

Modification and Bioactivities of Polysaccharide extracted from *Mangifera Indica* gum (Mango)

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Abstract

Natural polysaccharides own properties like biocompatibility, biodegradability, nontoxic and inexpensive material, gaining attention for biomedical applications. *Mangifera indica* gum is an excellent source of polysaccharides. The present research is aimed to investigate the impact of modification on polysaccharides extracted from *Mangifera indica* gum. Polysaccharide extracted from *Mangifera indica* gum was subjected to modification through the acrylamide grafting method to enhance the functionality of natural polysaccharide. It was noted that for 100 grams of dried mango gum, 12 grams of modified polysaccharide and 15 grams of crude polysaccharide were produced. Characterization techniques such as FTIR was used to determine the functional groups on the structure of polysaccharide. The surface morphology and crystalline structure were elucidated from SEM, EDX, and XRD. The antioxidant and antibacterial activity of native and modified polysaccharides was studied. The results thus obtained were statistically analyzed and reported. The modification of native polysaccharides was expected to find low antioxidant activity after modification but gum polysaccharides did not show any antibacterial activity against *Escherichia coli*, *Pseudomonas sp.*, *Bacillus sp.*, and *Staphylococcus aureus* in both native and modified polysaccharides.

Introduction

Polysaccharides consist of a number of monosaccharides (almost more than 10) linked by glycosidic bonds in branched or unbranched chains, usually referred to as one of the active compounds in medicinal plants (Ching, 2007). The hemiacetal group of one monosaccharide and the hydroxyl group of another monosaccharide join by a glycosidic bond to form building blocks (Clayden et al., 2001). There are many structural or storage-related biological functions of polysaccharides. For instance, storage polysaccharides are starch and structural polysaccharides are glycogen present in the plant's cell walls (Klemm et al., 2005). Polysaccharides are very complex polymers, generally referred to as "glycans". Polysaccharides exhibit different fine structures as "polydisperse molecules" due to the wide range of molecular weights. About 90% of total natural polysaccharides can be found in vegetables (Werpy et al., 2004). Polysaccharides play a critical role in many physiological functions of life and these are universally found in numerous parts of plants (guar gum, cellulose, hemicelluloses, and pectin), seaweed (carrageenan, alginate, and agar-agar), animals (chondroitin, chitin, heparin, and hyaluronan), fungi, and bacteria (Zong et al., 2012). The increasing demand of plant-based products is generating millions of tons of waste increasing the intensity of agro-industrial activities acreage, and agricultural production. Some wastes like bio-agro wastes by horticulture and agricultural activities during cultivation, postharvest, and processing of plants. Such waste biomass is constituted by vegetable transformation residues like exhausted seeds, pulps, peels, and unemployed parts of plants, like roots, cobs, straw, and leaves (Sanchez-Vazquez et al., 2013).

Plant-based polysaccharides have many properties such as low-cost materials, abundant, and renewable. These are non-toxic, biocompatible, and biodegradable polyfunctional, owing chemical reactivity, chelating, and absorptive capacities (Crini, 2005). Plant-based polysaccharides also have some

properties based on different functional groups present in their chemical structure. Some functional groups like primary hydroxyl ($-\text{CH}_2\text{-OH}$) and amino ($-\text{NH}_2$) groups, provide high chemical reactivity toward functionalization (Pillai et al., 2009). Some functional properties of polysaccharides are directly concerned by the primary sequence structure and the space-filling conformations adopted by the biopolymers. Some physicochemical properties of polysaccharides closely related to the biological activities are features of glycosidic linkages (configuration of glycosidic bonding, position of glycosidic bonding, arrangement of monosaccharides), ratios of constituent monosaccharides and molecular size (Ching, 2007). Polysaccharides consist of versatile renewable sources due to their diversity of structures and properties, which could be used as high-performance materials (Pillai et al., 2009).

Mangifera indica (MI), is also known as mango, aam (Shah et al., 2010). According to Ayurveda, different parts of the mango tree are attributed to many medicinal properties (Alaribe et al., 2012). Tannins and flavonoids are the rich components of the plant's organs. Leaves, fruit, seed kernel, gum, fruit pulp, roots, bark, and stem bark extracts are used widely for medicinal purposes in various countries (Nunez-Selles, 2005, Singh et al., 2005).

Natural gums obtained from plants are composed of monosaccharide units linked by glycosidic bonds. Gums are essentially cheap and easily available naturally occurring polysaccharides in plants. They are high molecular weights of hydrophilic carbohydrate polymers. Some gums are insoluble in organic solvents like hydrocarbons, ether, acetone, or alcohol (Goswami & Naik, 2014). Some of them give a viscous solution or jelly to swell up or disperse in cold water and some are soluble or absorb water (Wandrey et al., 2010). Polysaccharide gums are one of the most important industrial raw materials due to their sustainability, availability, biodegradability, and biosafety. 'Gels' are three-dimensional interconnected molecular networks. Mostly natural gums formed this network like gels. There are some factors such as ionic strength, pH, temperature, structure, and concentration that define the strength of the gel (Goyal et al., 2007).

In the present study, the increasing demand for polysaccharides for different industrial applications forces scientists to explore new sources of polysaccharides and improve their properties. So there is a need to modify polysaccharides in order to enhance their functionality and physicochemical properties and form new polymeric derivatives that are useful in a variety of applications. A lot of application of polysaccharides, it is carried out lead to the extraction, modification, and characterization of polysaccharides from the agro-waste *Mangifera indica* gum.

Materials and Methods

Chemical reagents

All chemicals and reagents were obtained from Sigma Aldrich and were of analytical grade (99.99% purity). The chemicals and reagents were mainly comprised of acetone ($(\text{CH}_3)_2\text{CO}$), trichloroacetic acid, ethanol sulphuric acid (H_2SO_4), Molisch's reagent, Fehling's reagent, Benedict's reagent, sodium citrate,

sodium carbonate (Na_2CO_3), Copper sulphate ($\text{CuSO}_4(\text{H}_2\text{O})$), Barfoed's reagent, copper acetate, glacial acetic acid (CH_3COOH), Iodine reagent, acrylamide ($\text{C}_3\text{H}_5\text{NO}$), ceric ammonium nitrate (CAN), DPPH radical, ascorbic acid ($\text{HC}_6\text{H}_7\text{O}_6$), phosphate buffer.

Collection of sample

Dried gum was collected from *Mangifera indica* trees by wounding the stem during June and July. Then *Mangifera indica* gum was isolated from fungal affected sides. It was desiccated, pulverized, and then changed into little sections using a mallet. Small pieces were dried in the shade at room temperature for a continuous one week. The acquired mass was ground in a mill to obtain a dry powder. Then, the dried powder was stored in an airtight closed container.

Extraction and purification of polysaccharide

Extraction and purification of polysaccharides was conducted according to the reported method by Samrot et al., 2021. In brief, native polysaccharide extract was prepared by taking 0.5 g of gum powder in 50 ml of distilled water under heating and then filtered. The polysaccharide was purified with a defatting technique that involved soaking the gum (20 g) in 50 ml of absolute ethanol and leaving it at room temperature for a whole night. A hot air oven (58°C) was used to gather the residue and dry it off. Using a magnetic stirrer, 10 g of defatted gum was measured and continuously stirred for 4 hours to dissolve in 100 ml of distilled water. Muslin cloth was used to filter the solution to get rid of anything that wouldn't dissolve in water. The filtrate was then added to 100 ml of distilled water and agitated for one hour at 100°C . The solution was filtered once more after cooling to room temperature. To precipitate out the proteins in the filtrate, an equal volume of 10% trichloroacetic acid was added. After that, the solution was centrifuged for 10 minutes at 10,000 rpm. Acetone was added in a 1 to 0.5 ratio (supernatant: acetone) after the supernatant had been put into a beaker. For 10 minutes, the solution was centrifuged at 10,000 rpm. The supernatant was put into dialysis tubes and dialyzed for five days against distilled water. After a 5-day dialysis operation, pure polysaccharide was produced.

Modification through Acrylamide grafting

Acrylamide grafting was carried out using microwave-assisted synthesis following the protocol reported by Mishra et al., 2011. Microwave-assisted synthesis of acrylamide grafting polysaccharide in which 1g of polysaccharide was dissolved in 400 ml distilled water using ceric ammonium nitrate (CAN) as the free radical initiator. Then desired amount of acrylamide was mixed in 10 ml water and this mixture was poured into a polysaccharide solution. The solution was mixed thoroughly and poured into the reaction vessel (1000 ml borosil beaker) and a catalytic quantity of CAN was added. The reaction vessel was then set on the microwave oven's tunable. At this point, microwave irradiation was carried out at 800W of power. Periodically, microwave irradiation was stopped just as the reaction mixture began to boil (about 65°C) and the reaction vessel was cooled by submerging in it ice water. This was done in order to minimize the competing reaction of homopolymer synthesis and stop the development of potentially harmful or cancer-causing vapors that could contain acrylamide. Repeating this microwave irradiation cooling cycle up to 3 minutes at a time resulting in the formation of a gel-like substance. The reaction

vessel and its contents were then ultimately cooled and left undisturbed for 24 hours to finish the grafting reactions. Then excess acetone was poured into the gel-like mass. The graft copolymer precipitate that resulted was collected and dried.

Characterization techniques for polysaccharide

3.8.1 FTIR analysis

FTIR was carried out to study the molecular structure and functional groups of polysaccharide gum. Fourier transform infrared (FT-IR) spectroscopy was monitored in the scan region of $4000\text{--}400\text{ cm}^{-1}$. This technique was used to illustrate and validate the information present in polysaccharides *Mangifera indica* gum. The oven-dried crude and modified polysaccharide gum was pressed on an IR press and mixed in KBr. The gum polysaccharide spectrum was verified by using an FTIR spectrophotometer (Perkin-Elmer)

3.8.2 XRD analysis

X-ray diffraction analysis of native and modified polysaccharide gum was performed in order to obtain information about phase transition. The X-ray diffraction pattern of crude polysaccharides and grafted polysaccharides was monitored at temperature 37° by using a technique such as an X-ray diffractometer (PAN analytical X'Pert Pro X-ray Diffractometer). The information was gathered in the 2θ Range $2\text{--}60^{\circ}$ with a particle mass of 0.02° and a calculating time of 5 s/step

3.8.3 SEM with EDX analysis

The surface morphology and elemental composition of native and modified polysaccharide samples were analyzed by SEM with EDX. Scanning electron microscopy of polysaccharide gum samples was recorded by technique (Nova Nano 450) behind the gold covering. Images of gum polysaccharides at various amplification levels such as 20000, 35000, 40000, and 55000 were observed by changeable the hurrying voltage of 15 KV SEM for crude and modified polysaccharides.

Bioactivities of polysaccharide

Antioxidant activity

The free radical scavenging activity of native and crude polysaccharide fractions was calculated by using the procedure highlighted by Raji et al., 2014 with some modifications. DPPH has the capacity to transfer hydrogen atoms using the DPPH assay. For the activity, take 3000 μL of the portion at variation concentrations (50–600 $\mu\text{g/mL}$). Then it was transferred into a beaker and 2000 μL solution was added to 500 μM DPPH and ethanol. The reaction mechanism was shaken and left reaction in room temperature for a period of 30 minutes. UV-VIS spectrophotometer (λ 25, Perin Elmer) was used to measure activity at absorbance 517 nm. Ascorbic acid was used as a standard in this assay. Control was formed by pouring 1 ml of methanol into 2 ml DPPH solution and a blank solution was prepared by using methanol.

The DPPH radical scavenging effect was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \frac{1 - (A - A_i)}{A_0} \times 100\%$$

Here A was the absorbance of the solution and DPPH, A_i was the absorbance of the gum polysaccharide without the DPPH solution and A_0 was the absorbance of the DPPH reaction.

Antibacterial activity

Antibacterial activity of native and modified polysaccharides was done by using the agar well diffusion method as reported by (Balouiri et al., 2016). The agar well diffusion method was used for the determination of the antibacterial activity of polysaccharides. Native and modified polysaccharides were tested against two Gram-negative bacteria (*Pseudomonas* sp. and *Escherichia coli*) and two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus* sp.) a cork borer was used to punch holes in the nutrient agar and broth culture was swabbed over the entire piece of agar. After that four various concentrations (100 µg, 200 µg, 300 µg, and 400 µg) of native and modified polysaccharides were put into the wells. As a positive control, a 5 g antibiotic disc of ciprofloxacin was employed, while one well was loaded with distilled water as a negative control. Then incubation of the agar plates was placed overnight at 37°C. The zone of inhibition was calculated after 24 hours.

Statistical analysis

The information obtained from chemical analysis, physicochemical analysis, and antibacterial, and antioxidant activities were reported as $M \pm SD$. Further, one-way ANOVA was performed to investigate statistical differences ($p < 0.05$).

Results

Extraction

Gum polysaccharide was brown in color, tasteless, odorless, and irregular in shape. It was observed that crude gum polysaccharides are soluble in hot water and slightly soluble in cold water. The observation showed that the crude *Mangifera indica* gum (MIG) polysaccharide contained galactose, furfural, and mannose content in the desired amount. The extraction process yielded 15 grams of polysaccharide per 100 grams of dried *Mangifera indica* gum.

Phytochemical screening tests were observed to detect the nature of *Mangifera indica* gum polysaccharide. The different carbohydrate contents such as flavonoids, phenolic, tannins, and alkaloids were found to be absent in gum polysaccharides. The other phytochemical screening tests were performed. The details of those tests are discussed briefly in the table given below

Table 1
phytochemical observations

Phytochemical test	Observations
Molisch's Test for carbohydrate	+
Fehling's Test for Reducing and Non Reducing sugar	
Benedict's Test for Reducing Sugar	
Barfoed's Test for monosaccharide	
Iodine test for starch	

Influence of Grafting Reaction

The yield of modified polysaccharide obtained around 12 grams per 100 grams of dried *Mangifera indica* gum. The modified *Mangifera indica* gum polysaccharide was generated via the reaction of acrylamide on the backbone of the polysaccharide under microwave irradiation (800 W). The microwave irradiation was carried out at different time intervals. Graft copolymerization was preferred because gum polysaccharides have high exposure to the grafting mechanism. It was observed that the grafting reaction occurred with the addition of the acrylamide group, and the reaction rate increased by enhancing revelation time to microwave irradiation about 4 minutes stepwise. After that, polar functional groups such as the OH group was broken down by the attack of free radical and the formation of a homo-polymer takes place. Grafted polysaccharides have a larger aqua phobic and less viscosity compared to native gum polysaccharides. It was concluded that modified gum polysaccharide was observed to be a useful way to enhance the efficacy, selectivity, productivity, and applications of natural polysaccharides (Anjum et al., 2015). These observations are closely related to the information reported by Anjum et al., 2015.

Characterization of polysaccharide

FTIR analysis

FTIR technique was monitored to obtain information on functional groups and the molecular structure present in MIG polysaccharides. The FTIR spectra of gum polysaccharides were recorded in the region of 4000- 400cm⁻¹. The FTIR spectrum of the *Mangifera indica* gum was observed at a peak of 2871 cm⁻¹ due to C-H stretching vibration of -CH₂ groups and some other peaks of O-H stretching vibration at 3327 cm⁻¹. The peak at 1703 cm⁻¹ represented the stretching of C = O groups. Normal polymeric O-H stretching vibrations at 3127 cm⁻¹ and 3106 cm⁻¹ and the samples represented H bonded or O-H stretching vibration at 3327 cm⁻¹ and owing to the presence of C = O groups is shown in Fig. 1a. On the other hand, after modification, the observed peaks were changed. Along with those previously observed peaks, polyacrylamide grafting of gum polysaccharide exposed some of the additional peaks. NH stretching frequency of the NH₂ group in the case of polyacrylamide showed an absorption band at 3350

cm^{-1} . Two strong bands of Amide I (CO stretching) and amide II (NH bending) bands at 1622 and 1558 cm^{-1} respectively, C-N stretching band at 1423 and N-H wagging in the range of 750 – 610 cm^{-1} shown in Fig. 2. In the spectrum (Fig. 1b) band with the maximum 3327 is due to the stretching vibration of OH and NH_2 . These peaks demonstrated that cross-linked grafting took place. This spectrum observed similar peaks as discussed in the previous literature (Iqbal et al., 2020).

SEM analysis

Scanning electron microscopy (SEM) was monitored to detect the morphology and texture of native and modified *Mangifera indica* gum polysaccharides. From the spectrum, it was confirmed that native MIG polysaccharides have an irregular and amorphous surface (El-Din *et al.*, 2018) shown in Fig. 2 (a, b). On the other hand, modified MIG polysaccharide was analyzed differently in structure compared to native MIG polysaccharide. The addition of the acrylamide group altered the morphology of polysaccharides. The images of modified MIG polysaccharide showed that the morphology of polysaccharide are slightly regular and there are large-sized pores due to interlinkages. The porosity enables the fostering of swelling and shows a hydrophilic nature. The modification of gum polysaccharide was analyzed with a regular structure and as a consequence, less viscosity of the aqueous sample was obtained. This information was relevant to the SEM images depicted in previous literature (Samrot et al., 2020). The SEM analysis of modified MIG polysaccharide is shown in Fig. 2 (c, d)

EDX analysis

Electron dispersive spectroscopy (EDX) was analyzed to identify the different elements analysis of MI gum polysaccharide. From the spectrum, the percentage composition of elements was determined. The native MIG polysaccharides consist of a high percentage of carbon (59.53%) and oxygen (53.09%). Other trace elements such as magnesium Mg, potassium K, and calcium are also present in minute percentages which showed the impurities shown in Fig. 3a. While after modification the percentage of gum polysaccharide was changed. From the spectrum, it was analyzed that the modified MIG polysaccharide contains a percentage of carbon (58.76%) and oxygen (49.57%). So it was concluded that after modification new derivatives were formed and showed variation in spectrum shown in Fig. 3b. These results showed similarity with the existing information in the literature (Samrot et al., 2020).

XRD analysis

X-ray diffraction (XRD) was the potent nontoxic technique for analyzing crystalline phase. Native and modified gum polysaccharides exposed variable properties or well-defined structures. Crude MIG polysaccharide has an amorphous structure and two peaks were examined at scattering angle (2θ) at 12.7° and 16.9° as depicted in Fig. 4a. Modified MIG polysaccharide was observed as low crystallinity that resulted due to intramolecular linkage and shift in the crystallinity pattern, because after modification structure of polysaccharides changed and new derivatives formed. So the XRD pattern of modified polysaccharide was recorded at an angle (2θ) consisting of the less intense peak at 13.0° and the

dominant peak at 27.5 ° (Hinz et al., 2007). Moreover, it was concluded that the modification moderately enhanced the crystallinity of MIG polysaccharides compared to native polysaccharides shown in Fig. 4b.

Bioactivities of polysaccharide

Antioxidant activity

According to calculation, DPPH scavenging activity was enhanced by increasing in concentration of both native and modified polysaccharides. The concentration at which the maximum percentage scavenging was found to be 100 (µg/ mL). The antioxidant activity of standard ascorbic acid was notably higher than crude and modified polysaccharides. A higher scavenging effect was observed in modified polysaccharides when compared with crude polysaccharides (figure). However, the scavenging activity of modified polysaccharides was observed as not significant due to the big difference in ascorbic acid scavenging percentage. Similar results were calculated by Samrot et al., 2021 who tested *Mangifera Indica* gum, in which the scavenging activity of crude polysaccharide was significantly lower than the control and magnified polysaccharide (Samrot et al., 2016). Samrot *et al.* conducted an experiment on *Azadirachta indica* gum, where the scavenging activity of the extracted polysaccharide was noticeably lower than the control. Additionally, Samrot *et al.* showed that a water extract of *Ficus iyrata* gum has substantial free radical activity (Omar et al., 2015).

Antibacterial activity

The lack of a zone of inhibition indicated that native polysaccharide was not able to inhibit the four organisms tested in this study. This study showed that antibacterial activity was not present in polysaccharides extracted from *Mangifera Indica* gum, because lack of bioactive compounds such as polyphenols, flavonoids, and tannins in MI gum. In this study, it was also observed that Modified polysaccharide has no antibacterial activity. However, another study demonstrated that the leaves of MI have antimicrobial activity due to their rich content of bioactive compounds (Samrot et al., 2016). A study showed by Yahia *et al.*, that due to significant amounts of bioactive components such as polyphenols, flavonoids, and tannins, in *Ziziphus lotus* and *Ziziphus mauritiana L.* leaves extract have strong antibacterial activity (Yahia et al., 2020). In contrast to the findings of this investigation, it has been shown that a number of plant species' gums have antibacterial properties. At a concentration of 20 g/ml, polysaccharides from *Azadirachta indica* gum can inhibit *E. coli* from growing (Samrot et al., 2020).

Conclusion

In summary, polysaccharide was extracted from the *Mangifera indica* gum and purified. The extraction process was done by using a chemical method. The extracted polysaccharides had some drawbacks, in order to overcome this, the process of modification such as acrylamide grafting was performed. After modification, new derivatives were formed which show better biological applications as compared to native polysaccharides. Further, a phytochemical screening test was observed in order to confirm the

presence of carbohydrate content. Both forms of polysaccharides were characterized using FTIR, SEM, EDX, and XRD. Native and modified polysaccharides exhibited weak antioxidant activity and neither of them showed antibacterial activity against organisms.

Declarations

Conflict of interest

The authors declare that there is no conflict of interest.

Author Contribution

first author did all the experimental work and writeup. second author proof read it.

Acknowledgment

None

Data availability

All datasets generated or analyzed during this study are included in the manuscript

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Figures

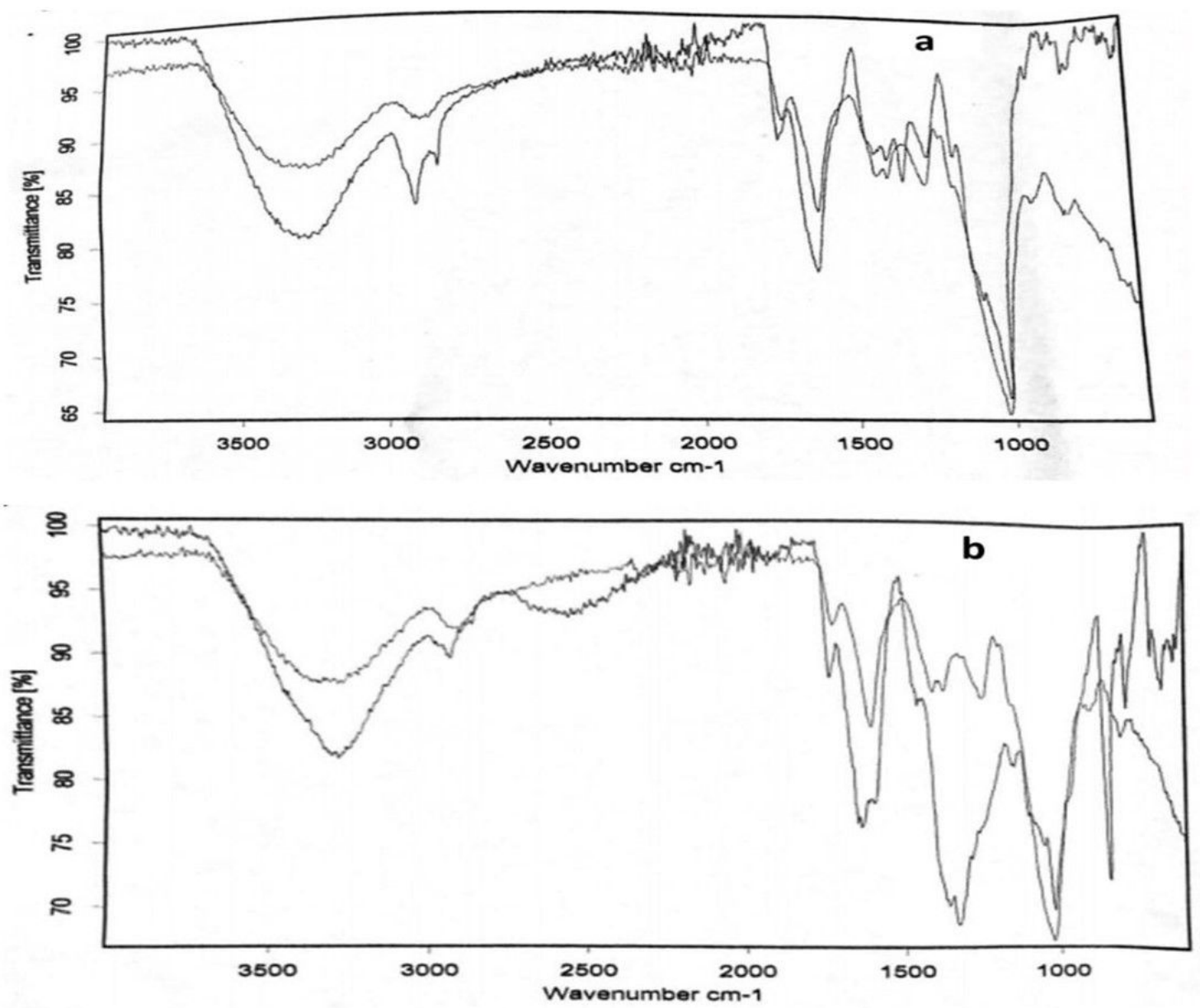


Figure 1

a) FTIR spectrum of native gum polysaccharide **b)** Modified polysaccharide

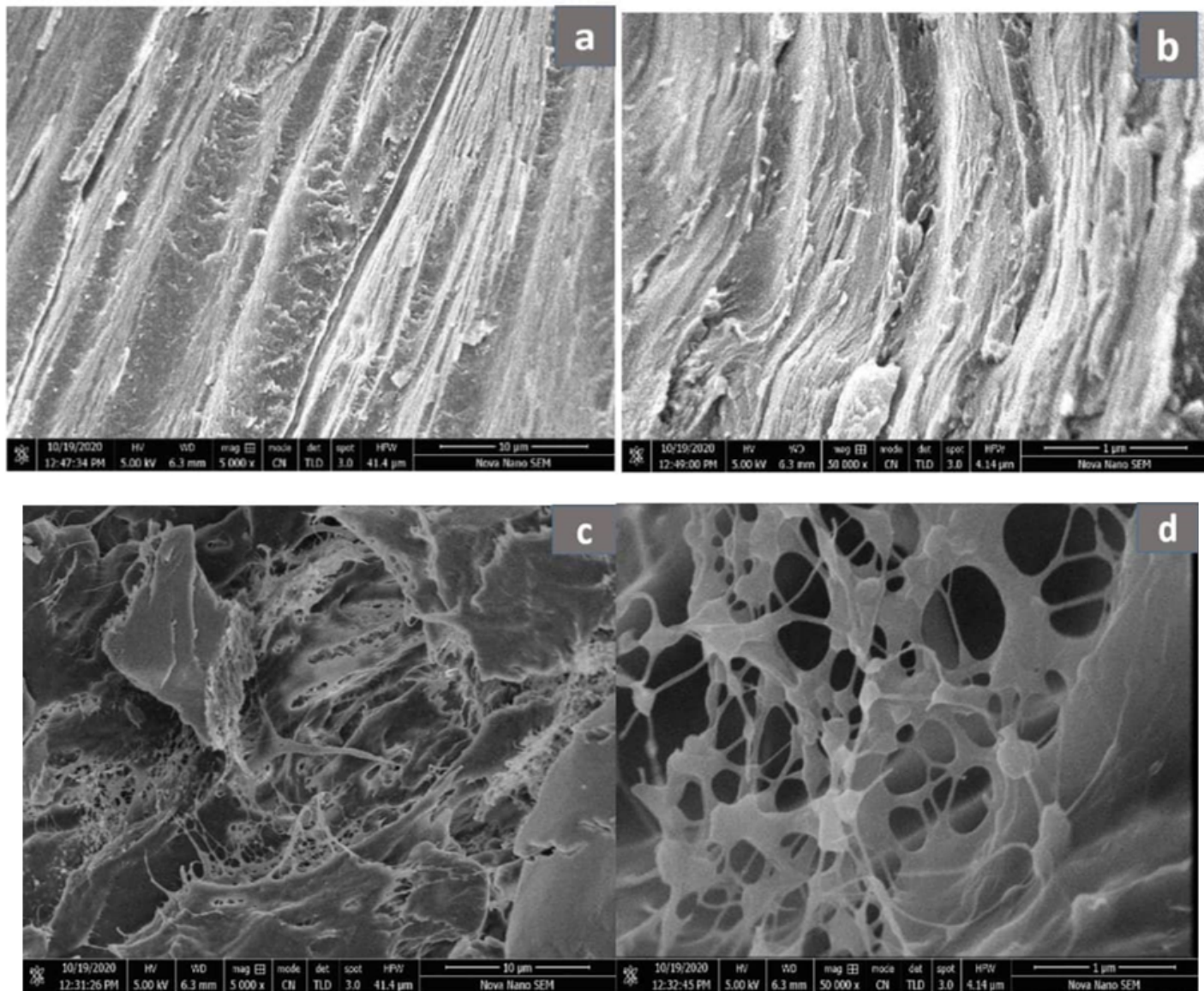


Figure 2

a, b) SEM images of native gum polysaccharide c, d) Modified polysaccharide

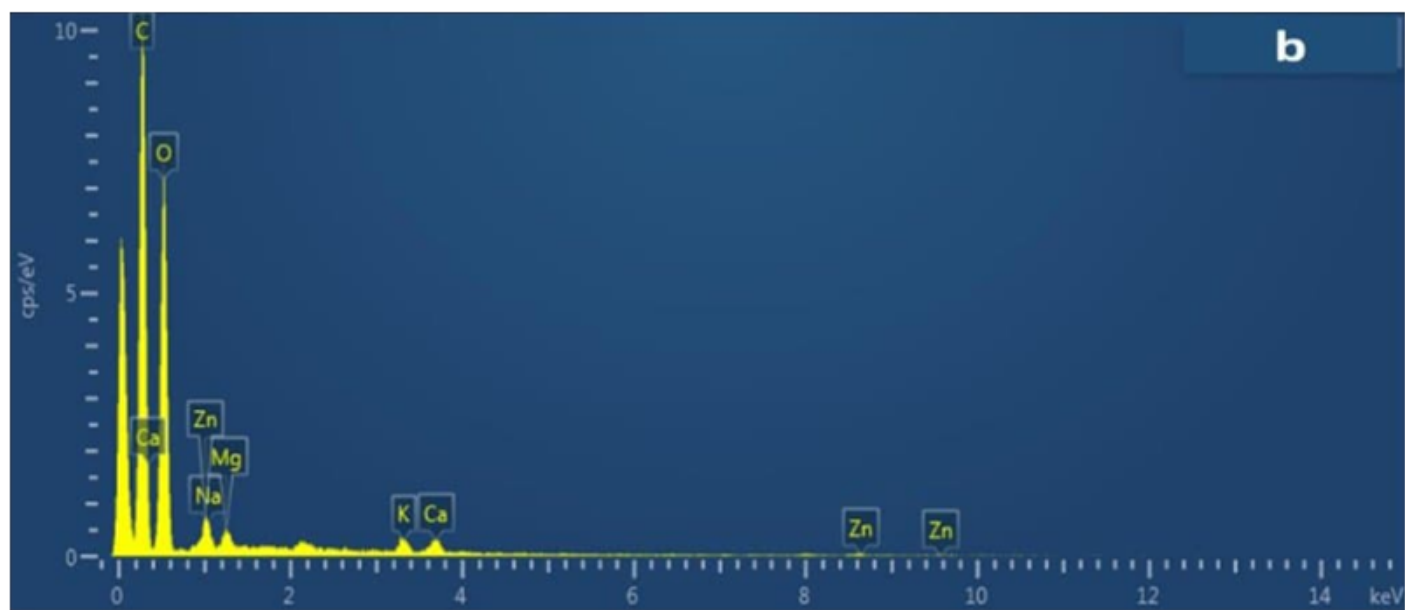
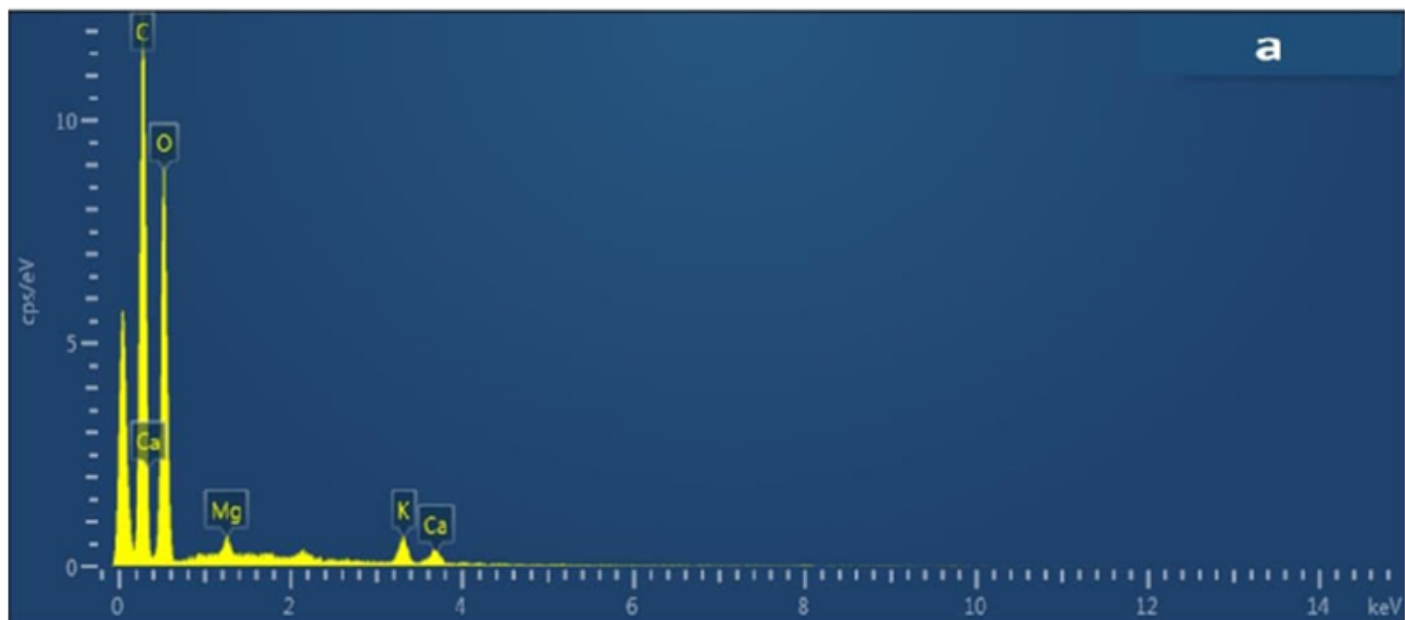


Figure 3

a) EDX spectrum of native polysaccharide **b)** Modified polysaccharide

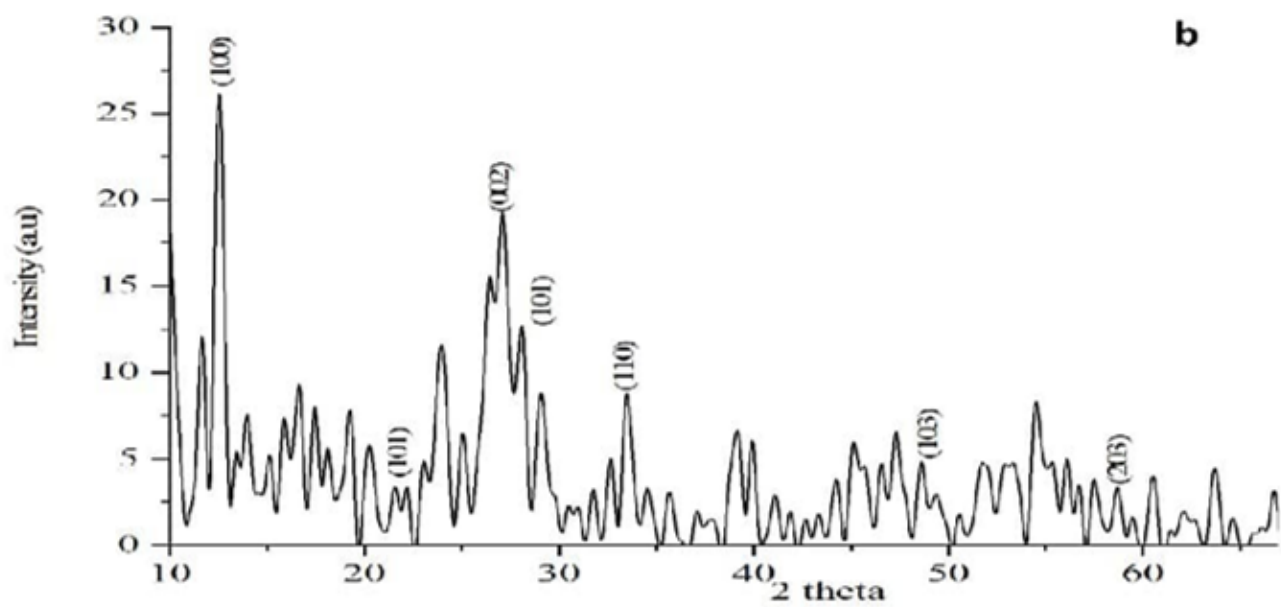
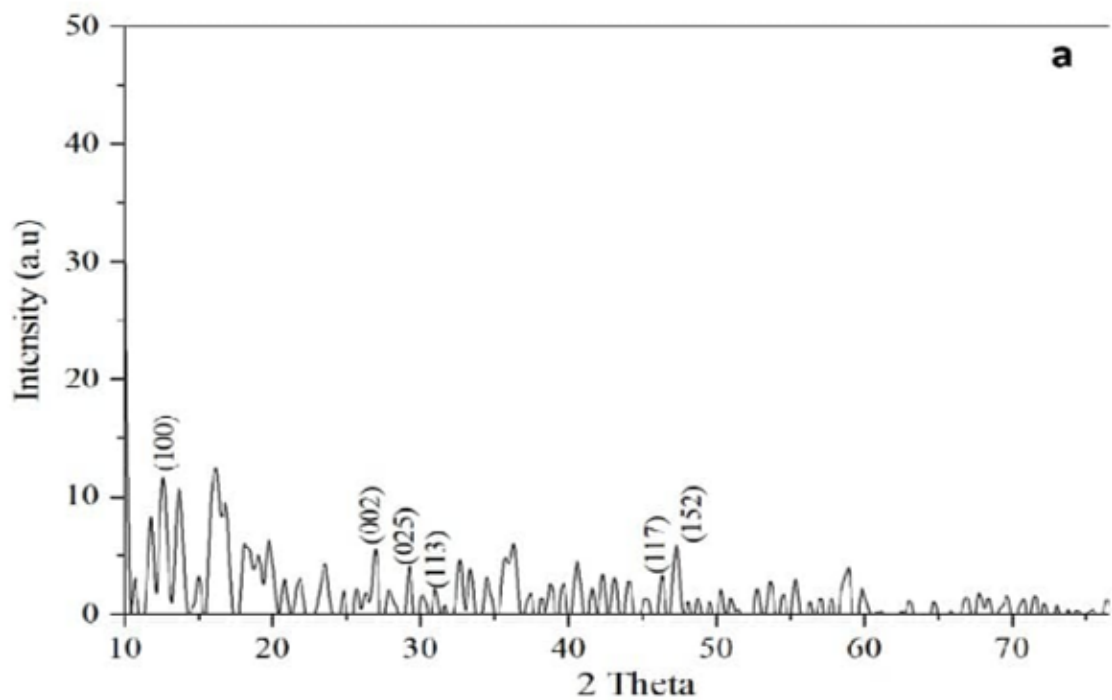


Figure 4

a) XRD spectrum of native gum polysaccharide b) Modified gum polysaccharide

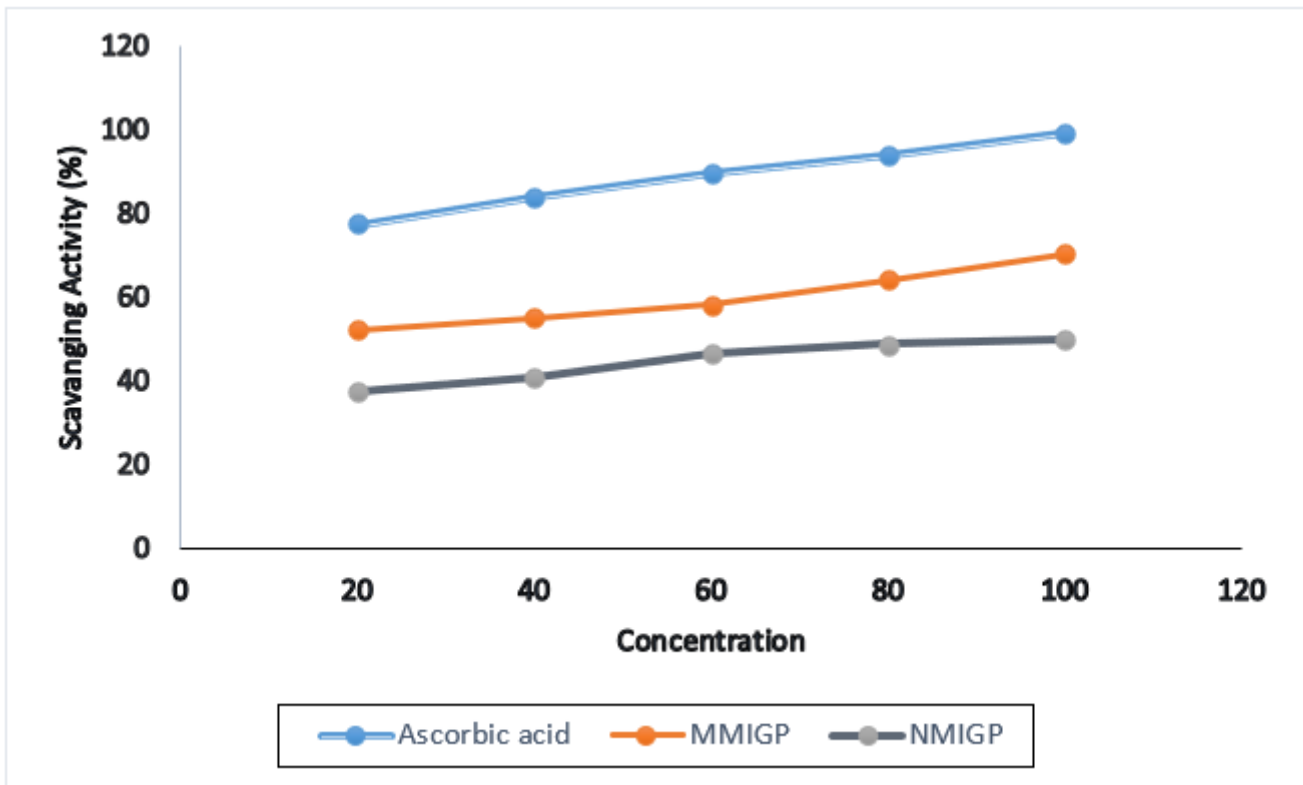


Figure 5

DPPH antioxidant activity. Data in the form of mean $\pm$ SD, $p < 0.05$, $p < 0.01$