Received: 22 January 2021 | Revised: 23 February 2021 | Accepted: 08 March 2021
DOI: 10.22034/ecc.2021.273357.1139
Eurasian Chemical Communications
SAMI

FULL PAPER

Characterization of chemical structure of water-soluble polysaccharides from Sudanese *Ziziphus spina Christi* fruits

Mohamed Aamer Abubaker[a,b] |Hamouda Adam Hamouda[c] |Lele Chen[a] |Ling Ding[a] |Sheng Yong Zhu[a] |Liang Wan Fu[a] | Ji Zhang[a,*]

[a]*College of Life Science, Northwest Normal University, Lanzhou 730070, China*

[b]*Department of Biology, Faculty of Education, University of Khartoum, Khartoum, 11111, Sudan*

[c]*College of Chemistry and Chemical Engineering, Northwest Normal University, Lanzhou, 730070, China*

Polysaccharides have multiple significant roles and possess extensive bioactivities in field of medicine, healthcare, food and cosmetic industries, because of their therapeutic effects and relatively minimal toxicity. For the first time, we investigated the chemical structure of *Ziziphus spina Christ* fruits polysaccharides fraction. The plant is classified as one of the most important Sudanese medical plant, still not adequately researched.
The *Z. spina Christi* polysaccharides fraction was extracted by using cold water extraction method, deproteinized via Savage's method, then after participated by using ethanol 80%, the precipitant fractions were dried via lyophilized for 24h. ZSCFPs was investigated by using colorimetric and analytic methods; the colorimetric assays were carried out to determine some functional groups in the polysaccharides fraction such as phenol-sulfuric acid, sulfuric acid - carbazole reaction, ferric chloride reaction. The analytical assays were carried out to confirm the chemical structure of the ZSCFP 80%, which were FTIR spectra, Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), and X-ray diffraction (XRD).

***Corresponding Author:**
Ji Zhang
Email: zhangj@nwnu.edu.cn
Tel.: +1321287662

KEYWORDS

Ziziphus spina Christi; polysaccharides; protein precipitation; lyophilized.

Introduction

Polysaccharides are considered as one of the most important constituents that exist in the organism, having several biological features, such as the source of energy, and exist in building of the living organism's body [1]. The polysaccharides are derived from carbohydrate; they are polymer macromolecule. They show bio-activity in plants, animals, and microorganism; therefore, they are considered as the most promising medicinal constituents, because of various biological activities [2]. Polysaccharides show therapeutic potent features against chronic diseases such as cancer, and act as anti-tumour, anti-diabetic, anti-inflammatory agents [3]. The biological influences of polysaccharides rely on their chemical components, molecular weight, and structure [3].

The Sudanese flora is rich in the variety of medicine plants[4], one of which is *Ziziphus spina Christi* tree considered as the most important species of Genus. It belongs to the Rhamnaceae family. In Sudan and the Middle East it is known as Sidder, and in English as Christ's thorn Jujube[5]. Morphologically it is a spiny shrub, with length 5-10 meters tall, and remains green throughout the year.

Geographically it is spread throughout tropical and subtropical countries precisely in North Africa, Middle East, and northwest India, In Sudan it grows over large parts of the country [6]. Several studies have reported that *Ziziphus spina Christi* aerial parts such as Leaves, Flowers, Fruits, and seeds possess a various nutritional and therapeutic lineaments, having been used in folk medicine because of anti-oxidants, and anti-microbial activities. Leaves, fruit and seed have a potent source of many phytochemical compounds precisely polyphenol, flavonoids, Tannins, Alkaloids, Terpenoids and Saponins [7]. *Z. Spina Christi* fruit is edible and wealthy with phytoconstituents [8]. Morphologically the fruit is semi globe shape, yellow or reddish colour; the pulp is usually sweaty, and eaten as fresh, and used in folk medicine for a long time. It has been used to relief of digestive, disorder, and treat many microbial infections [9]. Despite the existence of many reports that indicated the widespread of this wild edible plant, especially in some Sudanese regions like North Kordufan State, it still lacks research and documentation. It should be noted that there is no internationally documentation of Sudanese collected plant germplasm. Thus, many plants lack identification and documentation [10]. Accordingly, this study aimed to extract *Ziziphus spina Christi* fruit's pulp polysaccharides for the first time by using distil water, and screening the chemical structure of fraction by mean FT-IR and UV-vis spectroscopic analysis, and scanning electronic microscopy (SEM) techniques.

Materials and methods

Materials

Methanol, potassium bromide (KBr), sulfuric acid, phenol, carbazole, Ferric chloride, distilled water, Ethanol 95%, Chloroform, n-Butanol, were applied. All other chemicals were of analytical grade.

The fruits of *Ziziphus spina* was purchased from the market in Khartoum, Om-Dorman, Sudan, washed thoroughly with distilled water to remove the dirt and other contaminations. After being dried under ambient temperature, the pulp was removed carefully from seeds, and dried by using technique of freeze and drying.

Methods

Extraction of ZSCFP fractions

The extraction procedures of *Z. spina* fruits polysaccharide fractions were started by eliminate the lipids with methanol at 65 ^{0}C for 6h, by using Soxhlet apparatus (Figure 1), then the residual was filtered and washed *via* ethanol 95% several times to remove monosaccharides and other impurities.

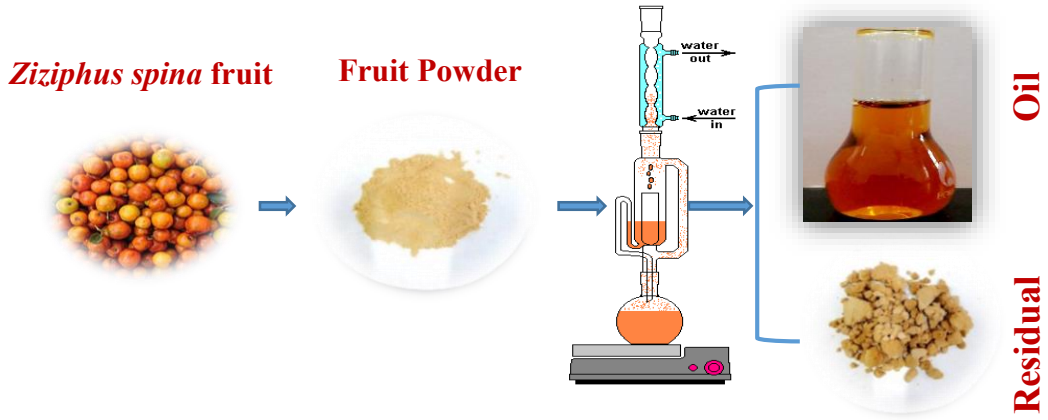

FIGURE 1 Processes of removing lipids from *Z. spina* fruit's pulp

The residual was dried under room temperature to remove the solvent ethanol 95%. It was prepared for extraction polysaccharides fraction by using distilled water in ratio solid to liquid (1:40) mg/ml stirred for 24 h, and then the extraction solution was removed *via* centrifugation at 6000 rpm for 15 minutes. The supernatant was kept for further experiments.

Deproteinization of ZSCFPs

The deproteinization of extracted crude polysaccharides was carried out by using Savage method as mentioned in the literature[11] with slight modifications; the ratio of Savage's reagents was chloroform: n-butanol = 4:1, V/V and centrifuged at 6000 rpm for each deproteinization was done to get rid of insoluble protein; the deproteinization was repeated eight to ten times until no protein sediment remained in the polysaccharides solution (Figure 2).

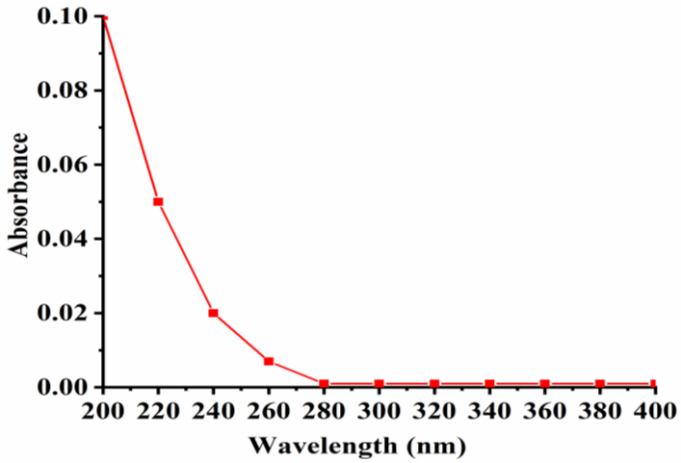

Figure 2. UV-vis spectra of Z. spina fruits polysaccharides fractions

Precipitation of ZSCFP fractions

The precipitation of *Ziziphus spina Christi* polysaccharides was carried out by using ethanol 80%. After 24 hours the precipitate was centrifuged at 4000g for 20 min at 10 °C (Figure 3) and lyophilised. The polysaccharides fractions have a various solubility in existence of alcohol such as ethanol, or acetone.

Purification of ZSCFP fractions by using ultracentrifugation method

The purification of Ziziphus spina Christi polysaccharides was carried out by using (Ultracentrifugation method). This method leads to separation of polysaccharides molecular weight via speed of the strong centrifugal force [11].

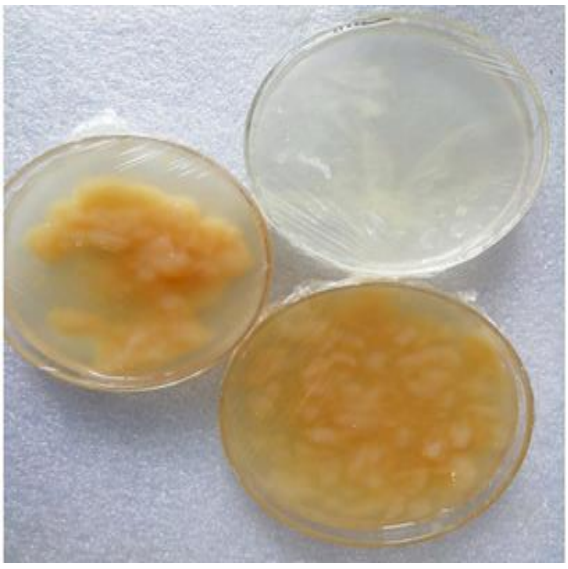

Figure 3. The precipitation of Z. spina fruits polysaccharides fraction at ethanol 80%

Chemical characterization of ZSCFPs

Colorimetric assays of chemical functional groups

Ziziphus Spina Christi fruits polysaccharides fractions were screening colorimetrically by using different reagents to identify the various chemical functional groups, the result shown in (Figure 4).

Phenol-sulfuric acid

The polysaccharides were hydrolysed into monosaccharides and formed furfural derivatives, which led to composing brownish red component with phenol, in the presence of sulfuric acid [13].

Sulfuric acid - carbazole reaction

The sulfuric carbazole reaction was used to confirm the existence of uronic acid in the polysaccharides; the uronic acid has a condensation reaction with carbazole in the presence of concentrated sulfuric acid [14].

Ferric chloride reaction

This reaction indicates the presence of phenolic hydroxyl group, by forming coloured coordination compounds [15].

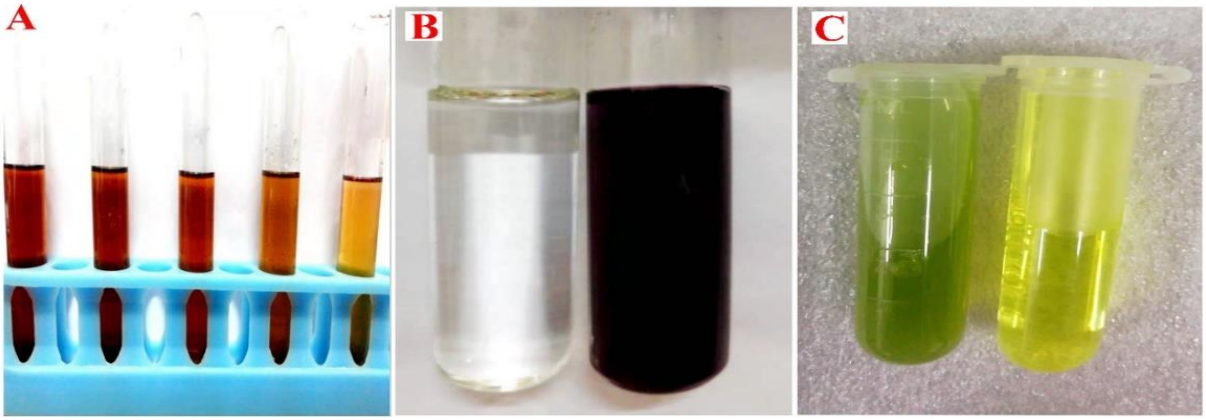

FIGURE 4 (A) Phenol-sulfuric acid reaction, (B) Sulfuric acid - carbazole reaction**, (C)** Ferric chloride reaction

Scanning electron microscopy (SEM)

Instrument model is Ultra Plus made in Germany named thermal field emission scanning electron microscope. It was used for surface characterization and microstructure of *Ziziphus spina Christi* fruits pulp polysaccharides before and after extraction, and after deproteinization, the scanning electron microscope was used as qualitative tool to analyse polysaccharides morphological surface [16] as shown in (Figure 5).

The result of SEM test showed differences in morphological of *Ziziphus spina Christi* fruit polysaccharides fraction surface, because of extraction and deproteinization procedures.

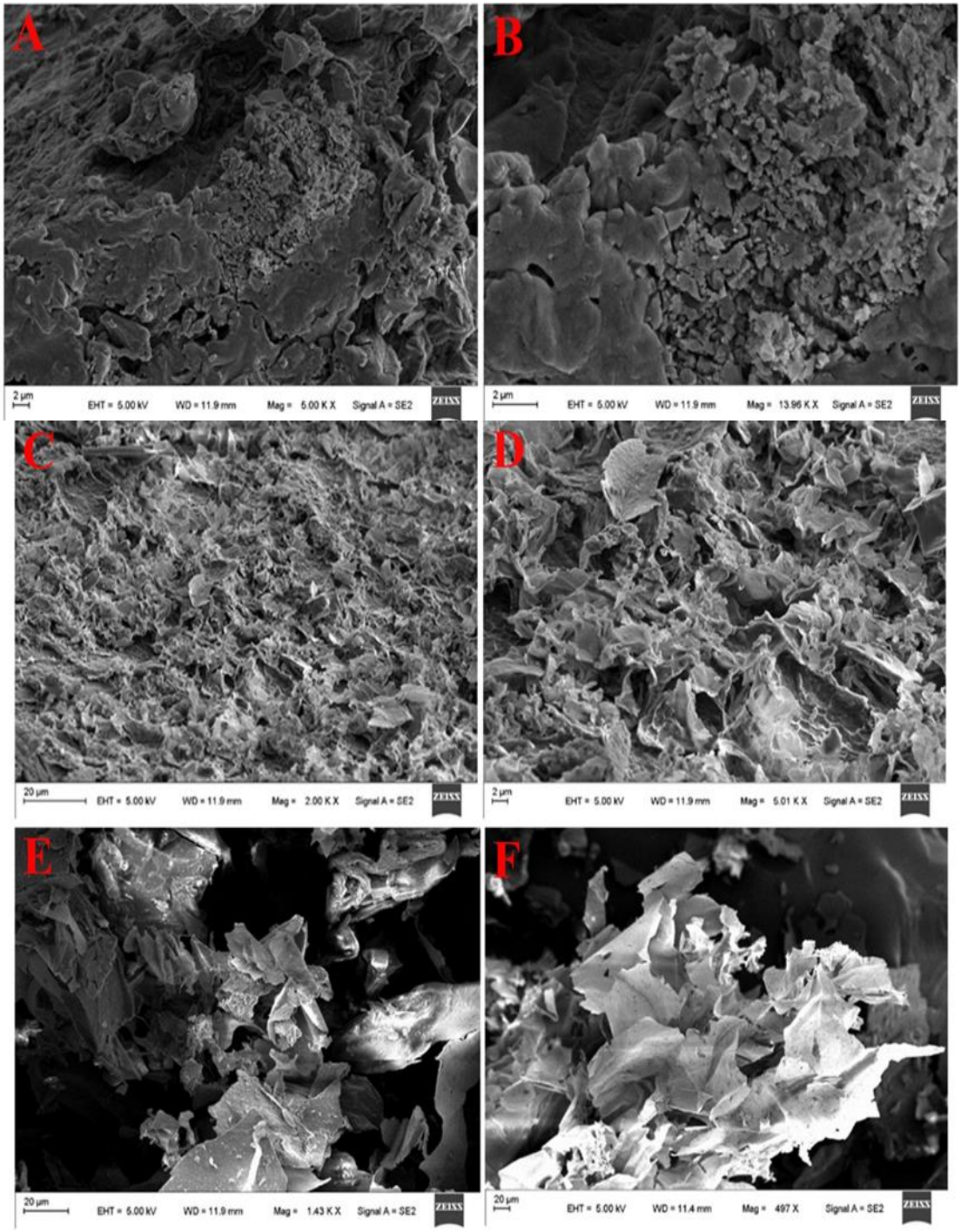

FIGURE 5 Surface characterisation of *Ziziphus spina Christi* fruit pulp (A, B) before extraction, (C, D) after extraction (E, F) after deproteinization

Size-exclusion chromatograph molecular mass ZSCFP fractions

Size-exclusion chromatograph combined with multi-angle laser photometer (Wyatt Technology Co., USA) and Optilab refractometer (wavelength of 690 nm) was used to determine the weight average molecular mass (MW) of ZSCFPs fractions, by using method of [17]. With slight modification, samples were dissolved in ultra- pure water at room temperature and filtered through a (0.45μm) cellulose filter to obtain

polysaccharide solution with the concentration of 1mg/mL. The Ultrahydrogel™ column (7.8×300 mm, Waters, USA) was used to perform sample elution (ultra-pure water as mobile phase, flow rate of 1 mL/min and injected mass of (50µL). The refractive index increment (dn/dc) value was determine to be 1.3 mL/g. The data was analysed by using ASTRA software, and the result is shown in Figure 6 and Table 1.

TABLE 1 Molecular mass and conformational parameters of ZSCP from SEC-MALLS

Sample	MW×10^5 (g/mol)	Mw/Mn	Mn×10^5 (g/mol)
ZSCFPs	4.23	1.37	3.09

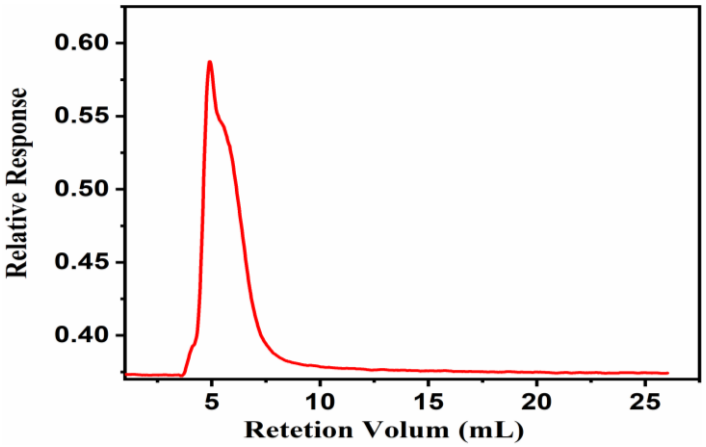

FIGURE 6 SEC-MALLS chromatograms of Ziziphus spina Christi fruit polysaccharides fraction

The results are shown in (Figure 6) and Table1. The weight average molecular weight (Mw), number average molecular weight (Mn) and poly disparity coefficient (Mw/Mn) of ZSCPS were 4.23×10^5 g/mol, 3.09×10^5 g/mol and 1.37, respectively.

Atomic force microscopic (AFM)

The AFM is considered as one of the advance technique used for studying Polysaccharides surface morphologies[18] ZSCFP dissolved in deionized water and diluted serially to concentration 1- 0.5 ng/mL. (20 µL) solution was deposited on the surface of newly cleaved mica at room temperature. Atomic force microscope (Veeco instruments, USA) was set in tapping mode, scanned and observed. AFM images were analysed by Nano Scope Analysis software (Digital Instruments, Santa Barbara, CA, USA).

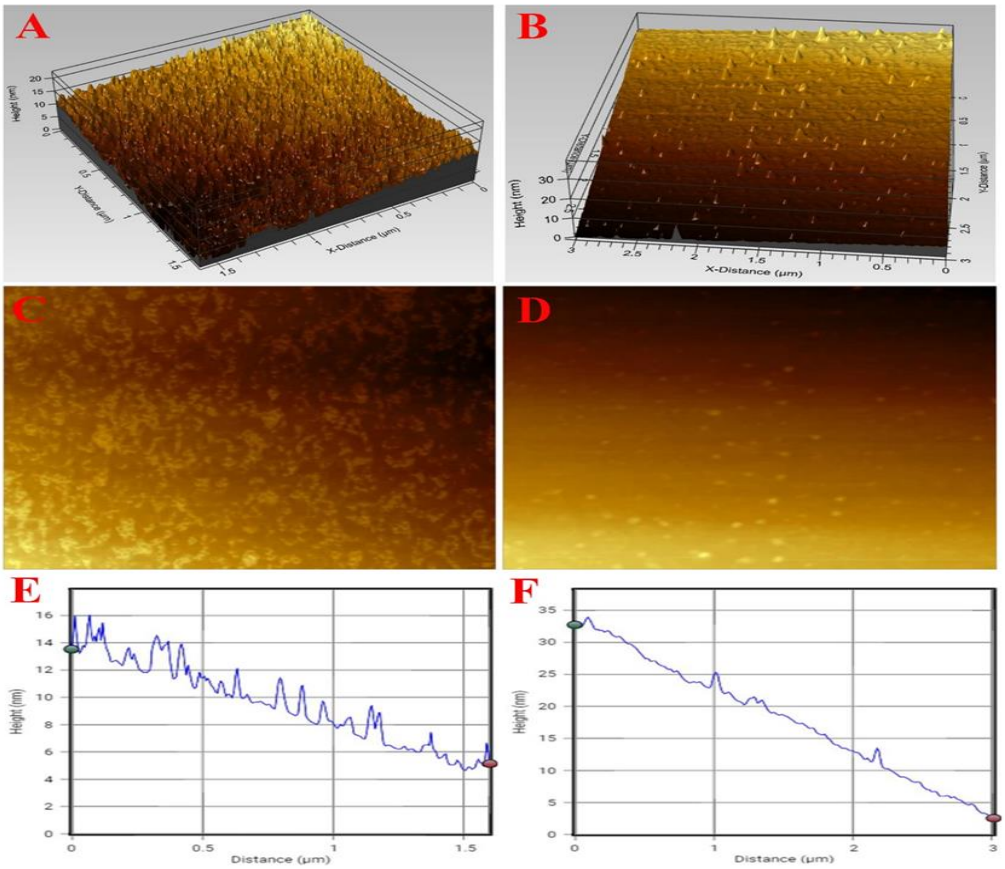

FIGURE 7 The AFM images ZSCFPs at the concentration of (A 0.5- B 1) ug/mL

The AFM images of ZSCFPs are shown in (Figure 7). There are obviously several spherical lumps in *Z. spina* polysaccharides fractions, which elucidate to molecular aggregation. This could indicate the hydrogen bonds in the aggregated side chains, which play a significant role in the aggregation of the polysaccharide molecules, because the hydroxyl groups on the polysaccharide chains can form strong intermolecular and intramolecular interactions with each other or with water molecules. Based on these morphological properties, we concluded that this aggregation has an evident effect on the conformation, molecular weight, distribution, and even the biological activities of *Z. spina* fruit polysaccharides.

Fourier transformer microscopy (FTIR)

The FTIR spectra technique was used for identification functional groups of compounds. The results of this test showed form of absorbance band regions, with every region corresponded to chemical bond types, and with distinct peak area region called fingerprint shown in (Figure 8). 100 microgram powder of ZSCFP was mixed with 10 microgram of KBr and adjusted to IR instrument [19].

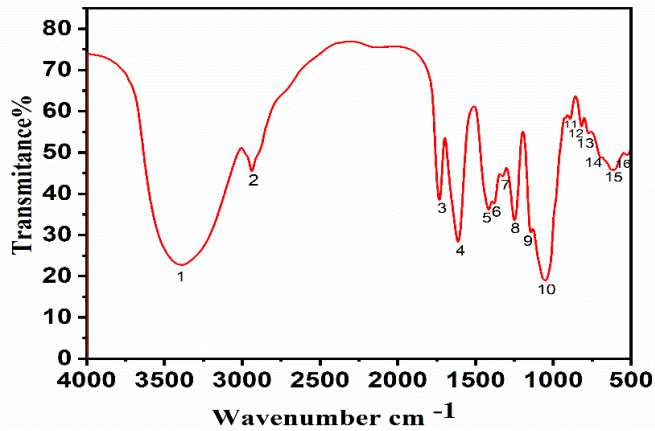

FIGURE 8 IR spectra of the ZSCFP fractions

TABLE 2 IR spectra of the ZSCFP fractions functional groups

Peak No	Wavenumber cm^{-1}	Functional groups	Identification
1	3384.82 cm^{-1}	OH, (v.br.)	alcohol
2	2938.24 cm^{-1}	C-H , (w)	aliphatic
3	1733.29 cm^{-1}	C=O , (m)	carbonyl
4	1612.21 cm^{-1}	C=O , (s)	carbonyl
5	1416.01 cm^{-1}	C=C , (w)	cis
6	1380.98 cm^{-1}	C=C, (w)	aromatic
7	1325.84 cm^{-1}	O-H, (w)	phenol
8	1249.38 cm^{-1}	C-N, (m)	amine
9	1144.62 cm^{-1}	C-O, (w)	aliphatic ether
10	1050.57 cm^{-1}	C-O, (s)	phenol
11	892.58 cm^{-1}	C=C, bending (w)	alkene
12	818.83 cm^{-1}	Unidentified	-
13	772.91 cm^{-1}	Unidentified	-
14	693.80 cm^{-1}	Unidentified	-
15	610.32 cm^{-1}	C-H , (br)	aromatic
16	527.07 cm^{-1}	C-H , (w)	aromatic

Key: m = medium, v = very, s = strong or sharp, w = weak, br. = Broad

The IR analysis was carried out by using Mid-IR in region (4000-500) cm^{-1} at FTIR (thermos Nicolet IS10, USA). *Z. spina* fruit polysaccharides spectrum result in (Table 2) show about sixteen absorbance bands, separated in at wavelength number. The first very broad peak appeared at 3384.82cm^{-1} indicating the presence of OH, alcohol chemical bond[20], and weak peak at 2938.24 cm^{-1} which is connected with C-H , aliphatic[21]. C=O, carbonyl functional group appeared at two regions with two absorbance bands 1733.29 cm^{-1}, and 1612.21cm^{-1} respectively**.** At the fingerprint in region (1500-500) cm^{-1} with about twelve peaks, there are nine peaks identified and three of them unidentified. Identification of fingerprint by using IR is considered as one of the most important methods in the investigation of medicinal features of the plant[22].

X-ray diffraction of ZCFP fractions

The x-ray diffraction (XRD) was conducted using a Rigaku D/Max-2400 diffractometer equipped with Cu Kα radiation (k = 1.5418 Å). The X-ray diffractogram of ZSCP was between **5°** and **50°**. The test proved that the water-soluble polysaccharides extracted from ZSC plant were semi crystalline polymer, in agreement with [23].

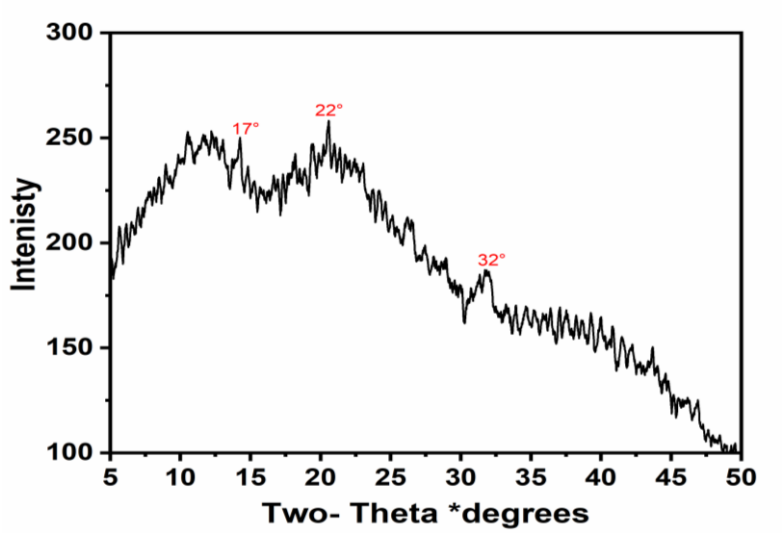

FIGURE 9 X-ray diffraction pattern of ZSCP fractions

The XRD analysis of ZSCP fractions in (Figure 9), showed sharp narrow diffraction peaks at **17°** and **32°**, crystalline polysaccharides, broad peak at **22°** showing amorphous components, and there is a probability of a peak at **40°** in XRD, related to the existence of uronic acid [24].

Conclusion

The results of chemical characterization by using colorimetric such as Phenol-sulfuric acid, Sulfuric acid - carbazole reaction, and Ferric chloride reaction showed the existence of uronic acid, and phenol functional groups. Besides, the analytical tests such as the SEM showed the change that was observed on the surface of ZSCFPs before extraction until purification. The AFM, GPC elucidated the molecular weight. The FTIR spectra showed about sixteen active functional groups in the ZSCFPS fractions. The XRD identified the ZSCFPS as semi crystalline polymer.

Acknowledgments

The authors would like to acknowledge Prof. Ji Zhang and the University of North West Normal - College of Life Science Staff for technical support, and thankful to Lecturer Widad Mudthir University of Khartoum Faculty of Education.

Orcid:

Mohamed Abubaker: https://orcid.org/0000-0002-0073-1407

Hamouda Adam Hamouda: https://orcid.org/0000-0002-6967-7269

Ji Zhang: https://orcid.org/0000-0003-2773-5245

References

[1] L. Chen, L., Huang, G. Hu, *Int. J. Biol. Macromol.*, **2018**, *108*, 408–411.

[2] L. Cai, B. Chen, F. Yi, S. Zou, *Int. J. Biol. Macromol.*, **2019**, *140*, 907–919.

[3] H. Wang, Y. Li, Z. Ren, Z. Cong, M. Chen, L. Shi, X Han, J. Pei, *Int. J. Biol. Macromol.*, **2018**, *112*, 473–482.

[4] Y. Hilmi, M.F. Abushama, H. Abdalgadir, A. Khalid, H. Khalid, *BMC Complement. Altern. Med.*, **2014**, *14*, 1–5.

[5] A. Dafni, Sh. Levy, E. Lev, *J. Ethnobiol. Ethnomedicine,* **2005**, *1*, 1-11.

[6] E.M. Abdallah, *Int. J. Curr. Microbiol. Appl. Sci.*, **2017**, *6*, 38–44.

[7] L. Baghazadeh-Daryaii, G.R. Sharifi-Sirchi, D. Samsampoor, *J. Appl. Res. Med. Aromat. Plants.*, **2017**, *7*, 99–107.

[8] M. Bahmani, A. Jalilian, I. Salimikia, S. Shahsavari, N. Abbasi, *Plant Science Today*, **2020**, *7*, 275–280.

[9] A. Fath El-Rahman, I. Munged, A. Tegani, A. Ibrahim, A. Mohamed, H. Mohamed, A.

Mubarak, I*nternational Journal of Engineering and Applied Sciences*, **2019**, *11*, 21–23.
[10] A.S. Saied, J. Gebauer, K. Hammer, A. Buerkert, *Genet. Resour. Crop Evol.*, **2008**, *55*, 929–937.
[11] L. Shi, *Int. J. Biol. Macromol.*, **2016**, *92*, 37–48.
[12] J. Xu, R-Q. Yue, J. Liu, H-M. Ho, T. Yi, *Int. J. Biol. Macromol.*, **2014**, *67*, 205–209.
[13] S. Zhang, X. Li, *Int. J. Biol. Macromol.*, **2018**, *115*, 811–819.
[14] H. Liua, Y. Fanb, W. Wanga, N. Liua, H. Zhanga, Z. Zhua, A. Liu, *Int. J. Biol. Macromol.*, **2012**, *51*, 417–422.
[15] S. Zhang, X. Li, *Int. J. Biol. Macromol.*, **2018**, *115*, 811–819.
[16] X. Ji, F. Liu, Q. Peng, M. Wang, *Food Chem.*, **2018**, *245*, 1124–1130.
[17] Sh. Zhua, J. Hua, Sh. Liua, Sh. Guoa, Y. Jiaa, M. Lia, W. Kong, J. Liang, J. Zhang, J. Wang, *Carbohydr. Polym.*, **2020**, *246*, 116545.
[18] K-ping Wang, J. Wang, Q. Li, Q-lin Zhang, R-xu You, Y. Cheng, L. Luo, Y. Zhang, *Food Res. Int.*, **2014**, *62*, 223–232.
[19] S. Sasidharan, Y. Chen, D. Saravanan, K.M. Sundram, L. Yoga Latha, *Lect. Notes Math.*, **2007**, *1902*, 121–130.
[20] S. Maiti, S. Mukherjee, R. Datta, *Int. J. Biol. Macromol.*, **2014**, *70*, 20–25.
[21] A.B.D. Nandiyanto, R. Oktiani, R. Ragadhita, *Indones. J. Sci. Technol.*, **2019**, *4*, 97–118.
[22] R. Vijayalakshmi, R. Ravindhran, *Asian Pac. J. Trop. Biomed.*, **2012**, *2*, S1367–S1371.
[23] S. Ben Slima, N. Ktari, I. Trabelsi, H. Moussa, I. Makni, R. Ben Salah, *Int. J. Biol. Macromol.*, **2018**, *106*, 168–178.
[24] X. Ji, C. Hou, Y. Yan, M. Shi, Y. Liu, *Int. J. Biol. Macromol.*, **2020**, *149*, 1008–1018.

How to cite this article: Mohamed Aamer Abubaker, Hamouda Adam Hamouda, Lele Chen, Shengyong Zhu, Ji Zhang*. Characterization of chemical structure of water-soluble polysaccharides from Sudanese *Ziziphus spina christi* fruits. *Eurasian Chemical Communications*, 2021, 3(3), 200-209. **Link:** http://www.echemcom.com/article_128059.html