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Original article

Green synthesis of *Moringa oleifera* leaf nanoparticles and an assessment of their therapeutic potential



Promy Virk^{a,*}, Manal A. Awad^{b,*}, Sarah Saleh Abdu-llah Alsaif^c, Awatif A. Hendi^d, Mai Elobeid^a, Khalid Ortashi^e, Rabia Qindeel^f, Manal F. El-Khadragy^{g,*}, Hany M. Yehia^{h,i}, Mohamed Ferkry Serag EL-DIN^{h,j}, Hatem Ali Salama^{h,k}

^a Department of Zoology, College of Sciences, King Saud University, P.O. Box 22452, Riyadh 11459, Saudi Arabia

^b King Abdullah Institute for Nanotechnology, King Saud University, Riyadh 11451, Saudi Arabia

^c Department of Botany and Microbiology, King Saud University, Riyadh 11451, Saudi Arabia

^d Department of Physics, College of Science, Princess Nourah Bint Abdulrahman University, Riyadh 11671, Saudi Arabia

^e Department of Chemical Engineering, King Saud University, Riyadh 11421, Saudi Arabia

^f Department of Physics and Astronomy, College of Sciences, King Saud University, P.O. Box 22452, Riyadh 11459, Saudi Arabia

^g Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia

^h Department of Food Science and Nutrition, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia

ⁱ Department of Food Science and Nutrition, Faculty of Home Economics, Helwan University, Cairo, P.O. Box 11611, Egypt

^j Department of Nutrition and Food Science, Faculty of Home Economics, Minufiya University, Minufiya, Egypt

^k Department of Food Technology, National Research Center, Dokki, Cairo 12622, Egypt

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ABSTRACT

The present study centered around the prospect of bio fabricating nanoparticles using *Moringa oleifera* leaf and investigating their therapeutic potential with an assessment of the antioxidant status. Synthesis of stable 'green' nanoparticles of *Moringa* leaf extract is successfully reported with an extensive characterization. The average particle size and polydispersity index (PDI) of the nanoparticles obtained by Dynamic light scattering (DLS) analysis were 141.6 nm and a polydispersity index (PDI) of 0.32 respectively. The UV–visible spectroscopy (UV–vis) revealed a strong red absorbance peak at 664 nm, characteristic of chlorophyll. Electron micrographs confirmed that particles were in the nano range and were spherical with minimal agglomeration. The constituent functional groups were assessed by Fourier transformed infrared spectroscopy (FTIR). The X-ray diffractograms of reflected both the amorphous and crystalline domains. Furthermore, the phytochemical screening showed a higher phenolic/ flavonoid content of the *Moringa* nanoparticles (NMo) in comparison to the bulk *Moringa* leaf extract (Mo). These results corresponded to a potent antioxidant status of the NMo which was assessed by three complementary antioxidant assays. The therapeutic potential of the nanoparticles was evaluated based on *in vitro* anti-diabetic assays and cytotoxicity assay on human cancer cell lines (MCF-7 and HepG-2). The key findings suggest a more profound therapeutic efficacy of NMo than the *Moringa* leaf extract.

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* Corresponding authors at: King Abdullah Institute for Nanotechnology, King Saud University, Riyadh 11451, Saudi Arabia

E-mail addresses: bvirk@ksu.edu.sa (P. Virk), mawad@ksu.edu.sa (M.A. Awad), mfelkhadragy@pnu.edu.sa (M.F. El-Khadragy).

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1. Introduction

Moringa oleifera, also known as drumstick tree is a member of the Moringaceae family and is also called the “Miracle Tree” (Mahato et al., 2022). Being a native to north-western India it has been well recognized both in the Ayurveda and Unani systems of medicine (Milla et al., 2021) and in ethnomedicine as well. Its diverse therapeutic benefits attributed to the constituent bioactive phytochemicals in its leaves, pods, and seeds have garnered attention in pharmacology (Palizban et al., 2015; Mahato et al., 2022). It is one of the most widely used functional foods in the current times being nutrient dense that adds to its therapeutic value.

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Additionally, *Moringa* is an important versatile tree species for agroforestry as it survives under a variety of meteorological conditions (Mridha, 2015) and is thus cultivated in many tropical and sub-tropical countries including Saudi Arabia (Alaklabi, 2015). The leaves and the pods have been a part of the Indian diet since ancient times. The pharmacological benefits of the leaves are extensively reported in previous literature (Irfan et al., 2021). During the past decade several experimental studies have evaluated the phytochemical profile of the *Moringa* leaves emphasizing on their antioxidant potency. The appreciable amount of ascorbic acid, vitamins A, B, α -tocopherol, β -carotene, β -sitosterol, protein, and specifically essential amino acids present in *Moringa* leaves and pods attribute to its status of an ideal nutraceutical (Ali et al., 2018). The dried leaves of *Moringa* are also endowed with polyphenols such as flavonoids and phenolic acids. The key concept of scientific development in the current times is human well-being and health. The buzz word is 'green' which in green technology refers to the use of plant products/phytocompounds. An offshoot of green technology is green nanotechnology, a key technology of the century. This includes a nanotechnological approach in phyto formulation of pharmacologically important bioactive constituents in herbs (Verma et al., 2019). The fundamental problem of some of these bioactive compounds being insoluble and prone to rapid degradation in the biological milieu is effectively solved which enhances their bioavailability and therapeutic efficacy. Literature is replete with studies on plant based, both metallic and non-metallic nanoparticles making the bio-based nano synthesis, environmentally safe, facile and cost effective (Verma et al., 2018). Furthermore, recent studies (Matinese et al., 2017; Moodely et al., 2018; Amina et al., 2019; Venkatachalam et al., 2021) have reported the use of *M. oleifera* extracts as capping and reducing agent in the biosynthesis of metallic nanoparticles. Nevertheless, the nanoformulation of the *Moringa* leaf extract in its pure form without the use of chemical surfactants and metals has not been reported.

Considering this premise, the current study included the novel synthesis of nanoparticles sans any chemical surfactants or stabilizers followed by *in vitro* bioassays to evaluate their anti-diabetic and anti-carcinogenic potency.

2. Materials and methods

2.1. Synthesis of moringa nanoparticles

Moringa leaves were dried and 400 mg dried leaf powder was then added to 20 mL of methanol. Thereafter, this solution was sprayed at a rate of 0.2 mL/min for 5 min into 50 mL of boiling water under ultrasonic conditions (power of 750 W and a frequency of 20 kHz). After sonicating for 5 min the contents were stirred at room temperature at 200–800 rpm for approximately 20 min. The solution was concentrated and then freeze-dried.

2.2. Characterization of the nanoparticles

An extensive characterization of nanoparticles included the following techniques; UV-visible spectrophotometer (Perkin Elmer, Japan) within a wavelength range of 200–800 nm, Dynamic light scattering (DLS) technique by Zeta sizer (ZEN 3600, MALVERN, United Kingdom), Fourier-transform infrared spectroscopy (FTIR) (Perkin-Elmer FTIR-1600, USA) within the range of 500–4000 cm^{-1} and a resolution of 4 cm^{-1} . X-ray diffraction (PAN analytical X PRT PRO, D-8, Advanced Brucker instrument (Netherlands) and Transmission electron microscopy (TEM) (JEM-1011, JEOL, Japan).

2.3. Phytochemical screening

Phytochemical examinations were carried out for the *Moringa* leaf extract and the nanoparticles as per the standard tests. Total phenol content was quantitated using the Folin Ciocalteu reagent with gallic acid as the standard (Siddiqui et al., 2017). Colorimetric assays were used to quantitate the total flavonoid (Fattahi et al., 2014). Total flavonoids were expressed as catechin and rutin in milligrams equivalents per gram of sample (mg/g) in the two assays.

2.4. Determination of *in vitro* antioxidant activity

The antioxidative status of the *Moringa* leaf extract and its nanoparticles was measured by three complementary tests.

2.4.1. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical scavenging activity

ABTS radical-scavenging activity of the *Moringa* leaf extract and its nanoparticles was evaluated in accordance with the protocol given by Re et al. with some modifications (1999). Trolox was used as a standard for the assay and the optical density (OD) was recorded at 734 nm. Results were expressed in terms of g trolox equivalent per g of dry weight (g eq. trolox/g).

2.4.2. α , α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging activity

This test was in accordance with the standard protocol by Adebiyi et al. (2017). The OD of the solutions was recorded at 515 nm. The DPPH radical scavenging activity was illustrated as percentage inhibition and computed as follows:

DPPH scavenging activity (%) = $(A_0 - A_1) / A_0 \times 100$, where A_0 is the OD of the control and A_1 is the OD of the sample.

2.4.3. Ferric reducing antioxidant power (FRAP) assay

A modified form of the FRAP assay as previously reported by Benzie and Strain (1996) was used in the study. Trolox was used as a standard for the assay and the absorbance of the samples was measured at 593 nm. The FRAP results were expressed in terms of g trolox equivalent per g of dry weight. (g eq. trolox/g).

2.5. Cytotoxicity assay

The human cancer cell lines (MCF-7; human breast cancer and HepG-2; human hepatocellular carcinoma) were procured from VACSERA Tissue Culture Unit (Giza, Egypt).

Cytotoxicity was evaluated by the viability assay as per the standard protocol (Riss et al., 2013). Cell control without the tested compounds used to compare the treated cells. The optical density (SunRise, TECAN, Inc, USA) assessed the number of viable cells and their percentage was computed as $[(OD_t/OD_c)] \times 100\%$ where OD_t is the mean optical density of wells treated with the tested sample and OD_c is the mean optical density of control cells. The dose response curve estimated the IC_{50} (50 % inhibitory concentration) using the GraphPad Prism software (San Diego, CA, USA).

2.6. *In-vitro* antidiabetic assays

α -glucosidase inhibitory activity of *Moringa* leaves and the nanoparticles was evaluated following the standard method with minor modifications (Shai et al., 2011). Further, α -amylase inhibitory activity was determined in accordance with the standard protocol (Heidari et al., 2005). Acarbose at various concentrations 1000 to 7.81 $\mu\text{g/mL}$ was the standard for both the assays. Inhibitory activity (%) = $(1 - As/Ac) \times 100$ was calculated, and expressed

as mean ± standard deviation and IC₅₀ values were quantified using GraphPad.

3. Results

3.1. Characterization of *Moringa oleifera* nanoparticles

UV-vis spectroscopy was scanned in the range of 200–800 nm. The absorption spectra of moringa leaf extract (Mo) (as a control) and synthesized nano *Moringa* (NMo) showed a strong red absorbance peak at 664 nm which is distinctive of chlorophyll common to both nano *Moringa* and *Moringa* leaf extract (Fig. 1). The formed nanoparticles demonstrate mean diameter of 141.6 nm with a polydispersity index (PDI) of 0.32 (Fig. 2). The TEM micrographs (Fig. 3 A and B), illustrate spherical nanoparticles with diverse sizes. In addition, the nanoparticles (NMo) were covered by a layer with minimal agglomeration. Fig. 4, shows the FTIR spectra for the bulk *Moringa* leaves and the nanoparticles. The absorption peaks at 3401.36 cm⁻¹, 3401.10 cm⁻¹, 2925.92 cm⁻¹ and 2925.88 cm⁻¹ respectively, were elongated and sharpened, this could be due to the O–H, C–H stretching off –C=O and/or –CH₃ groups. At 1655.04 cm⁻¹ and 1654.52, respectively, an elongation was observed due to the C=O group of the carboxylic acids being

stretched. It was also observed that the peak at 1059.93 cm⁻¹ was shifted to 1062.07 cm⁻¹ and the peak at 1239.69 cm⁻¹ to 1236.79 cm⁻¹ which could be due to the stretching of C–O. Peak at 1319.02 cm⁻¹ also was elongated due to the bending in the N=O (Fig. 4).

To assess the crystalline nature of the Mo and, nano-moringa NMo powder, the XRD analysis was conducted. The X-ray diffractograms of Mo and NMo showed broad five low intensity distinct diffraction characteristic peaks at 2 Theta (2θ) values of 21°, 37°, 43°, 77°, and 810°, reflecting both the amorphous and crystalline domain (Fig. 5).

3.2. Phytochemical profile and in vitro antioxidative activity

The analysis shows that the phytochemical content; total phenols, total flavonoids was higher in the NMo than the leaf extract (Mo) (Table 1). Furthermore, the ability of the Mo and the NMo to scavenge the reactive oxygen species (ROS) was evaluated by three complementary *in vitro* antioxidant assays. In the DPPH assay, the NMo showed a notably higher radical scavenging activity with a percent inhibition of 80.630 ± 0.545 in comparison to the leaf extract (Mo)(37.611 ± 0.472). A higher antioxidant activity was observed in the ABTS assay for NMo; 57.750 ± 2.385 g equiv.Trolox/g sample as compared to the Mo; 4.983 ± 0.101 g equiv.Trolox/g sample. The results of the FRAP assay showed a higher value for NMo(8.989 ± 0.671 equiv.Trolox/g sample) as compared to Mo (1.989 ± 0.123 g equiv.Trolox/g sample). The values observed for the Mo and nanoparticles NMo were consistent within all three assays (Table 1).

3.3. Bioassays

3.3.1. Cytotoxicity

As illustrated in Figs. 6 and 7, a cell survival significantly declined in both MCF-7 and HepG-2 cancer cell lines when treated with Mo and NMo. For MCF-7, an IC₅₀ of 168 ± 27.04 µg/ml was observed for Mo (Fig. 6A). The inhibitory activity of MoN was significantly higher when compared to *Moringa* leaf powder, with an IC₅₀ of 65.6 ± 13.97 µg/ml (Fig. 6B). Additionally, for the HepG-2 cell line, an IC₅₀ of 113.5 ± 6.59 µg/ml was observed for *Moringa* leaf powder (Mo). The inhibitory activity of NMo showed a signif-

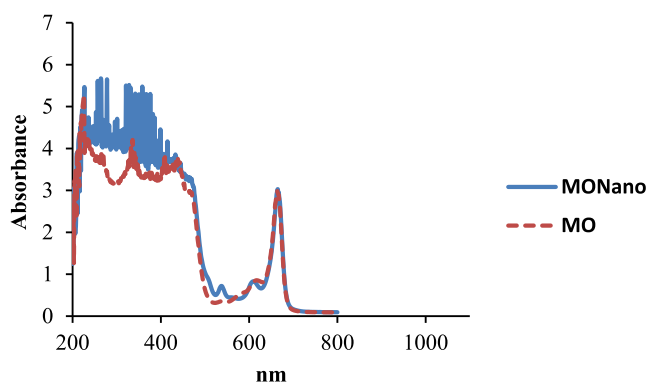


Fig. 1. UV-vis spectra of synthesized nanoparticles moringa and moringa leaf extract.

	Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 141.0	Peak 1: 145.8	94.9	57.33
Pdl: 0.324	Peak 2: 19.82	5.1	4.006
Intercept: 0.910	Peak 3: 0.000	0.0	0.000

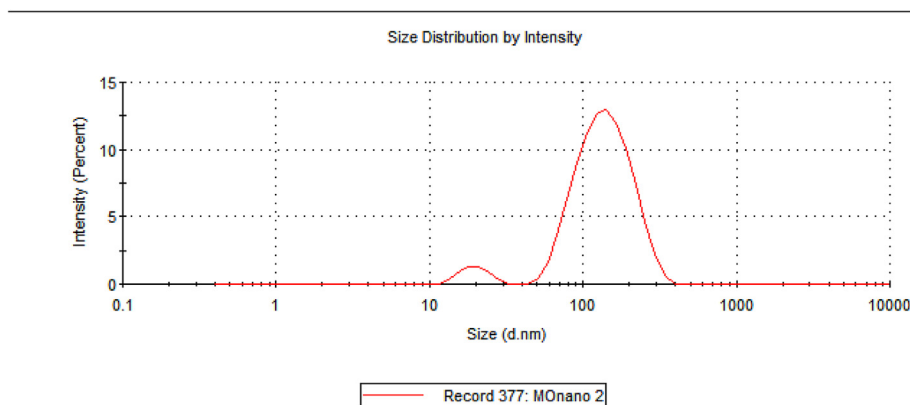


Fig. 2. The DLS graph showing the average particle size of the nano particles with size distribution.

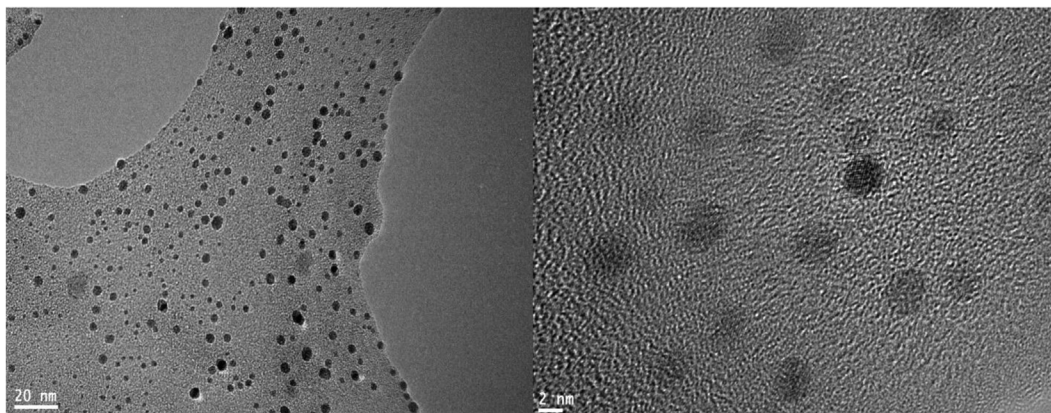


Fig. 3. TEM micrographs showing the *Moringa* leaf nanoparticles.

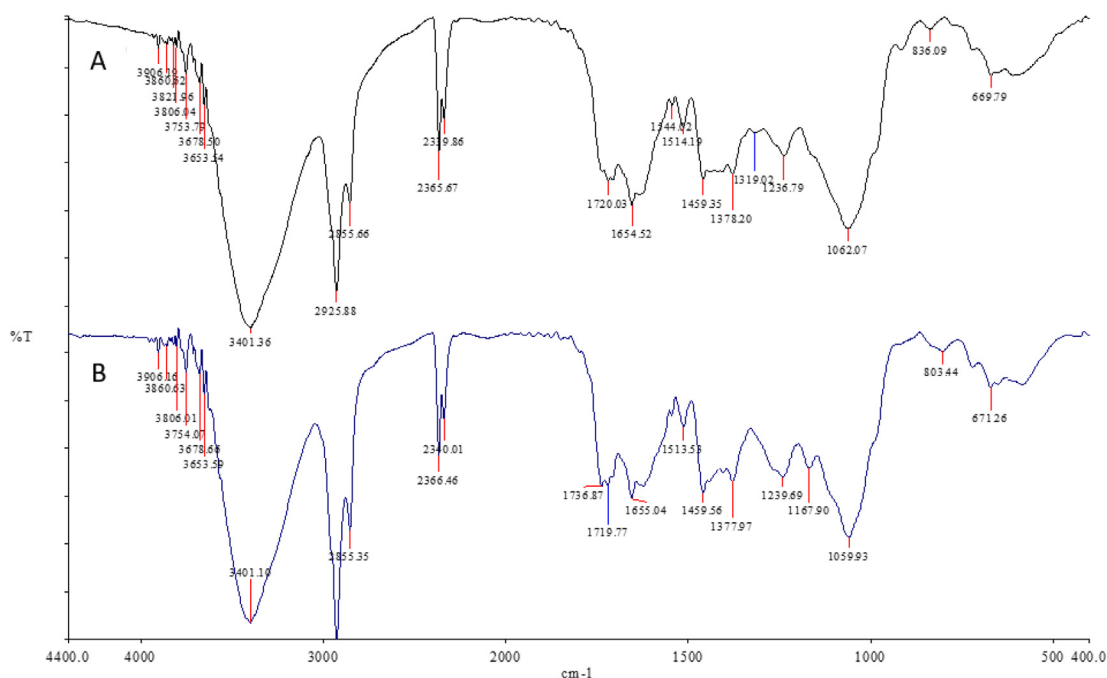


Fig. 4. FTIR spectra of (A) *Moringa* nanoparticles and (B) *Moringa* leaf extract.

icant increase in comparison to Mo, with an IC_{50} of $53.4 \pm 1.93 \mu\text{g/ml}$ (Fig. 7 A and B). Taken together, the results the study strongly suggest a “switching off” of cancer cell survival mechanisms under the *Moringa* leaf treatments, being more profound with the nanoformulation.

3.3.2. Anti-diabetic activity

Inhibitory potency of *Moringa* leaf extract (Mo) against α -amylase activity was observed with an IC_{50} value of $719.92 \pm 4.4 \mu\text{g}$. While the nano formulation,(NMo) showed significantly more lower IC_{50} value of $115.9 \pm 1.11 \mu\text{g/ml}$ Fig.8A and B. Further, inhibitory potency of *Moringa* leaf extract against α -glucosidase activity was observed with an IC_{50} value of $231.08 \pm 3.73 \mu\text{g/ml}$. While the nano *Moringa* (Mo N), was significantly more efficacious as is evident from the IC_{50} value, 59.38 ± 1.42 (Fig. 9A and B).Thus, based on the results of both the complementary anti-diabetic assays *Moringa* nanoparticles (NMo) exhibited a more potent anti-diabetic activity.

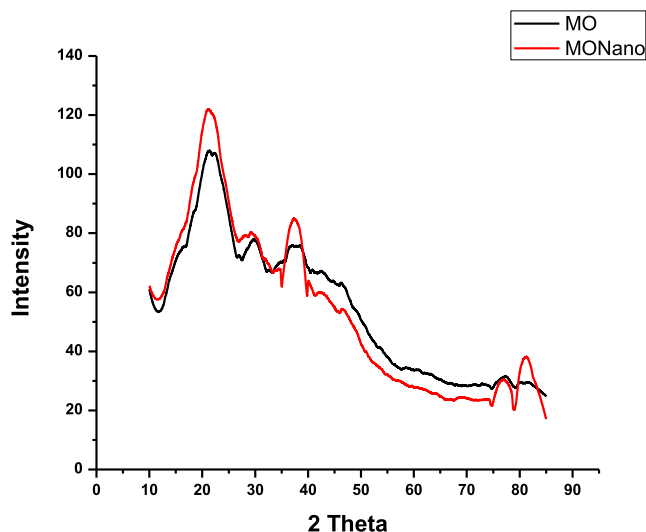


Fig. 5. X-ray diffractograms *Moringa* nanoparticles and *Moringa* leaf extract.

Table 1
Phytochemical profile and the free radical scavenging ability of the *Moringa* leaf extract(Mo) and nanoparticles(NMo).

	T.Phenols (mg Gallic acid /g sample)	T. Flavonoids (mg Catachin /g sample)	T. Flavonoids (mg Rutin /g sample)	DPPH (%) inhibition	ABTS (g Trolox/g sample)	FRAP (g Trolox/g sample)
Mo	149.702 ± 1.510	0.303 ± 0.119	3.809 ± 1.319	37.611 ± 0.472	4.983 ± 0.101	1.989 ± 0.123
NMo	455.621 ± 2.008	6.777 ± 0.207	57.130 ± 2.285	80.630 ± 0.545	57.750 ± 2.385	8.989 ± 0.671

Values are means of four replicates. Results are given as mean ± SD.

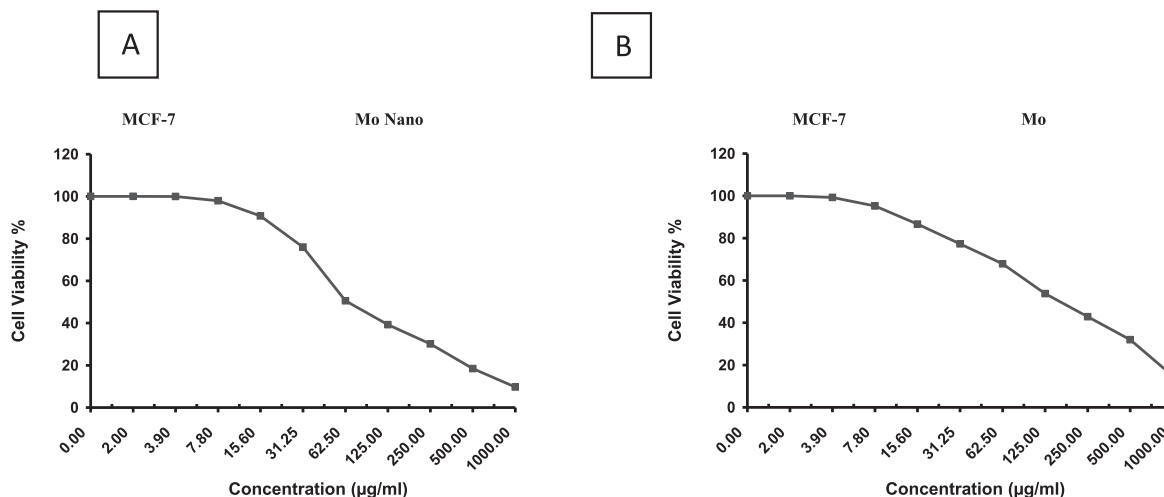


Fig. 6. Effect of variable concentrations of nanoparticles of (A)*Moringa* leaf extract(NMo) and (B)*Moringa* leaf extract(Mo) on the cell viability (%) of MCF-7 cell line.

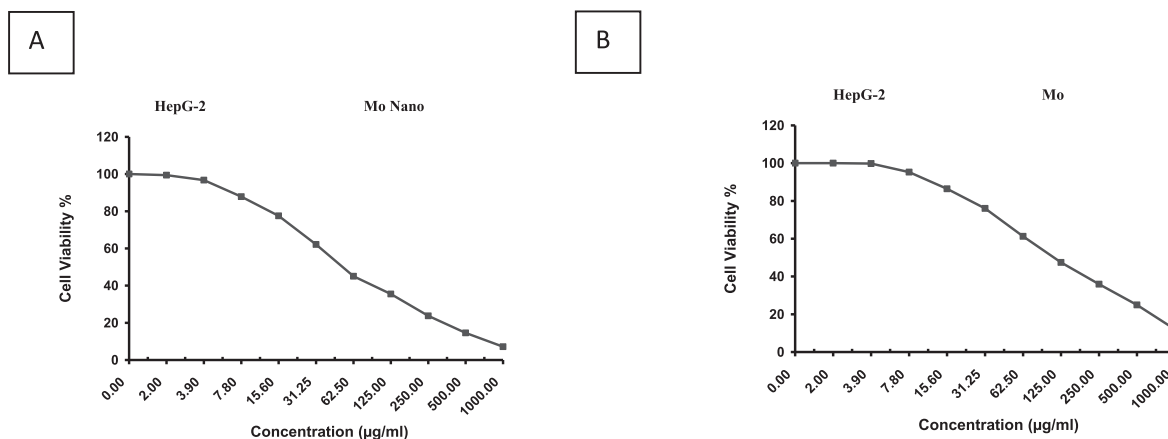


Fig. 7. Effect of variable concentrations of nanoparticles of (A)*Moringa* leaf extract(NMo) and (B) *Moringa* leaf extract(Mo) on the cell viability (%) of HepG-2 cell line.

4. Discussion

Green nanotechnology has become popular over the past decade and has the potential of being successfully commercialized as an industry with strong green credentials. The present study focused on fostering the concept with the ‘green’ synthesis of non-metallic nanoparticles of *M. oleifera* leaves. Nanoparticles of *Moringa* leaves which were spherical in shape, 141.6 nm in size with a (PDI) 0.32 were successfully synthesized. It has been reported that a homogenous distribution is demonstrated at values of 0.3 and below and are considered acceptable (Danaei et al., 2018). A close observation of this agglomeration shows that the NPs were enveloped in a faint thin layer of constituent biomolecules in the *Moringa* leaf; protein or cellulose. This is in concurrence with the study by Mallikarjuna et al. (2011). The absorption spectra Mo and NMo showed a strong absorbance peak

at 664 nm which is the distinctive for chlorophyll, a feature of the leaves used. The spectrum for the *Moringa* nanoparticles showed smaller absorbance peaks possibly due to nanosization and modulation in the concentration of some of the bio components (Alexandre-Tudo and Toit, 2018). The broader pattern of the FTIR spectra of both the Mo and the nanoparticles was similar reflecting related constituent functional groups which were not altered on being nano scaled. However, a slight shift in the spectra was evident with the presence of distinguishable peaks for the nanoparticles. These findings are in consensus with previous reports (Saleem et al., 2020; Prasetyaningrum et al., 2022). In comparison to other previous studies, the variability observed in the results, could be possibly due to the different source of *M. oleifera* leaves used. Overall, series of sharp peaks are characteristic of crystalline materials while a broad pattern is illustrated by amorphous substances (Khatri et al., 2018). The extraordinarily small diffraction patterns

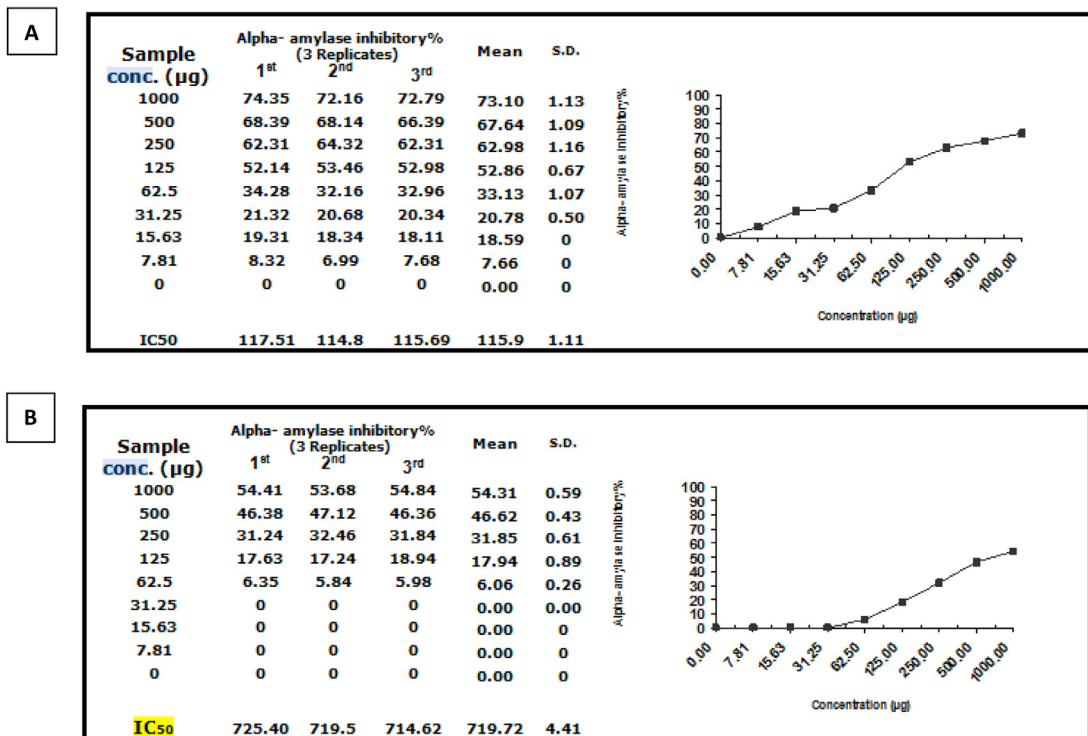


Fig. 8. α-glucosidase inhibitory activity(%) of (A)*Moringa* nanoparticles and (B)*Moringa* leaf extract.

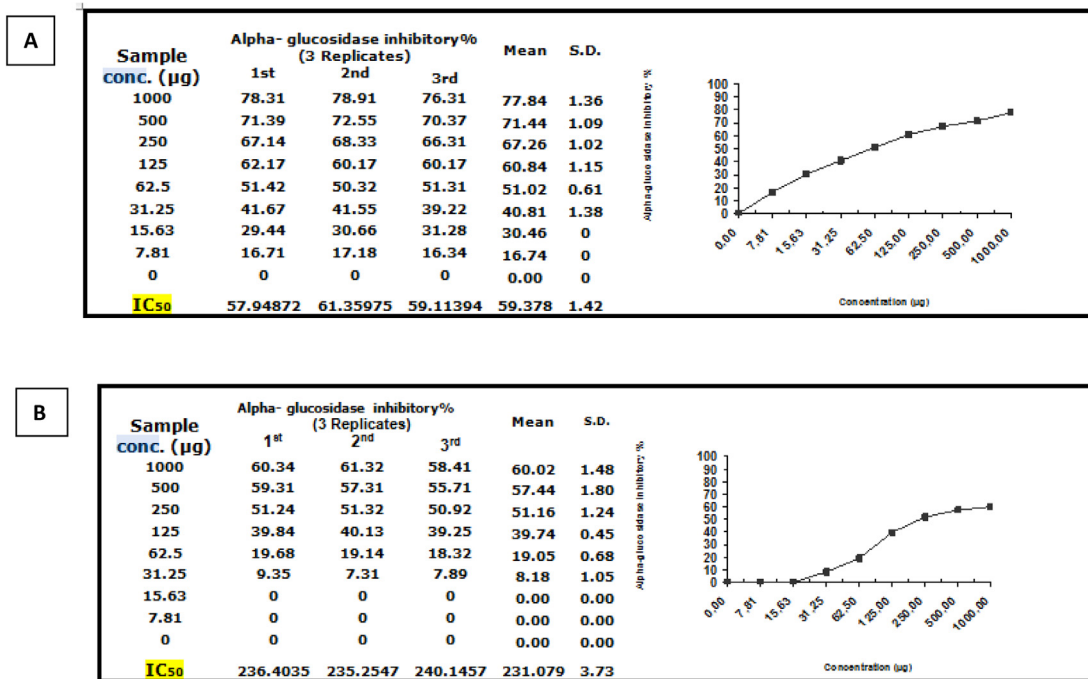


Fig. 9. α-amylase inhibitory activity(%) of (A)*Moringa* nanoparticles and (B)*Moringa* leaf extract.

of NMo suggested a small crystallite size (part of crystalline cellulose and hemicellulose). Furthermore a higher protein content of the NMo (Gopalakrishnan et al., 2016), contributes to the prevalence of amorphous nature of native proteins, corresponding to the α-helix and β-sheet secondary conformations. Furthermore, the presence of lipids with polyunsaturated fatty acids results in high background base and broader pattern due to their amorphous

nature. Similar results were reported in the literature previously (Sharma, 2019; Ferreira et al., 2021).

There have been previous studies that reported the phytochemical profile of *Moringa* leaves emphasizing on the various constituent pharmacologically active phytochemicals in the leaves with (Bhalla et al., 2021; Reminus and Cornelius, 2019). The current study is among the very few studies where the phytochemical

profile coupled with the potency to scavenge free radicals has been reported for Mo and its nanoparticles. The antioxidant status of a phytocompounds is of vital importance and adds to the therapeutic potential. The results clearly showed a higher total content of phenols and flavonoids in the NMo compared to the aqueous Mo. Further, based on the results of the *in vitro* antioxidant assays, NMo reflected higher free radical scavenging ability in comparison to the aqueous extract of Mo. This mirrors the results on the phytochemical screening and could be attributed to the higher amount of the polyphenolic components in the nanoparticles contributing towards higher scavenging potency. Consistent with this, a study reported that the incorporation of Ag-NPs into crude extract of moringa leaves increased the antioxidative ability, total polyphenolic content, and free radical scavenging activity in addition to enhanced cytotoxic effect on colon cancer cells (Shousha et al., 2019; Mohammad et al., 2022). This could be best explained by an enhanced bioavailability of the active phytocompounds. Oxidative stress has been incriminated in the pathogenesis of several human disorders including diabetes mellitus (DM) and cancer. The anti-diabetic effect of *M. oleifera* leaves has been reported as its potential ability to lower blood sugar levels after ingestion attributed to the presence of polyphenols; quercetin-3-glycoside, rutin, kaempferol and glycosides (Al- Malki and El Rabey, 2015). In addition, Villarruel-López et al. (2018) reported that was blood glucose levels and enterobacteria counts were improved in diabetic rats administered with *M. oleifera* powdered leaves. These reports clearly support the anti-diabetic activity of the Mo and its nanoparticles reported in the current study. The obtained results of both *in vitro* anti-diabetic assays showed that the *Moringa* nanoparticles were more efficacious as illustrated by lower IC₅₀ values. Additionally, the antioxidant status of the *Moringa* leaves was demonstrated as the leaf extract (Mo) and the nanoparticles (NMo) proved to be effective in the cytotoxicity assay in both cancer cell lines (MCF-7; HepG-2) used. Nevertheless, the higher content of phenols and flavonoids in the nanoparticles (NMo) attributed to a more profound inhibitory activity in comparison to the leaf extract (Mo). A docking analysis of *M. oleifera* leaves showed several constituent ligands demonstrated good docking scores with BRCA-1 (Balogun et al., 2021).

5. Conclusions

The study included the synthesis, characterization and an assessment of the phytochemical profile, antioxidant status of the nanoparticles with the response to the bioassays. The mode of synthesis of nanoparticles kept the 'green' credential as the cornerstone which was facile, environmentally benign and safe. The nanoparticles were therapeutically more potent than the leaf extract owing to the increased bioavailability of the phytocompounds on nanosization. Nevertheless, further in-depth studies and *in vivo* testing of the nano formulation as a nutraceutical is imperative to open a new horizon in nanomedicine.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jksus.2023.102576>.

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