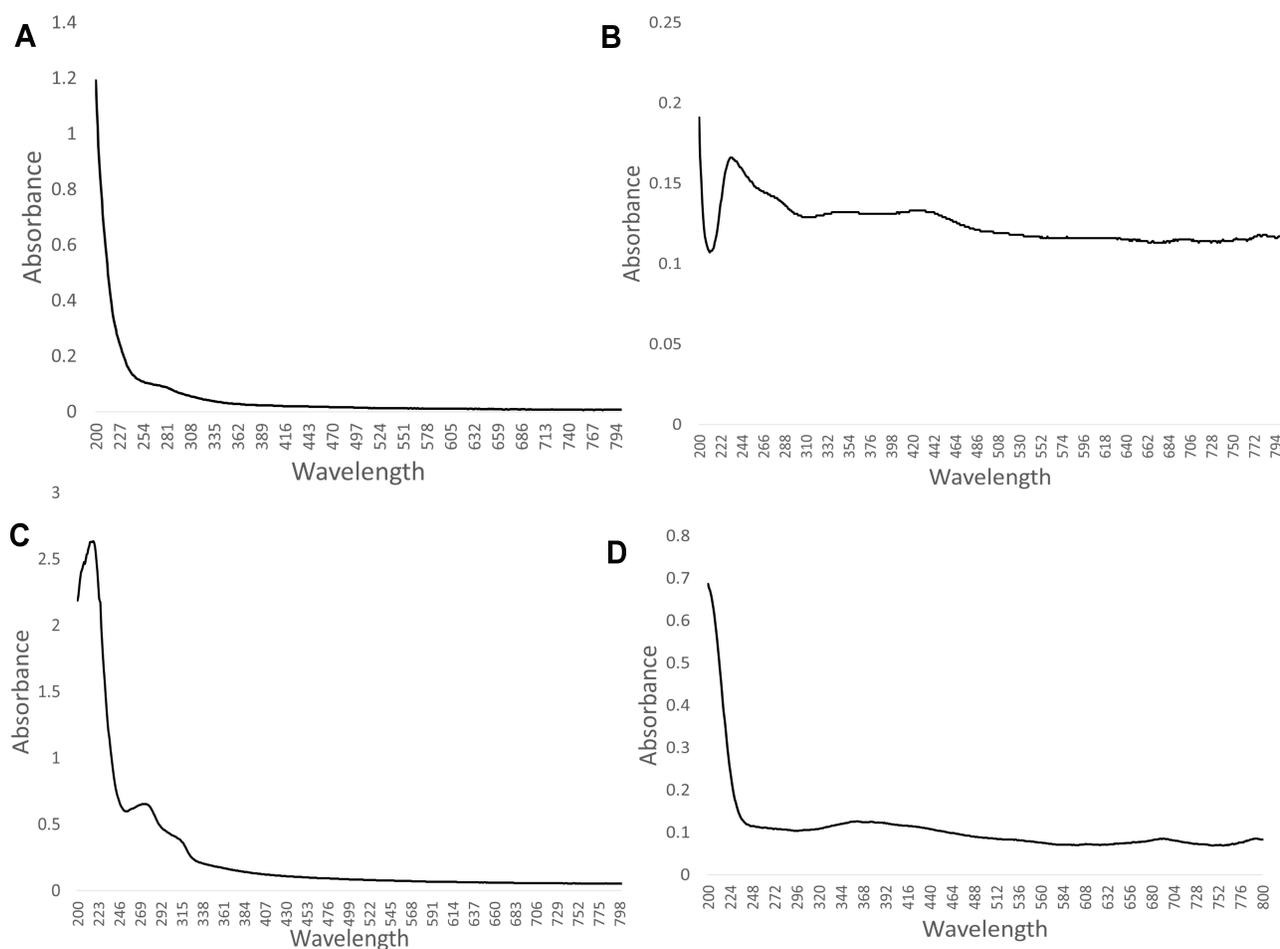
Swarming motility activity of *A. heterophylla* and *P. chilensis* against *Bacillus* sp was determined (Table 3) and both the polysaccharides were found to inhibit swarming motility and having biofilm inhibition. A poor biofilm development over the substrate layer on the plate showed that the polysaccharide was able to prevent the motility of the organism.<sup>46</sup>



**Figure 11** DPPH activity of *A. heterophylla*.



**Figure 12** DPPH activity of *P. chilensis*.



**Figure 13** UV-Vis spectroscopy of unloaded and drug-loaded nanocarriers: (A) Unloaded *A. heterophylla*, (B) Drug-loaded *A. heterophylla*, (C) Unloaded *P. chilensis*, and (D) Drug-loaded *P. chilensis*.

## Antioxidant Assay (DPPH)

As the concentration of the extract increased, the percentage of scavenging activity increased. The percentage of scavenging activity was 47.03% and 45.34% at 500  $\mu\text{g}$  concentration for *A. heterophylla* and *P. chilensis* respectively (Figures 11 and 12). As the concentration increases, the amount of free radical scavenged was increased. From the results obtained, both the polysaccharides were found to have an increased radical scavenging activity against DPPH with the increase of the concentration.<sup>4,47</sup>

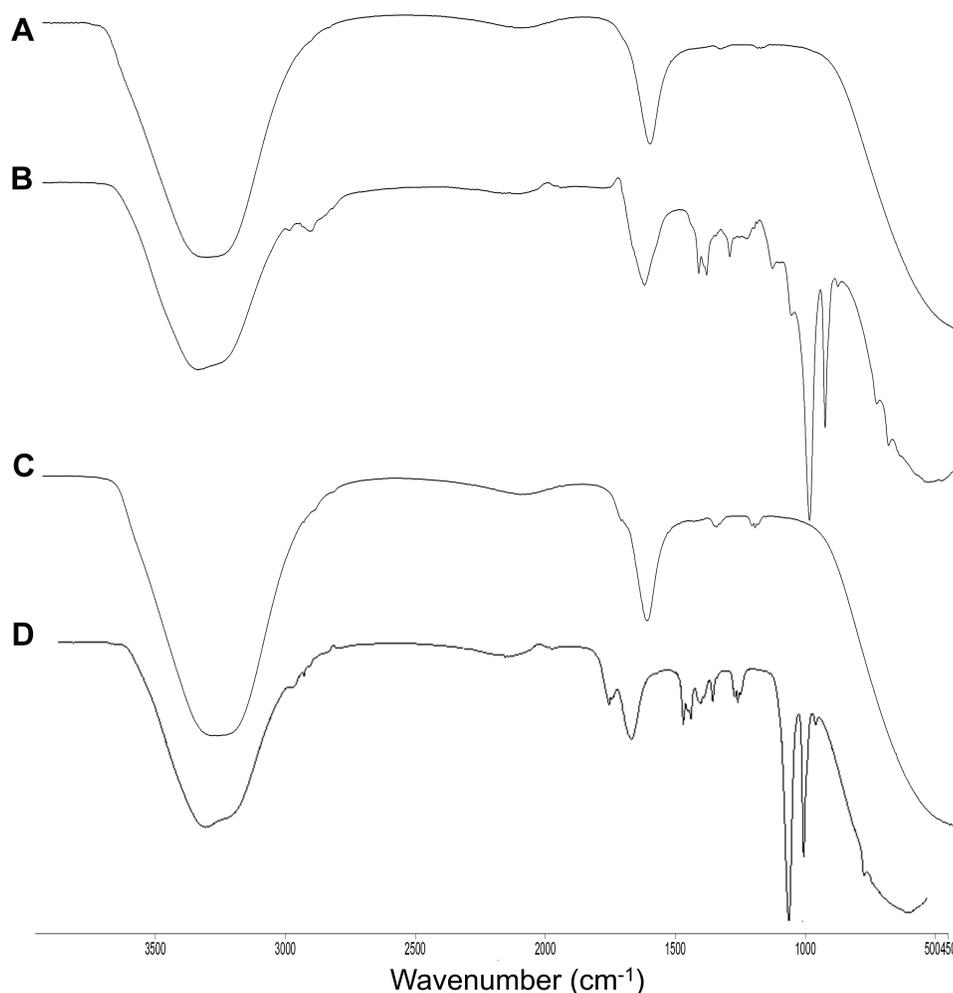
## Characterization of Loaded and Unloaded Nanocarrier

The fabrication of carboxymethylated gum into nanoparticles was attained by using STMP (sodium trimetaphosphate). STMP acted as crosslinker which initiated the carboxymethylated gum to form nanoparticles. Here, the reaction

was brought by formation of phosphoester linkage between two carboxymethylated biopolymer chains (with STMP as a crosslinker) and release of sodium pyrophosphate as the by-product. This mechanism of crosslinking the carboxymethylated biopolymers resulted in a 3-dimensional network of nanoparticulates which are used as nanocarriers to load drugs. The size of these nanocarriers can be controlled with the ratio of STMP and carboxymethyl agents used.<sup>48</sup> Loading of the drug is achieved by a simple precipitation of curcumin onto the nanocarriers in acidic pH.<sup>49,50</sup>

## UV-Vis Analysis

Figure 13 shows the UV-Vis analysis of unloaded nanocarrier and loaded nanocarrier of *A. heterophylla* and *P. chilensis* respectively. For both the drug-unloaded and -loaded nanocarriers of *A. heterophylla* and *P. chilensis* showed absorbance around 210 nm and 265 nm, which indicated the xylose and glucose of polysaccharide (Figure 13 A, B, C and D).<sup>51</sup> The absorbance



**Figure 14** FTIR analysis of: (A) Unloaded nanocarrier of *A. heterophylla*, (B) Drug-loaded nanocarrier of *A. heterophylla*, (C) Unloaded nanocarrier of *P. chilensis*, and (D) Drug-loaded nanocarrier of *P. chilensis*.

peak for drug-loaded nanocarriers were showing around 400 nm, a characteristic of curcumin (Figure 13B and D).<sup>3</sup>

### FTIR Analysis

The unloaded nanocarriers showed peaks at  $3307\text{ cm}^{-1}$ ,  $2144\text{ cm}^{-1}$ ,  $1635\text{ cm}^{-1}$ , and  $1217\text{ cm}^{-1}$  for polysaccharides and STMP (Figure 14A, B, C and D). For the loaded sample, carbonyl group and enolic C-O group of curcumin was observed in loaded nanocarrier at peaks  $1645\text{ cm}^{-1}$  and  $1217\text{ cm}^{-1}$  (Figure 14B and D) which were absent in the unloaded nanocarrier.<sup>52</sup> Peaks at  $3447\text{ cm}^{-1}$  represent the OH stretching of the polysaccharides, and  $870\text{ cm}^{-1}$  for C-H bending.<sup>4</sup>

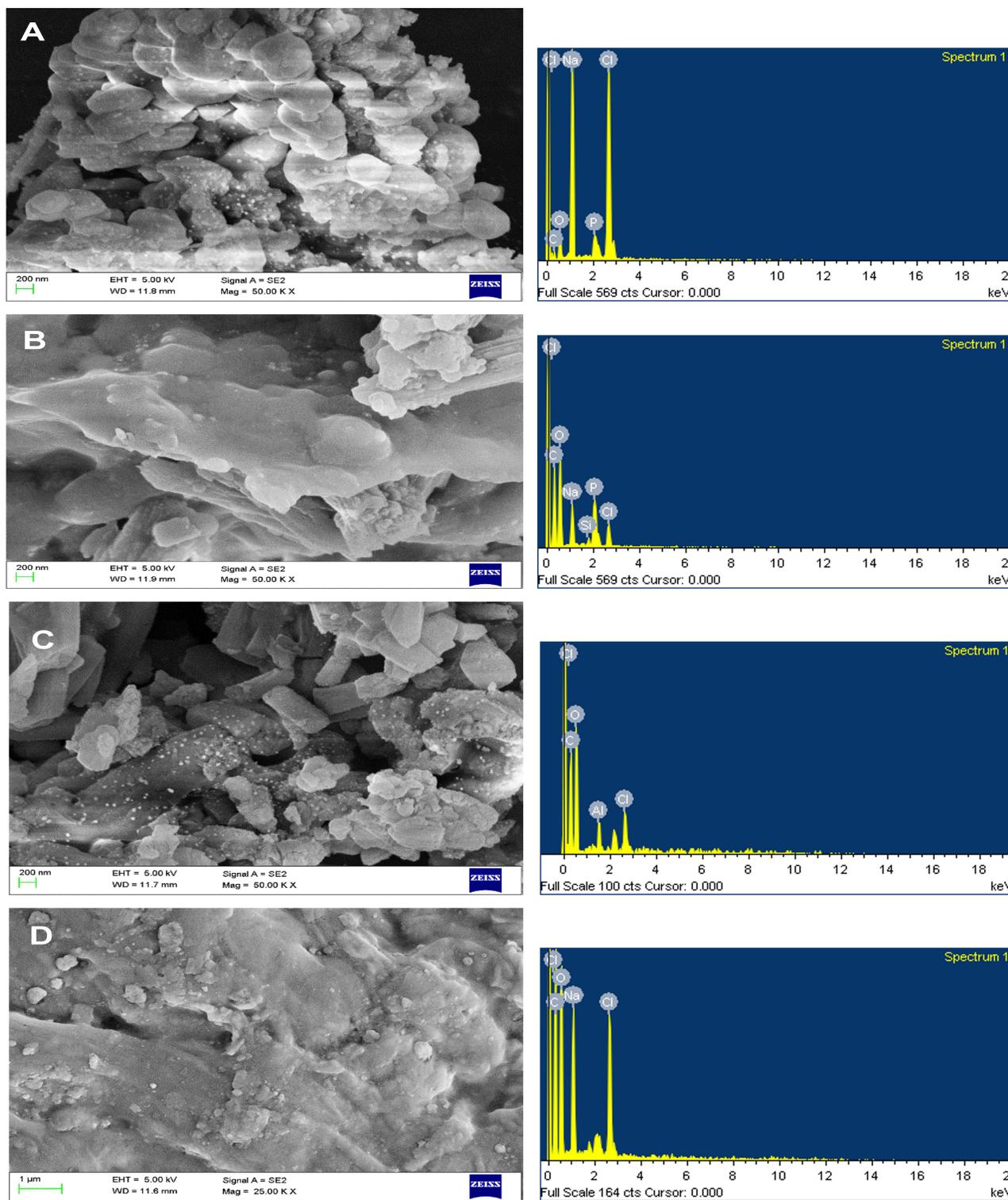
### Scanning Electron Microscopy

The size of unloaded nanocarriers was found to be below 100 nm but the shape was irregular (Figure 15A and C), where the drug-loaded nanocarrier of *A. heterophylla* and

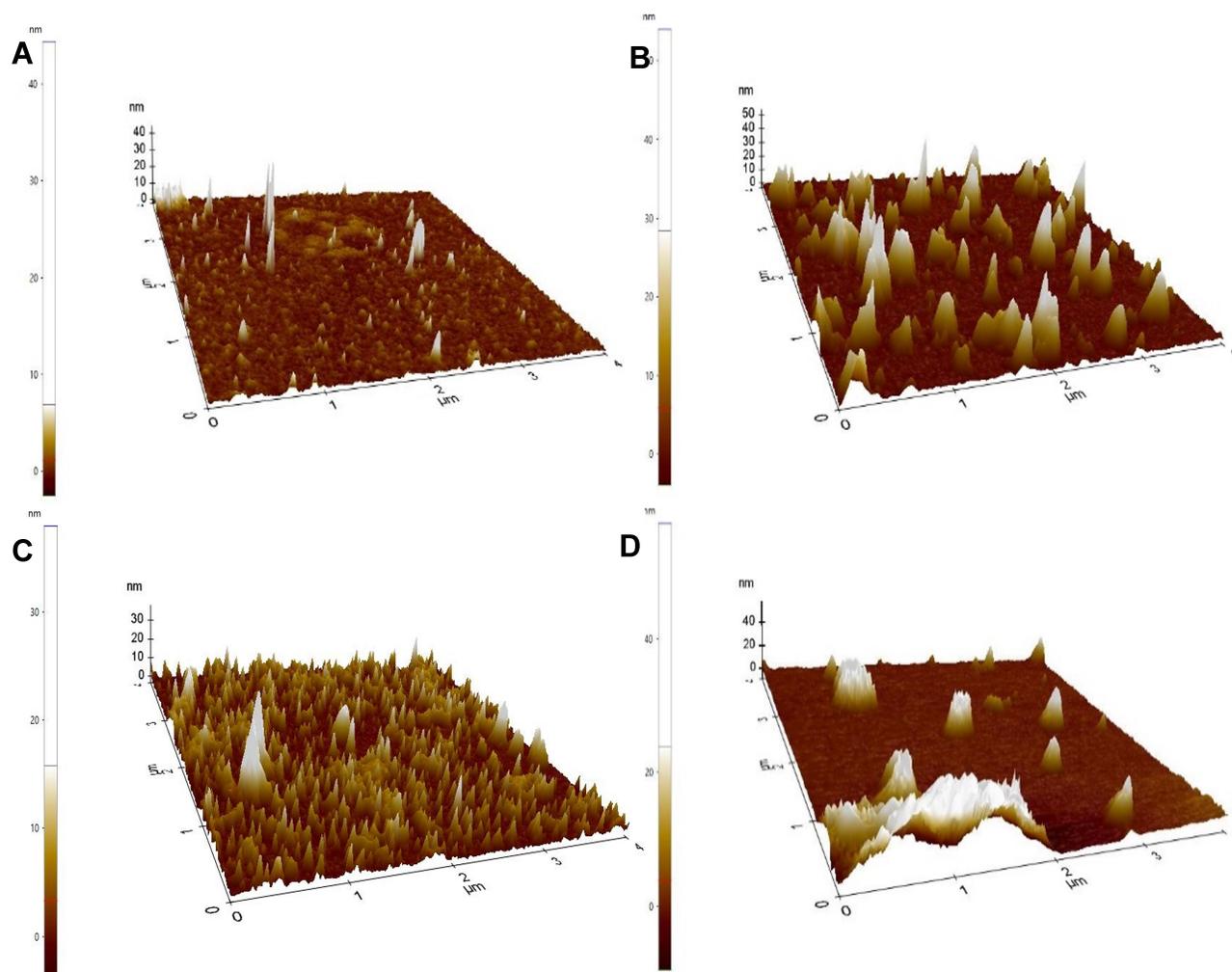
*P. chilensis* was around 200–500 nm respectively. Increase in size happened as the drug was encapsulated (Figure 15B and D). Even Samrot et al,<sup>8</sup> found increased size in drug-loaded nanocarriers. EDX analysis for both unloaded and loaded nanocarriers showed peaks for carbon, oxygen and other trace elements which was contributed to by the polysaccharide nature of the nanocarrier, water, and chelator used.<sup>8</sup>

### Atomic Force Microscopy

The size of unloaded nanocarriers of *A. heterophylla*, was below 100 nm and the size for loaded nanocarriers was about 200 nm (Figure 16A and B) whereas the unloaded nanocarriers of *P. chilensis* were below 100 nm, however size increased to 400 nm in drug-loaded nanocarriers (Figure 16C and D). Thus, the results that were found were on a par with the SEM analysis.



**Figure 15** SEM -EDX analysis of: (A) Unloaded nanocarrier of *A. heterophylla*, and (B) Drug-loaded nanocarrier of *A. heterophylla*. (C) Unloaded nanocarrier of *P. chilensis*, and (D) Drug-loaded nanocarrier of *P. chilensis*.



**Figure 16** AFM analysis of: (A) Unloaded nanocarrier of *A. heterophylla*, and (B) Drug-loaded nanocarrier of *A. heterophylla*. (C) Unloaded nanocarrier of *P. chilensis*, and (D) Drug-loaded nanocarrier of *P. chilensis*.

## Antibacterial Activity of Loaded Nanocarriers

The antibacterial activity for unloaded and drug-loaded nanocarriers was performed using water and ethanol as solvents (Figures 17 and 18). Tables 4 and 5 show the antibacterial activity of unloaded and loaded nanocarriers of *A. heterophylla* and *P. chilensis*. There was antibacterial activity against the bacteria while water and ethanol were used as the solvent. When just ethanol was used as the solvent, there was increased antibacterial activity which may be because of its bipolar solvent nature.

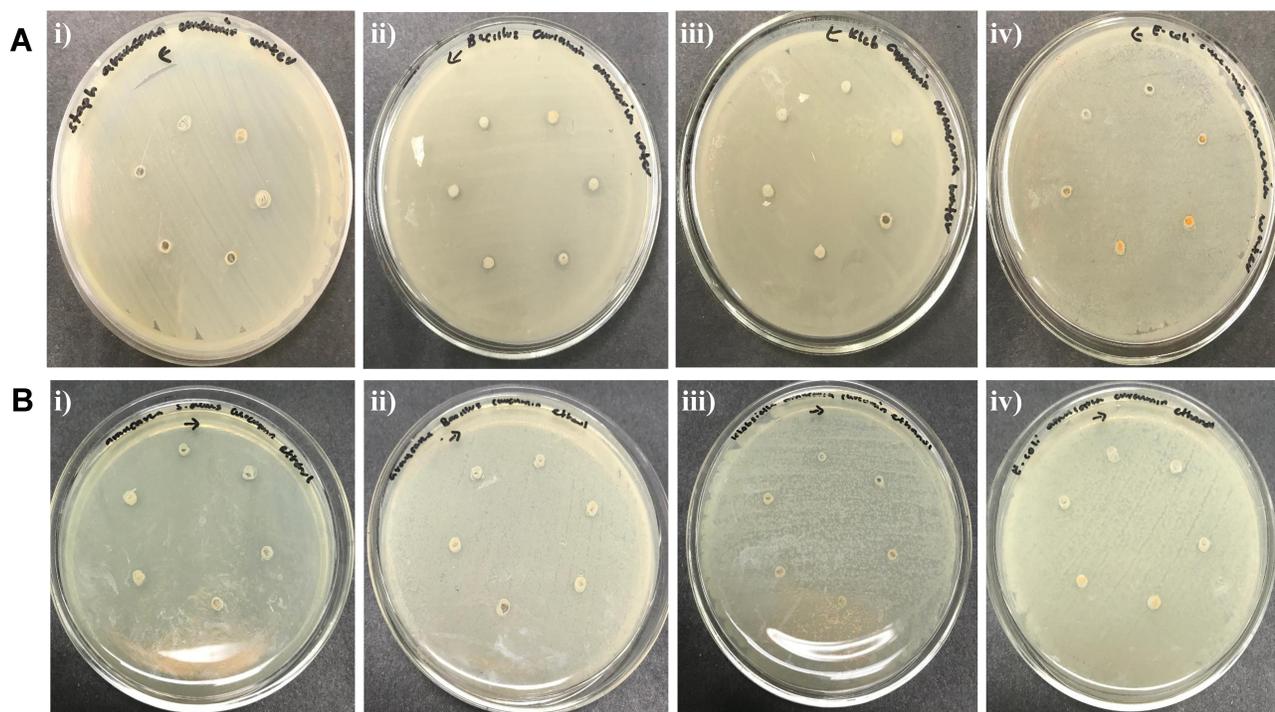
## Drug Release Kinetics

The release of drug molecules from nanocarriers were detected spectrophotometrically at 420 nm for every aliquot sample with a 15-minute time interval. The absorbance was found to increase as the incubation period was extended.

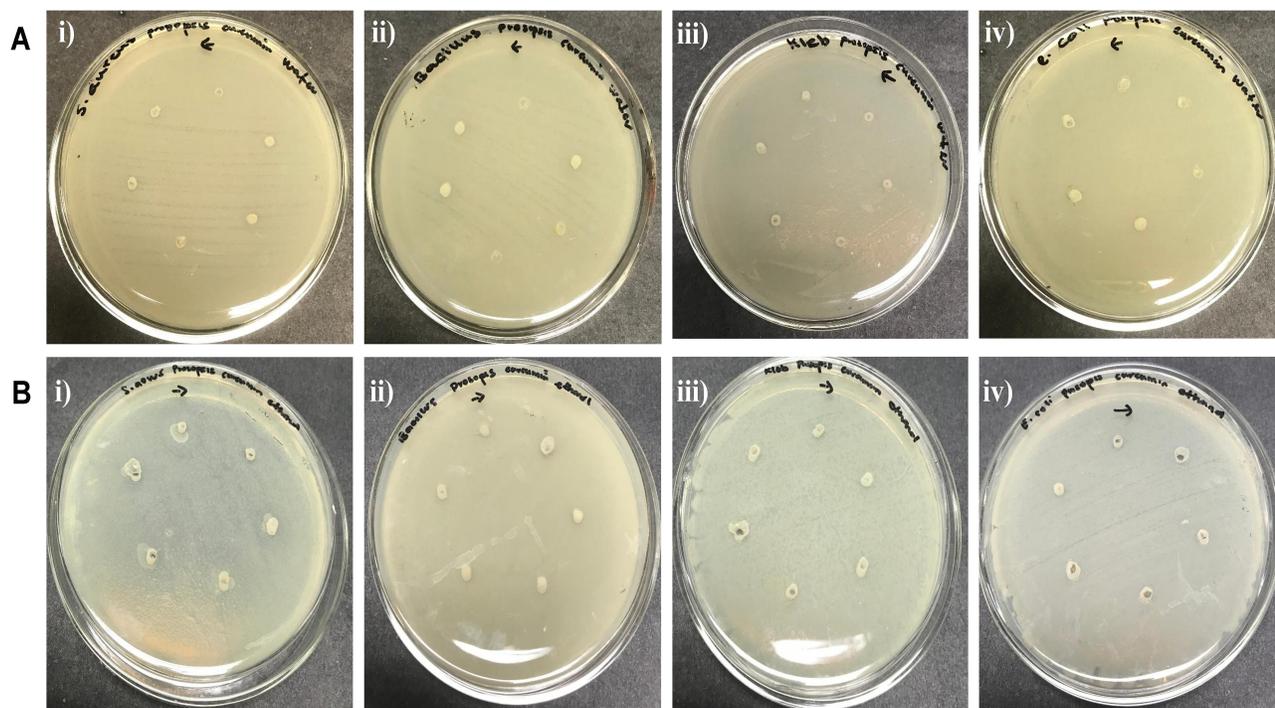
This confirmed that the curcumin molecules were gradually released from the nanocarriers. Bashir et al<sup>53</sup> also photometrically analyzed the activity of theophylline from loaded hydrogels. Figure 19 shows the result for drug release kinetics of loaded polysaccharides *A. heterophylla* and *P. chilensis*. Throughout the incubation time (a 3-hour period), curcumin was released slowly. The curcumin release was steady with the case of loaded *P. chilensis* nanocarriers comparatively. Polysaccharide-based nanoparticles and hydrogels were reported to release drugs - efficiently.<sup>8,54-57</sup>

## Conclusion

In this study, polysaccharides from *A. heterophylla* and *P. chilensis* gum were extracted, purified and characterized. Bioactivities were performed for both of the gums. Purified



**Figure 17** Antibacterial activity of curcumin-loaded *A. heterophylla* nanocarrier. (A) Water as solvent, and (B) Ethanol as solvent: i) *Staphylococcus aureus*, ii) *Bacillus* sp., iii) *Klebsiella* sp., iv) *Escherichia coli*.



**Figure 18** Antibacterial activity of curcumin-loaded *P. chilensis* nanocarrier. (A) Water as solvent, and (B) Ethanol as solvent: i) *Staphylococcus aureus*, ii) *Bacillus* sp., iii) *Klebsiella* sp., iv) *Escherichia coli*.

**Table 4** Antibacterial Activity of Unloaded and Drug-Loaded Nanocarrier of *A. heterophylla*

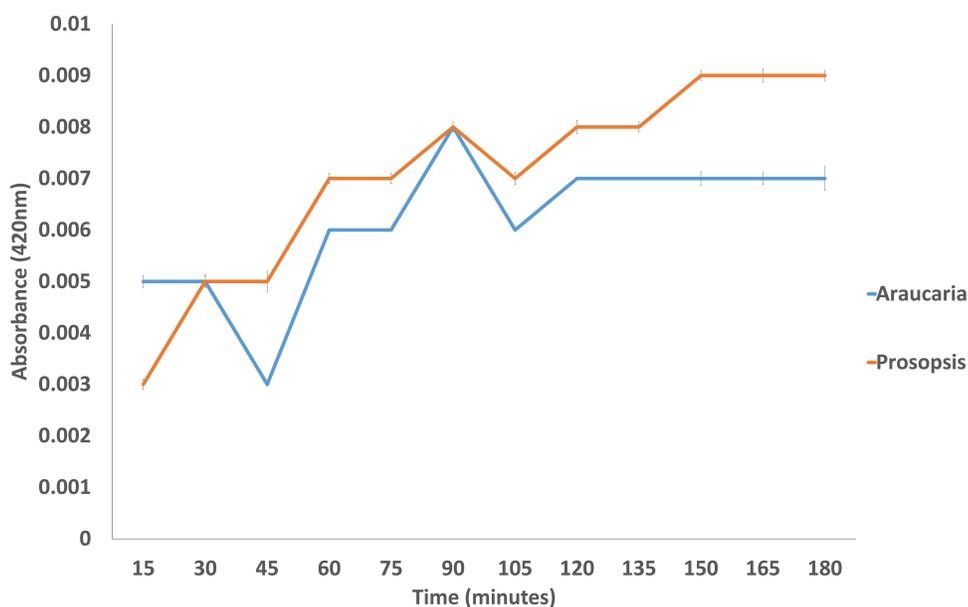
Organism	Zone of Inhibition (cm)											
	Solvent Control		Unloaded Nanocarrier		2µL		4µL		6µL		8µL	
	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol
<i>S. aureus</i>	–	–	0.1	0.2	–	0.2	–	0.2	–	0.2	0.1	0.2
<i>Bacillus</i>	–	–	0.1	0.2	–	–	0.1	–	0.1	–	0.2	0.1
<i>Klebsiella</i>	–	–	0.1	0.1	–	–	–	0.1	–	0.1	–	0.2
<i>E. coli</i>	–	–	0.1	0.1	–	0.1	–	0.1	–	0.1	–	0.1

**Table 5** Antibacterial Activity of Unloaded and Drug-Loaded Nanocarrier of *P. chilensis*

Organism	Zone of Inhibition (cm)											
	Solvent Control		Unloaded Nanocarrier		2µL		4µL		6µL		8µL	
	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol
<i>S. aureus</i>	–	–	0.1	0.2	–	0.2	–	0.2	0.2	0.2	0.3	0.3
<i>Bacillus</i>	–	–	0.1	0.2	–	0.1	–	0.1	–	0.1	0.2	0.1
<i>Klebsiella</i>	–	–	0.2	0.2	–	–	0.1	–	0.1	0.1	0.1	0.1
<i>E. coli</i>	–	–	0.2	0.1	0.1	–	0.2	–	0.3	0.1	0.3	0.1

polysaccharides were found to have a good antioxidant property and also antibacterial activity against *Bacillus* sp. Purified polysaccharide was made to favor nanocarrier synthesis, where STMP chelator was used for the synthesis. Thus, the produced nanocarrier was loaded with curcumin and characterized by Fourier transform infra-red spectroscopic (FTIR), Scanning electron microscopy (SEM) and Atomic force microscopy (AFM) analysis. The size of drug-

loaded nanocarriers was found to be below 200–500 nm for *A. heterophylla* and *P. chilensis* respectively. There was efficient drug release even against bacterial activity in vitro. Plant gum-based polysaccharides are natural components which are biocompatible and biodegradable. Hence, formulation of nanocarriers from these non-toxic natural materials can be a favorable option for drug delivery agents.



**Figure 19** Drug release kinetics of drug-loaded polysaccharides of *A. heterophylla* and *P. chilensis*.

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## Disclosure

The authors report no conflicts of interest in this work.

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