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Research Paper

Chitosan-based nanoparticles of *Moringa oleifera* with enhanced bioactivities

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Abstract

Moringa oleifera is also known as a miracle tree because of its nutritional and physiological benefits. The objective of the current research was to assess the bioactivity efficacy of chitosan-based nanoparticles as compared to crude extracts. To understand does chitosan encapsulation enhances the bioavailability of *Moringa oleifera* phytochemicals, the microwave-assisted method was used to prepare the *n*-hexane, methanolic, and aqueous extracts of *M. oleifera*. Biochemical characterization was done by antioxidant (total phenolic content, total flavonoid content and DPPH radical scavenging assay), anti-diabetic (alpha amylase inhibition assay), cytotoxic (percentage hemolysis) and anti-inflammatory (albumin denaturation method) activities. Chitosan-based nanoparticles of methanolic extract were prepared using the ionic gelation method. One-way ANOVA was used to analyze the data. Chitosan-based nanoparticles of methanolic extract were almost 100nm in size with a positive charge. A comparison of bioactivities revealed that nanoparticles were less toxic (1.99±0.86%) with higher antioxidant (65.67±0.26%), anti-diabetic (85.87±6.64%) and anti-inflammatory potential (83.55±0.76%) than extracts. Different functional groups in extracts were identified by Fourier transform infrared spectroscopy such as primary and secondary amines, amides, carboxylic acid, alkanes, aldehyde, alkene, alcohol, ether, ester and sulphates. High-performance liquid chromatography confirmed the presence of kaempferol, p-coumaric acid, salicylic acid and chlorogenic acid. These functional groups were involved in bioactivities as well as in chitosan polymerization. It is concluded that nanoparticles showed enhanced therapeutic activity as compared to extracts. Further research to optimize the process in animal models is warranted to determine potential applications.

Introduction

Approximately 80% of people worldwide rely only on traditional medicine which makes medicinal plants a vital part of human healthcare systems in many regions (Asigbaase *et al.,* 2023). Numerous higher plants are grown in various parts of the world in order to extract valuable compounds for use in the medical field (Salmeron *et al.,* 2020).

Moringa oleifera, commonly referred to as "drumstick," (family *Moringaceae*) has been used for culinary and medicinal purposes. Its phytochemicals such as carbamates, thiocarbamates, nitriles, glycosidic glucosinolates and isothiocyanates, confer anti-inflammatory, antioxidant, antihyperglycemic, antihypertensive and chemopreventive effects. Moringa roots are used in Ayurvedic medicine to treat anxiety, hysteria and epilepsy (Kaur *et al.,* 2023; Younas *et al.,* 2023). As an unorthodox feed, *Moringa oleifera* can enhance the growth, reproduction, immunity, quality of animal products and boost the financial rewards of breeding (Jiang *et al.,* 2023).

Nanoparticles have gained popularity as they can exhibit different yet enhanced properties as compared to their large counterparts. A combination of plant extracts and natural biopolymers like chitosan excel in performance (Namakka *et al.,* 2023). *M. oleifera* extract-capped hydroxyapatite nanorods and metal-based nanoparticles showed excellent antifungal, anti-inflammatory and antioxidant activity (Kalaiselvi *et al.,* 2018; Ahamad *et al.,* 2023; Muhammad *et al.,* 2023).

There is limited research available on chitosan-based nanoparticles of *Moringa oleifera.* Although the literature review highlights various studies on chitosan polymerized nanoparticles and *Moringa oleifera* extracts, research combining both is limited. The objective of the current research, potentially the first of its kind, was to assess the bioactivity efficacy of chitosan-based nanoparticles as compared to crude extracts. To understand does chitosan encapsulation enhances the bioavailability of *Moringa oleifera* phytochemicals, numerous bioactivities and structural characterization by zeta sizer, zeta potential, FTIR and HPLC were done.

Materials and Methods

Sample Collection

Collection of plant sample was done from UAF. Identification and authentication of plant was done from the Department of Botany, University of Agriculture, Faisalabad, Pakistan. The voucher specimen number given by the Department of Botany, University of Agriculture, Faisalabad, Pakistan was 209-2-2023 dated on 14-9-2023.

Preparation of Plant Extract

After drying, the plant was ground to a fine powder. Extraction was carried out by using distilled water, methanol and *n*-hexane as a solvent. Beakers containing samples and solvent at 1:5 were enclosed with aluminium foil and heated for 30s by using microwave and let it cool down, repeated this process thrice before separation of extracts by filtration. Then filtered samples were placed at room temperature to evaporate solvent. Percentage yields were calculated and the extracts were used for further evaluation of bioactivities (Noreen *et al.*, 2020).

Preparation of Nanoparticles

Chitosan is a heteropolysaccharide whose molecular weight is 10-20KDa. The source of chitosan was a crab shell (9012-76-4 CAS number). Chitosan-based nanoparticles of methanolic extract were prepared using the ionic gelation method. For the preparation of nanoparticles, 25mL of 3% acetic acid and 50mg chitosan were taken in a clean beaker and placed on a hot plate magnetic stirrer at 25^{o}C and 1500rpm. When chitosan was properly dissolved, then 15mg of tripolyphosphate was added to it. When the mixture was homogenized, 50mg of methanolic extract was dissolved in 1mL of methanol and then added dropwise with the help of an insulin syringe. The resulting mixture was placed on a hot plate magnetic stirrer for 3 to 4 hours at 40^{o}C. Now these nanoparticles were centrifuged at 14000rpm (15 minutes) at 25^{o}C. The supernatant was separated and the pellet (nanoparticles) was vortexed along with distilled water and sonication was done (3 hours) to get a homogenized sample (Hannan *et al*., 2023).

Extracts and nanoparticles were used for various biochemical assessments as given below.

Antioxidant Profile

The following methods were used:

Total Phenolic Content (TPC)

Briefly, Na_2CO_3 solution (100 µL), test samples (25 µL) and folin-ciocalteu reagent reagent (125 µL) were mixed and incubated for two hours. Then 200 µL of solution was taken in an ELISA microtiter plate. At 765 nm, the absorbance was taken. TP contents were expressed as mg GAE /mL (Hussain *et al.,* 2021a).

Total Flavonoid Contents (TFC)

For the assessment of TFC, the mixture of test samples (25 µL each), 75 µL methanol and 140 µL distilled was incubated for 10 minutes. Then 5 µL of 20% $AlCl_3$ and 5 µL of 1M potassium acetate were added to the solutions and incubated for almost 5 minutes. Absorbance at 415 nm was used to measure TFC as µg CE/mL (Hussain *et al.*, 2021b).

DPPH Radical Scavenging Assay

The ability to scavenge 2, 2-diphenyl-l-picrylhydrazyl (DPPH) was used to assess antioxidant capability. Incubation for 30 minutes was done to a mixture of 250 µL of 0.004% DPPH solution and 2.5 µL test sample. Absorbance was observed at 570nm. Ascorbic acid was control. It was expressed as % inhibition (Hussain *et al*., 2021c). The radical scavenging capabilities were calculated as:

$$\frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

Antidiabetic Evaluation

The antidiabetic activity was assessed by alpha-amylase inhibition assay. The source of alpha amylase was *Bacillus subtilis*. In a 96-well plate, 10µL of samples (smpl) and 10µL enzyme solution were added and incubated it for 10 minutes. Starch solution (1%) was added and incubated for 30 minutes. Then 10µL 1M hydrochloric acid and 40 µL iodine solution were added. Acarbose standard solution (90 µL of 1%w/v) was considered as a positive control (cntrl). The absorbance was measured at a wavelength of 570 nm using an ELISA reader. It is expressed as % inhibition (Hussain *et al.* 2021d). Percentage inhibitory activity was calculated as:

$$\frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

Cytotoxicity Activity - Hemolytic Assay

The cytotoxicity of samples (S) was assessed by evaluating their hemolytic activity. Blood samples (3mL) were centrifuged for five minutes at 1000 rpm to isolate liquid and cellular portions. The pellets were washed thrice with phosphate buffer saline (PBS; pH 7.4) and centrifugation was done (1000 rpm, 5 minutes) and 1.3 mL PBS was added to maintain volume. The test sample (20 µL) and this solution (180 µL) were incubated (30 minutes) at 37 °C. The samples were centrifuged for 3 minutes at 1000 rpm. The supernatant (100 µL) and PBS (900 µL) were mixed and absorbance (Ab) was noted at 576 nm (Shahzadi *et al.,* 2019). The positive control (PC) for the test was 0.1 percent Triton X-100 and the negative control

(NC) was PBS. Hemolytic activity was expressed as: % hemolysis = [Ab (S) – Ab (NC)/Ab (PC)] ×100

Anti-inflammatory activity

BSA (bovine serum albumin) denaturation method was used for this activity. In the test tube, 2 mL (1%) BSA and 20µL test samples (S) were mixed. In the negative control, 20 µL PBS (phosphate buffer saline), *n*-hexane, methanol and distilled water were taken instead of test samples. In positive control (C), 20 µL of diclofenac sodium was taken instead of the sample. After incubation (20 minutes) at 37°C, samples were given heat treatment in a water bath at 72°C (5 minutes). Absorbance (Ab) was noted at 255nm (Williams *et al.,* 2008). It was expressed as inhibition%: [Ab (C) – Ab (S)/Ab (C)] x 100

Structural Characterization of Extracts

Fourier Transform Infrared Spectroscopy (FTIR)

The structural characteristics were described using FTIR. A Bruker Tensor 27 FTIR spectrometer was used to conduct the FTIR analysis. The sample was finely powdered with KBr (potassium bromide), converted to pellets by compression dye then observed in the range of 400 - 4000 cm^{-1} (Khalid *et al.,* 2023).

High-Performance Liquid Chromatography (HPLC)

For HPLC in the High-Tech laboratories, University of Agriculture, Faisalabad, the dried and hydrolyzed samples were used. A mixture of 0.5g sample with 40 mL of methanol containing 2g/L BHT was used. Then 10mL 1M hydrochloric acid was added and ultrasonification was done for 5minutes. In a water bath, the mixture was then incubated for two hours at 90^{o}C. Sample (20µL) was injected and at 280 nm reading was noted with UV-visible detector (model SPD-10AV). For the analysis, gradient HPLC with mobile phase of acetic acid and acetonitrile was used. Column was Shim-Pack CLC-ODS (Shimadzu, Japan. Several compounds were identified using their retention time (Khalid *et al.,* 2023).

Structural Characterization of Nanoparticles

In morphological characterization and particle size analysis, photon correlation spectroscopy was used to measure the size (polydispersity index) and charge (Zetaanalyzer, Beckman Coulter Inc., Delsa Nano 4C). The size distribution study was carried out using

samples diluted with distilled water (0.2 m filter, Minisart, Germany) at a scattering angle of 90 degrees and at a temperature of 25 degrees. A sample of the nanoparticle suspension (5–10 µL) was placed onto copper grids covered with formvar. The samples were dried with 2 percent w/v phosphotungstic acid after they had completely dried. The picture acquisition and analysis, which included particle size, were done using litesizer and soft imaging viewer software at Hi-Tech Laboratories, University of Agriculture Faisalabad (Jahan *et al.* 2016; Akhtar *et al*., 2020).

Data analysis

Data (n=3) is expressed as mean ± SE or percentage (%). Analysis was performed by using Minitab (version 21) statistical software.

Results

Yield of extracts

After the preparation of plant extracts, percentage yields were calculated. The highest percentage yield (8.86%) was observed in methanolic extracts. The yield of *n*-hexane and aqueous extract was 2.54% and 8.42% respectively.

Characterization of nanoparticles

Nanoparticles were characterized through zeta sizer and zeta potential by using litesizer 500. According to zeta sizer, the nanoparticles were 100nm in size as shown in Figure 1 and according to the zeta potential; nanoparticles were positively charged with 10.5mV potential as shown in Figure 2.

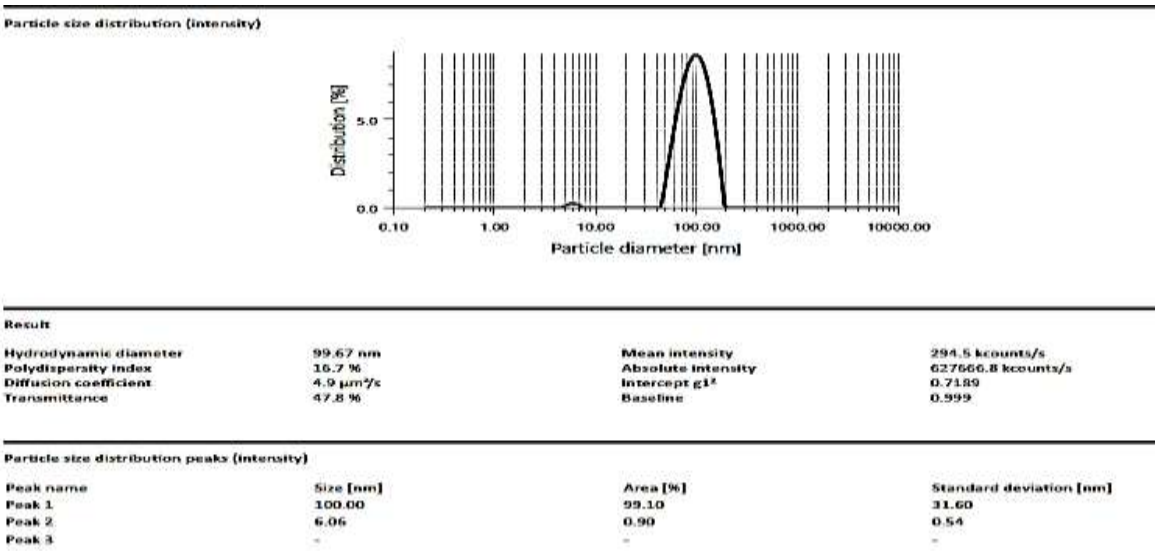

Figure 1: Results of zeta sizer

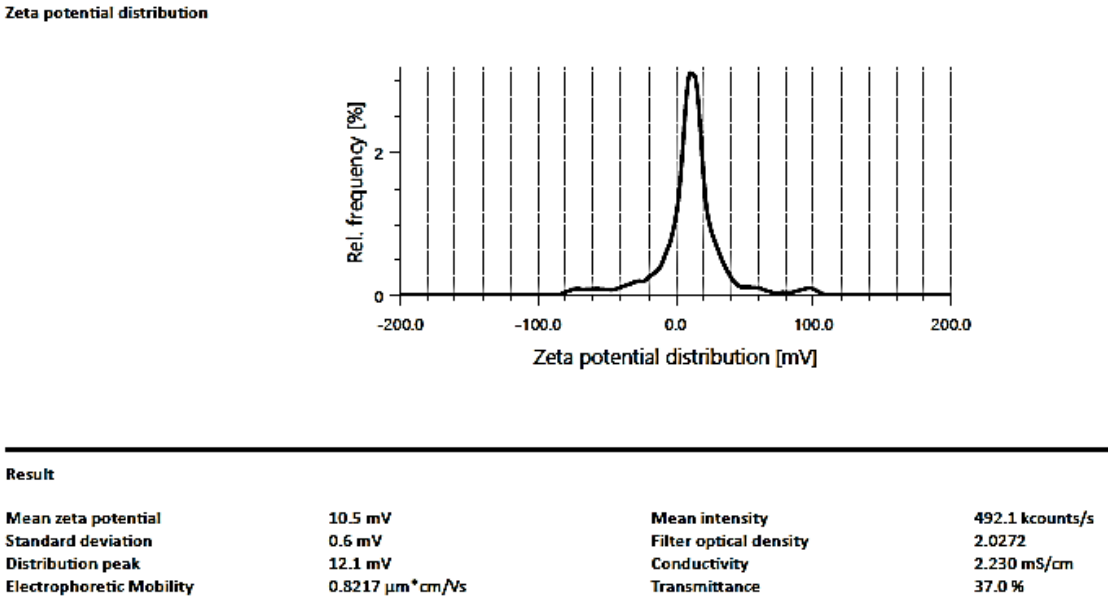

Figure 2: Results of zeta potential

Antioxidant profile

Antioxidant activity was assessed by TPC, TFC and DPPH radical scavenging assay. The observed results showed that nanoparticles had the highest antioxidant potential and among the extracts methanolic extract showed highest antioxidant potential as shown in Table 1.

Antidiabetic evaluation

Alpha amylase inhibition assay was used to assess anti-diabetic activity. The nanoparticles had higher antidiabetic potential than the extracts, while, among the extracts, aqueous extract showed highest antidiabetic activity (Table 1).

Cytotoxicity - Hemolytic activity

Hemolytic activity was determined by the percentage hemolysis which showed that the hemolytic activity of nanoparticles was the lowest. Among the extracts, methanolic extract showed lowest percentage hemolysis and *n*-hexane extract had highest percentage hemolysis because of toxicity present in *n*-hexane solvent (Table 1).

Anti-inflammatory activity

Anti-inflammatory activity was determined by BSA (Bovine serum albumin) assay (Table 1). Nanoparticles showed highest anti-inflammatory activity. Among the extracts, *n*-hexane

and methanolic extracts showed highest anti-inflammatory potential while least anti-inflammatory effect was showed by an aqueous extract.

Table 1: Comparative assessment of bioactivities by extracts and nanoparticles

Activities	Samples				
		Extracts			Nanoparticles
	Control	*n*-hexane	Aqueous	Methanol	
Antioxidant profile					
TPC (mg GAE /mL)	-	84.96±0.18	212.96±0.48	453.14±2.44	480.47±0.10*
TFC (µg CE/mL)	-	407.76±0.69	62.32±0.15	591.18±0.45	671.71±0.69*
DPPH (%)	97.16±0.13	22.37±2.13	23.52±0.35	26.74±1.49	65.67±0.26*
Anti-diabetic profile (% inhibition)					
Alpha-amylase inhibition (%)	88.01±3.97	47.19±2.38	65.99±3.02	40.60±3.80	85.87±6.64*
Cytotoxicity (% inhibition)					
Hemolytic activity (%)	90.50±0.08	44.08±1.21	7.50±0.30	2.81±0.44	1.99±0.86*
Anti-inflammatory (%)	90.10±0.50	80.34±1.57	9.09±4.54	67.11±1.53	83.55±0.76*

Data are represented as mean ± SE or percentage (n=3). * Significant at $p < 0.05$. Controls: DPPH assay: ascorbic acid, alpha-amylase inhibitory assay: acarbose, hemolytic assay: Triton X-100, anti-inflammatory activity: diclofenac sodium.

Structural characterization

Fourier-transform infrared spectroscopy

When compared to a typical spectrophotometer, FTIR offers an infrared (IR) spectrum. It is used to identify several functional groups and it determines the sample material's structure. FTIR analysis results are given in Figure 3 and Table 2.

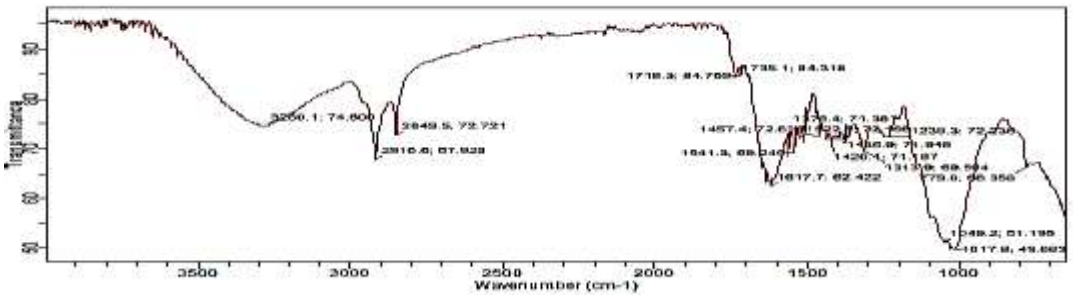

Figure 3: FTIR spectra of *M. oleifera*

X-axis: Absorption frequency (wavenumber) as cm^{-1}. Y-axis: Transmittance as percentage.

Table 2 FTIR spectra for *Moringa oleifera*

Sr. No	Characteristic Peak	Compounds	Functional group	Sr. No	Characteristic Peak	Compounds	Functional group
01	3280.1	Alcohols, phenols H Bounded Carboxylic acid Primary and secondary amines	O-H C=O N-H stretch	10	1436.9	-CH_3	C-H bend
02	2916.6	Alkane	C-H stretch	11	1420.1	bend CH3 Nitro(R-NO2)	C-H N=O
03	2849.5	Aldehyde	C-H	12	1375.4	bend CH3 Nitro(R-NO2) Sulfones, sulfonyl chlorides, sulfates, sulfonamides	C-H N=O S=O
04	1735.1	Carbonyl group	C=O	13	1313.9	Amines Sulfones, sulfonyl chloride, sulfates. Sulfonamides, fluoride	C-N S=O C-X
05	1718.3	Carbonyl group	C=O	14	1239.3	Alcohols, ethers, esters, carboxylic acid, anhydrides Sulfones, sulfonyl chloride, sulfates. Sulfonamides Fluoride	C-O S=O C-X
06	1617.7	bend primary and secondary Amines and amides carbonyl group	N-H C=O	15	1049.2	Alcohol, Ethers, Esters, Carboxylic acid, Anhydride Amines	C-O C-N
07	1541.3	Aromatic Nitro (R-NO_2)	C=C N=O	16	1017.6	Alcohol, Ethers, Esters, Carboxylic acid, Anhydride Amines	C-O C-N
08	1522.6	Nitro(R-NO2)	N=O	17	779.0	Chloride, Alkenes, Aromatic bend	CX
09	1457.4	Nitro (R-NO_2)	N=O				

Table 2 has the absorption values for the *Moringa oleifera*'s various functional groups, a

peak at 3280.1cm^{-1} showed the presence of alcohols, phenols, H-bounded compounds, carboxylic acid and amines. Peaks at 2916.6 cm^{-1}, 2849.5 cm^{-1} and 1718.3 cm^{-1} showed the presence of alkane, aldehyde, carbonyl group respectively. The peak at 1735.1 cm^{-1} also showed the presence of carbonyl group. The presence of bend CH_3, nitro(R-NO_2), sulfones, sulfonyl chlorides, sulfates, sulfonamides were indicated at band 1375.4 cm^{-1}. Peak at 1457.4 cm^{-1} showed the presence of nitro group while the peak at 1541.3 cm^{-1} showed the presence of aromatic and nitro group. Bend primary and secondary amines and amides and carbonyl group were indicated at 1617.7 cm^{-1} band while alcohol, ethers, esters, carboxylic acid, anhydride and amines were indicated at 1017.6 cm^{-1}. A peak at 1049.2 cm^{-1} showed the presence of alcohol, ethers, anhydride and amines while a peak at 779.0 cm^{-1} showed the presence of chloride, alkenes and aromatic bend. Amines, sulfones, sulfonyl chloride, sulfates, sulfonamides and fluoride were indicated at 1313.9 cm^{-1} band while bend CH_3 and nitro group were indicated at 1420.1 cm^{-1} band. The peaks at 1436.9 cm^{-1} and 1522.9 cm^{-1} showed the presence of CH_3 and nitro group respectively. Alcohols, ethers, esters, carboxylic acid, anhydrides, sulfones, sulfonyl chloride, sulfates, sulfonamides and fluoride were indicated at 1239.3 cm^{-1} band.

High Performance Liquid Chromatography

The results are represented in Figure 4 and Table 3. Chlorogenic acid was observed at retention time 2.839min. The retention time for p-coumeric acid was observed at 3.234min with 773,930.8 peak area. Kaempherol was observed at retention time 11.005min with peak area 88,101.6. Similarly, retention time for salicylic acid was observed 15.525min with 101,818 peak area.

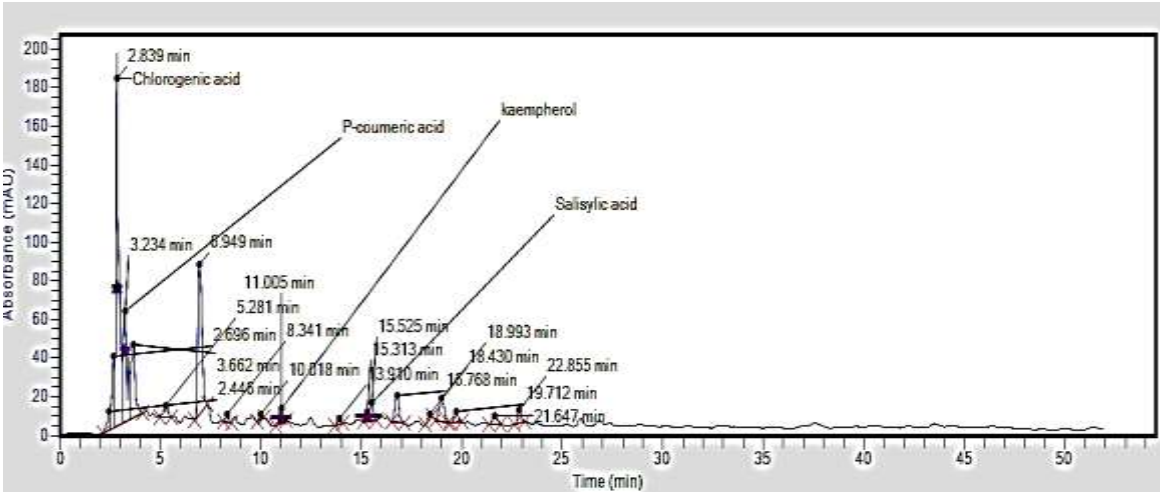

Figure 4: HPLC spectra of *M. oleifera*

Table 3 FTIR spectra for *Moringa oleifera*

Sr No.	Compound	Retention Time (min)	Peak Area [mV.s]
1	Chlorogenic acid	2.839	1,889,165.5
2	P-coumericacid	3.234	773,930.8
3	Kaempherol	11.005	88,101.6
4	Salicylic acid	15.525	101,818.1

Discussion

In this study, the structural and biochemical analysis of different extracts of *M. oleifera* was done. According to Pathak *et al*. (2020), the *n*- hexane extract of *Moringa oleifera* had a yield of 5.5% and Ukpe *et al*. (2024) mentioned a 7.4 % yield in the ethanolic extract of *M. oleifera* flower. In our research, methanolic extract showed the highest percentage yield (8.75%) and *n*-hexane extract showed the lowest percentage yield (2.54%). The disparity in yield is justifiable as extraction solvents and extraction methods employed are variable among studies. Solvents exhibit different polarities that determine phytoconstituents removed from any sample (Zakari *et al*., 2024). Susanto *et al.* (2024) reported that chitosan encapsulated nanoparticles prepared from *n*-hexane and ethanolic extracts of moringa had 115nm and 106.8nm size respectively. The potential was 5.9mV and 4.6mV with positive charges respectively. Current results are partially in agreement with this study as the size and potential of nanoparticles were 100nm and +10.5mV respectively.

According to Hani *et al.* (2016), the TPC of *M. oleifera* nanocapsules was 0.03 mg GAE /mL while the TPC of our methanolic nanoparticles were 480.47 mg GAE /mL. Kalauni *et al*. (2023) documented that methanol and hexane extracts showed the presence of significant quantities of total phenolics (207.75± 2.75 mg GAE/g, and 137.09 ± 1.1 mg GAE/g). In our study, the TPC of methanolic extract was the highest (453.14± 2.44 mg) and *n*-hexane extract was the lowest (84.96± 0.18 mg GAE /mL). According to Hussain *et al*. (2018), the TFC of silver nanoparticles were 1.1µg/mL while the TFC value of our nanoparticles was 671.71 µg CE/mL.

Saleem *et al.* (2020) observed the TFC value of aqueous extract was 53.28±1.27mg. However, in current research, TFC values of methanolic and *n*-hexane extracts range from 400 µg CE/mL to 600 µg CE/mL. Contrary to this, Kalauni *et al*. (2023) reported lower total flavonoids (94.56 ± 1.88 mg QE/g, and 82.71 ± 1.47 mg QE/g) respectively in methanol and hexane extracts. Regarding antioxidant activity, chitosan-based nanoparticles in the present

study showed 65.67% inhibition. Segwatibe *et al.* (2023) observed that the % inhibition of DPPH of *n*-hexane and methanolic extracts of *M. oleifera* ranges from 20% to 26%. In our research percentage inhibition of DPPH of *n*-hexane, methanolic and aqueous extracts of *M. oleifera* ranges between 23% and 26%. The discrepancy in data on antioxidant activity and contents in the current study and previous studies may be due to differences in solvents, extraction techniques used, experimental conditions and units of measurement.

According to Asuquo *et al.* (2024), the silver nanoparticles of *Moringa oleifera* showed 65.6% alpha-amylase inhibition while in our study chitosan-based nanoparticles of methanolic extract showed excellent anti-diabetic potential (85.87% inhibition). Magaji *et al.* (2020) found that among the various extracts, methanolic extract showed the highest alpha-amylase inhibition (23.163 ± 0.748%) and *n*-hexane extract showed less inhibition (18.983 ± 0.729%). In our research, among all extracts, the aqueous extract showed the highest anti-diabetic potential i.e., 65.99±1.74% inhibition.

According to Elhalabi *et al.* (2024), the *moringa* composite showed almost 40% hemolysis inhibition while in our study nanoparticle of methanolic extract showed only 1.99% hemolysis. Differences in these results may be due to different extraction and particle synthesis procedures. Current inferences are in agreement with those presented by Surendra *et al.* (2016). In that study, methanolic extract showed less hemolysis (1.20±0.09% hemolysis). Leave extracts did not exhibit cytotoxicity against human blood mononuclear cells and caused a maximum hemolysis of 2.08% in a previous study (Alves *et al*. 2024). In our research, aqueous and methanolic extracts proved to be non-toxic because their value was below 20%.

According to Bessalah *et al.* (2024) the gelatin-chitosan-moringa biopolymer showed 40% inhibition while in our study chitosan-based nanoparticles of methanolic extract showed 80-83.55% anti-inflammatory effect. Analogous to our results, Pandey and Bajpai, (2024) reported 85.8% inhibition compared to the standard drug's 99.9% inhibition when they studied inflammation inhibition by protein denaturation method.

Structural characterization was done by FTIR and HPLC. In FTIR, Khalid *et al*. (2023) identified alcohol, phenols stretching intermolecularity hydrogen bonded, aldehyde C-H stretching vibration, nitrogen compounds, carbonyl compounds, amine, alkane and aromatic compounds at peaks of 3450-3550.9, 2500-3200, 2700-2930, 2365 and 1700-1740, 1500-1650, 1445-1485, 1020-1280 respectively. Pavia *et al*. (2015) reported that band 1375.4 cm^{-1}, 1017.6 cm^{-1}value showed the presence of esters, alcohol, carboxylic acid, ethers, anhydride and

amines, 1049.2 cm^{-1} value showed the presence of ethers, alcohol, anhydride, esters, carboxylic acid and amines while 779.0 cm^{-1} value showed the presence of chloride, alkenes and aromatic bend, amines, sulfones, sulfonyl chloride, sulfates, sulfonamides and 1436.9 cm^{-1} value showed the presence of CH_3. Bello *et al*. (2017), reported that peak at 3280 cm^{-1} showed the presence of bonded – OH groups and according to the Atolani *et al*. (2020), bend CH_3 and nitro group were observed at 1420.1 cm^{-1} band. Nwokorie *et al*. (2023), reported that the band at 1457 cm^{-1} showed the presence of nitro group. According to the Aisida *et al*. (2021), band at 1618 cm^{-1} indicated the presence of bend primary and secondary amines and amides. According to the Adebisi *et al*. (2014), 2916.6 cm^{-1} value showed the presence of alkane. All these results are almost similar to our research.

In the case of HPLC, Ramesh (2018) described that the retention time for kaempherol was 10.507min which was near to our study in which the retention time for kaempherol was 11.005min. Salem *et al.* (2021) stated that the retention time for chlorogenic acid was 9.672min and salicylic acid was not observed. Abo El-Fadl *et al.* (2020) stated the presence of p-coumeric acid and salicylic acid in *M. oleifera* leaf extract. The retention time in our study for chlorogenic acid was 2.839min and for salicylic acid it was 15.525min.

Some potential limitations of the study include a lack of feasibility for large-scale nanoparticle production, optimization of stability, size and charge, limited characterization and *in vivo* testing to assess biocompatibility. Future research should focus on characterization by x-ray diffraction analysis, scanning electron microscopy, and *in vivo* trials to ensure safety and efficacy along with mechanistic insight.

Conclusion

Chitosan-based nanoparticles of *M. oleifera* showed maximum medicinal potential than the extracts. Encapsulation with biodegradable natural compound enhanced the bioavailability of phytoconstituents thereby improving bioactivities. Future research can focus on *in-vivo* studies, toxicology and pharmacokinetics to further explore the potential of *M. oleifera*. After further research, an effective herbal medicine may be formed from *M. oleifera.*

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