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Experimental Evaluation of The Effectiveness of Orange Peel/ Chitosan Nanoparticles

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ABSTRACT

Chitosan nanoparticles (CSNPs) are a useful vehicle for incorporating the highlysensitive components of orange peel extract. The aims of this study were to produce OPE-loaded CSNPs and characterize their physical, structural, antioxidant, and antibacterial properties. Chitosan ratios of 2:1 (v/v) allowed for the effective synthesis of physically stable CSNPs. A good mean diameter and a consistent zeta potential were seen in the size distribution profile of the OPE loading at chitosan nanoparticles. The OPE was physically entrapped within NPs because Fourier Transform Infrared (FTIR) examination of the OPE and OPE-CSNPs revealed no obvious spectrum changes, writing down no probable chemical interaction between the OPE and OPE-CSNPs. Furthermore, FTIR spectra of pure OPE and chitosan nanoparticles CSNPs loading of orange peel extract revealed that there was a slight shift in the peaks of the chitosan nanoparticles CSNPs loading of orange peel extract as compared to orange peel extract (OPE), as well as a slight decrease in the disparity of those peaks in the chitosan nanoparticles loading of orange peel extract, and it is clearly evident that the OPE-loaded with CSNPs, however, had more inhibitory antibacterial action as compared to OPE alone. This could mean that the OPE leakage from NPs is successfully constrained by loading of OPE into CSNPs.

1. Introduction

Researchers have shown that orange peel, a byproduct of fruit processing, is a good source of bioactive compounds (Mamta and Parminder, 2013). Peels and other orange debris are produced in enormous amounts annually. The orange peel waste produced during the production of orange juice and other products will accumulate and damage the environment. It is essential to find applications for these peels. Orange peels can be used to manufacture a variety of meals and medicines due to their nutritional value and high phytochemical content (Hegazy and Ibrahim, 2012). The citrus fruit crop, which has an annual production of 100 million cubic tones and is the largest fruit crop in the world, is 60% oranges (Oreopoulou and Tzia, 2006). The remaining amount of orange peel makes up about 45% of the total weight. As a result, a sizable amount of orange peel is produced as a byproduct. Due to the presence of biomaterials like essential oil and pectin in the orange peel, if it is treated as waste, it may cause environmental issues, most notably water pollution. phenols, flavonoids, carotenoids, and vitamins (Dhanani et al., 2017). As a surfactant, orange peel extract might be useful. A chemical called a surfactant lowers the surface tension of liquids. Orange peel extract may lessen the liquid in the stomach's surface tension, reducing the likelihood that it will splash up into the oesophagus. Orange peel extract has been found to suppress the growth of cancer cells. In laboratory studies, orange peel extract prevented breast, skin, liver, lung, pancreatic, colon and stomach cancers (Yano et al.,1999). Most agricultural wastes as well as municipal solid waste are left unused and create problems for the environment and municipal solid waste management (Gupta et al., 1998).

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Organic fruits like oranges have waste in the form of peels, seeds, and membrane residue, which accounts for 50–60% of their overall weight. According to (Miran et al., 2016), orange peels include 17.5% cellulose, 8.5% hemicellulose, and pectin. Citrus peel can be used as a dietary supplement for human or animal feed, an element in pharmaceutical product formulations for the creation of antidiarrheal and detoxifying medications, and in functional meals. Citrus peel phenolic chemicals have a wide range of pharmacological actions, from antiproliferative to antioxidant (Ghasemi et al., 2009). About 70% of all citrus species produced are of the orange fruit, or Citrus sinensis. Citrus sinensis peels, which account for about 50% of the fruit's mass and are either eaten fresh or used to make orange juice, are thrown away after usage (Mandalari et al., 2006). According to studies, orange peel extract stops cancer cells from reproducing and spreading. In lab tests, orange peel extract has been found to protect against cancers of the breast, skin, liver, lung, pancreas, intestine, and stomach. Additionally, it has been demonstrated that the phenolic and flavonoid compounds in orange peels contain anti-inflammatory and antibacterial effects that are advantageous to human health (Harris, 2001).

Recently, more research on nanotechnology has been conducted. One example is the rising use of polymeric nanoparticles, which are widely used in many facets of life and health. These nanoparticles are biocompatible and biodegradable polymers with demonstrated effects as coadjuvant antimicrobial agents (Islan et al., 2015), anticancer agents (Badran et al., 2017), and antioxidants (Tapia-Hernández et al., 2018), this gives them a certain level of dependability when used in terms of health. As immune system modulators, polymeric nanoparticles have been investigated for their potential in medicine, either directly through their composition or indirectly as active component transporters (Kubackova et al., 2020).

Many nano-materials have special qualities that are useful in various biotechnology applications, leading to their use in the creation of incredibly efficient diagnostic and therapeutic tools (Rafi et al., 2016). Interest in biological methods that do not employ dangerous chemicals as byproducts has increased due to the demand for ecologically acceptable, nontoxic nanoparticle manufacturing techniques. The biological method has a better chance because it is helpful from both an environmental and financial standpoint (Abebe Alamineh, 2018).

Chitosan is a linear polysaccharide with cationic nature and high potential to encapsulate natural ingredients. This would result in developing different forms of chitosan matrix (nanoparticles, nano emulsions, nanofibers, hydrogels, films and coatings). These systems can be used for the encapsulation of medicinal herbal extracts and essential oils for potential applications in the food industry, pharmaceutical and cosmetics (Sreekumar et al., 2018). Chitosan owing to its general recognition as safe (GRAS), has some advantages, such as nontoxicity, biocompatibility, and antimicrobial properties (Keawchaoon and Yoksan, 2010), which makes it suitable for in vivo use in biomedical treatments. It is simple to create chitosan nanoparticles (CSNPs) using the ionic gelation process between anionic tripolyphosphate and cationic linear chitosan polymer (Dehghani et al., 2019). The CSNPs, due to their higher surface-to-volume ratio, supplies the advantage of carrying natural extracts, which reinforce the functionality and compatibility of the nanoparticles (Vahedikia et al., 2019).

From a technological point of view, nanoparticles also serve as strengthening fillers in the polymeric matrix by enhancing their barrier properties. Moreover, nanomaterial fillers can be regarded as suitable carriers for antimicrobial and antioxidant agents in preserving food quality. In many biopolymeric applications in food packaging, CSNPs, like other nanoparticles, can act as nano-reinforcing carrier materials (Keawchaoon and Yoksan, 2011).

The goal of this study is to determine the effectiveness of orange peel extract (OPE) loading at chitosan nanoparticles in comparison to OPE as an antioxidant and antibacterial activity.

2. Materials and Methods

Plant material collection

Gathering of plant matter During the month of October 2022, ripe and recently harvested orange (Baladi) fruits were purchased in the adjacent supermarket. The orange was well scrubbed with tap water, the skin was scraped off by breaking it up into little pieces, and it was dried in an oven for 6-7 days at 30°C. The dried peel was crushed into a powder using an electric blender.

Preparation of orange peel extract (OPE)

By combining 25 g of orange sinensis peel with 1 L of double-distilled water and stirring constantly at 25°C for 40 minutes, the plant extract concentration was created. The unwanted material was eliminated by centrifuging the aqueous solution at 4,000 rpm for five minutes and filtering it through Whattman No. 1 filter paper after allowing it to cool. The supernatant was stored at 4°C prior to the experiments being conducted.

Preparation of Chitosan Nanoparticles (CSNPs) Loaded with OPE

Orange peel extract was mixed with chitosan in a 1:2 (v/v) ratio as a coating agent. The mixture was first homogenized for five minutes, then hydrated at 4°C for eighteen hours before being again homogenized for 30 seconds (Du et al., 2009). The Nanotechnology and Advanced Materials Central Lab (NAMCL) was the site of this experiment. Egypt's Giza Agriculture Research Center.

Physicochemical Characterization of Nanoparticles

Malvern Instruments' UV- Zeta sizer nano series (Nano ZS, ZEN 3600) was used to measure the size of the particles and their zeta potential. Samples were distributed in water and measured using the automated mode for zeta potential measurements. (Hernández-Téllez and coworkers, 2021).

Characterization of OPE and Chitosan nanoparticles Loaded with OPE

Fourier Transform Infrared (FTIR) Spectroscopy was used to examine the samples (German manufacturer: Bruker-OPTIK GMBH-FTIR). OPE and Chitosan nanoparticles loaded with OPE were used to construct an FTIR spectrophotometer with a range of 500 cm-1 to 4000 cm-1.

Quantification of total phenol content

An incubation process was carried out using test tubes with an amount of 50 µL of each sample incubated with 3.0 mL of distilled water and 250 µL of Folin–Ciocalteu reagent,750 µL of 20% Na2CO3, and 950 µL of distilled water. After 3 min, 1.0 mL of a sodium carbonate solution was added and allowed to react for 60 min in the dark (Júnior et al., 2021). Using a UV-VIS spectrophotometer, the solution's absorbance (A) at 765 nm was determined. The total phenolic content of the peel extracts was calculated by using standard curve prepared from gallic acid. The total phenolic content of the sample was expressed as mg/g of gallic acid equivalents.

Quantification of total flavonoids

The aluminum chloride (AlCl3) assay was used (Chen et al., 2022). Each sample (0.5 mL) was mixed with 2 mL of distilled water and 0.15 mL of 10% (w/v) AlCl3. After 10 min of incubation in the dark at room temperature, 1 mL of 1 M sodium hydroxide (NaOH) and 1.2 mL of distilled water were added to the mixture. After 15 min incubation in the dark at room temperature, the mixture was measured the absorbance (A) of the solution was measured at 430 nm with a UV-VIS spectrophotometer. The standard used was DPPH radical-scavenging activity (%) =Abs Control-Abs sample / Abs control x100.

Antimicrobial activity of OPE and chitosan nanoparticles in cooperated with OPE

It was analyzed using the agar disk diffusion method. Escherichia coli (E. coli), Salmonella cyphimurium as a model for Gram-negative bacteria; Staphylococcus aureus (S. aureus), Bacillus ceries as a model for Gram- positive bacteria and Candida albicans (C. albicans) as a model for yeast, were used. A negative (distilled water). After incubation at 37 °C for 24-48 hours, clear zone diameter (in mm) (Gullon et al., 2016).

Statistical analysis: The obtained data were exposed analysis of variance. Duncan's Multiple range tests at ($p \leq 0.05$) level was used to compare between means. The analysis was carried out using the PRO-ANOVA procedure of Statistical Analysis System SAS. Duncan's Multiple range tests at ($p \leq 0.05$) level was used to compare between means. The analysis was carried out using the PRO-ANOVA procedure of Statistical Analysis System SAS.

Evaluation of the antioxidant activity of the extract and Chitosan nanoparticles Loaded with OPE

Using DPPH radical scavenging assay: DPPH (2,2-Diphenyl-1-picrylhydrazyl) is a stable organic free radical that has been employed to study free radical activities and thus antioxidant activity in a variety of natural products. It was determined using the method of (Hazim et al.,2020). DPPH radical-scavenging activity (%) =Abs Control-Abs sample / Abs control x100.

Antimicrobial activity of OPE and chitosan nanoparticles in cooperated with OPE

It was analyzed using the agar disk diffusion method. Escherichia coli (E. coli), Salmonella cyphimurium as a model for Gram-negative bacteria; Staphylococcus aureus (S. aureus), Bacillus ceries as a model for Gram- positive bacteria and Candida albicans (C. albicans) as a model for yeast, were used. A negative (distilled water). After incubation at 37 °C for 24-48 hours, clear zone diameter (in mm). (Gullon et al., 2016).

Statistical analysis: The obtained data were exposed analysis of variance. Duncan's Multiple range tests at ($p \leq 0.05$) level was used to compare between means. The analysis was carried out using the PRO-ANOVA procedure of Statistical Analysis System SAS.

3. Results and Discussion

Chitosan was used as a coating agent, and orange peel extract was incorporated into it at a ratio of 2:1 (v/v). The proportion was chosen since it was the best one for the sample's appearance and its resistance to sedimentation. OPE loading at chitosan nanoparticles had a mean diameter of 105.7 nm with a narrow size distribution (Intercept: 0.926 d. nm; poly dispersity index: 0.518). The size distribution profile of the for nanosuspensions was given in Table 1 and Figure 1. These observations revealed that the chitosan nanoparticles we generated here had good OPE loading.

Table 1. Effect of OPE loading at chitosan on average diameter and poly disparity index (PDI).

Chitosan: OPE 2:1(v/v)	ZAverage nanometer (d. nm)	poly disparity index (PdI)	Intercept (d. nm)	Size (d. nm)	% Number	StDev (d. nm)	Result quality
	438.4	0.518	0.926	105.7	100	1.32	Good

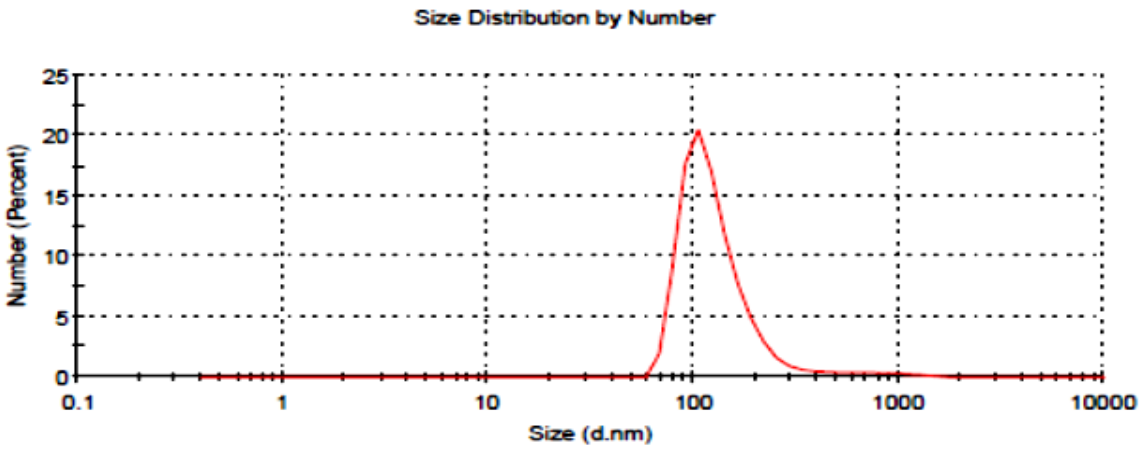

Figure 1. Particle size of chitosan nanoparticles incorporated with orange peel extract

Zeta potential is a crucial characteristic parameter, as indicated in Table 2 and Figure 2, the OPE loading at chitosan nanoparticles exhibited a zeta potential of 19.7 mV. Zeta potential (ZP) values > 30 mV correspond to high stability, ZP of around 20 mV indicates a moderate or short-term stability, and ZP values around 5 mV would result in low stability, and therefore a fast aggregation of NPs (Muller et al., 2001) for a physically moderately stable nanosuspension solely stabilised by electrostatic repulsion. All these findings indicated that the OPE loading at the locally produced chitosan nanoparticles was steady.

Table 2. Effect of OPE loading at chitosan on zeta potential (ZP).

Chitosan: OPE (v/v) 2:1	Zeta potential (mv)	Zeta deviation (mv)	Conductivity (mS/cm)	Mean (mV)	Area (%)	St Dev (mv)
	19.7	3.78	1.71	105.7	100	3.78

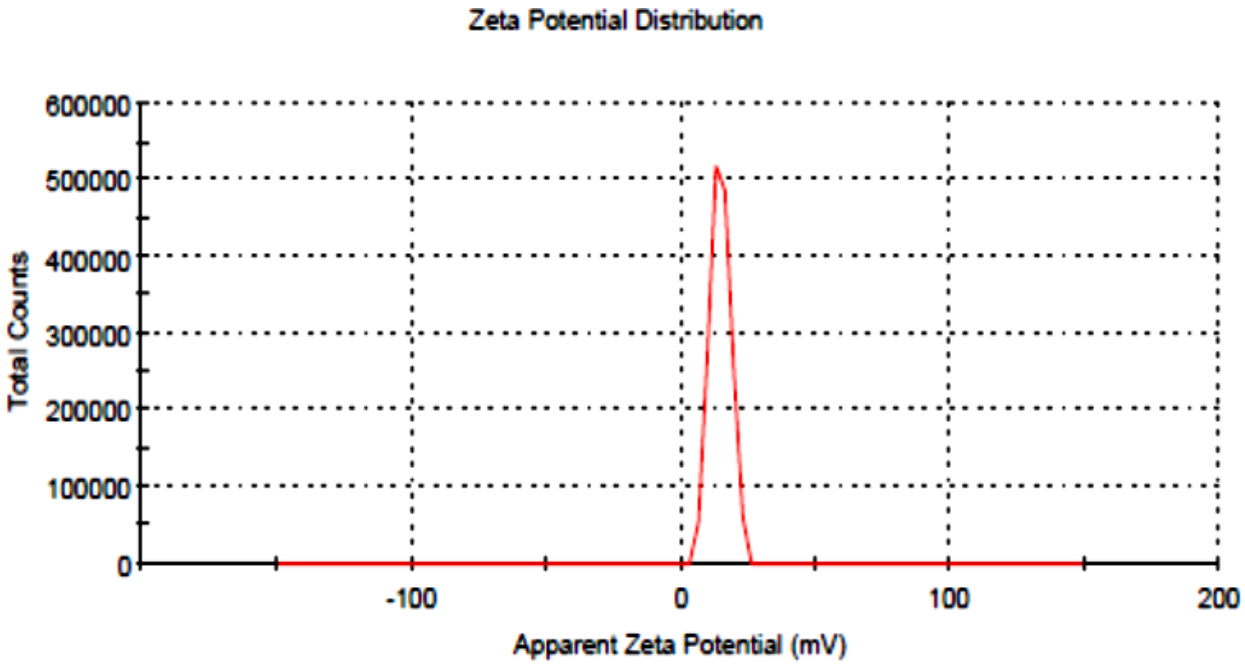

Figure 2. Zeta potential distribution of chitosan nanoparticles loading of orange peel extract.

To identify the chemical groups that are contained in the orange peel extract and chitosan nanoparticles loading FTIR spectra, which span from 4000 cm-1 to 500 cm-1, were depicted in Figure 3. Orange peel extract (OPE) and chitosan nanoparticles loading (Arockianathan et al., 2012) spectra revealed a broad band between 3500 cm-1 and 3100 cm-1 allocated to O-H stretching (hydroxyl groups) in this finding. The peak at 2992 cm-1 wave number was identified as an alkene's -C-H- stretching vibration. The C=O stretching present in hemicelluloses (Arthanarieswaran et al., 2015) and -C=C- stretching, respectively, were indicated by the absorption bands at 1741 cm-1 and 1622 cm^{-1}.

The stretching of the C=C ring (Aromatic group) is what causes the 1521 cm 1. Due to increased carbonyl stretching in lignin, a peak of 1438 cm-1 was found (Li et al., 2019).Ashok et al. (2018) and (Arockianathan et al., 2012) both confirmed the presence of glucosidic connections between hemicelluloses and cellulose sugar units in the finger print region of the bands at 1016 cm-1 and 838 cm-1, respectively. In cellulose, the C-OH bending was seen to peak at 598 cm-1 (Santhanam et al., 2016; Rahman et al., 2015).

From the Figure 3. it is conspicuous that there is slight shift in the peaks of the chitosan nanoparticles CSNPs loading of orange peel extract as compared

to orange peel extract (OPE) such as 3331.91 to 3331.05 cm^{-1}, 1640.92 to 1640.86 cm^{-1}, 732.72 to 732.39 cm-1. likewise, the intensity of those peaks slight decrease in the chitosan nanoparticles loading of orange peel extract and it clear that the minor shift in peaks does not affect the functional groups present in the OPE during loading of chitosan nanoparticles of orange peel extract.

As a result of the O-H bond in phenolic, flavonoids, and organic acids being stretched, the data in Figure 3 also indicate the band at 3331.91 and 3331.05 cm-1. At 1640.92 and 1640.86 cm 1, there are two noticeable bands that are caused by the aromatic C=C stretching vibration and the carbonyl group's C=O stretching. Due to the presence of glycosidic connections between cellulose sugar groups and hemicelluloses, a band may be seen from 732.72 to 732.39.

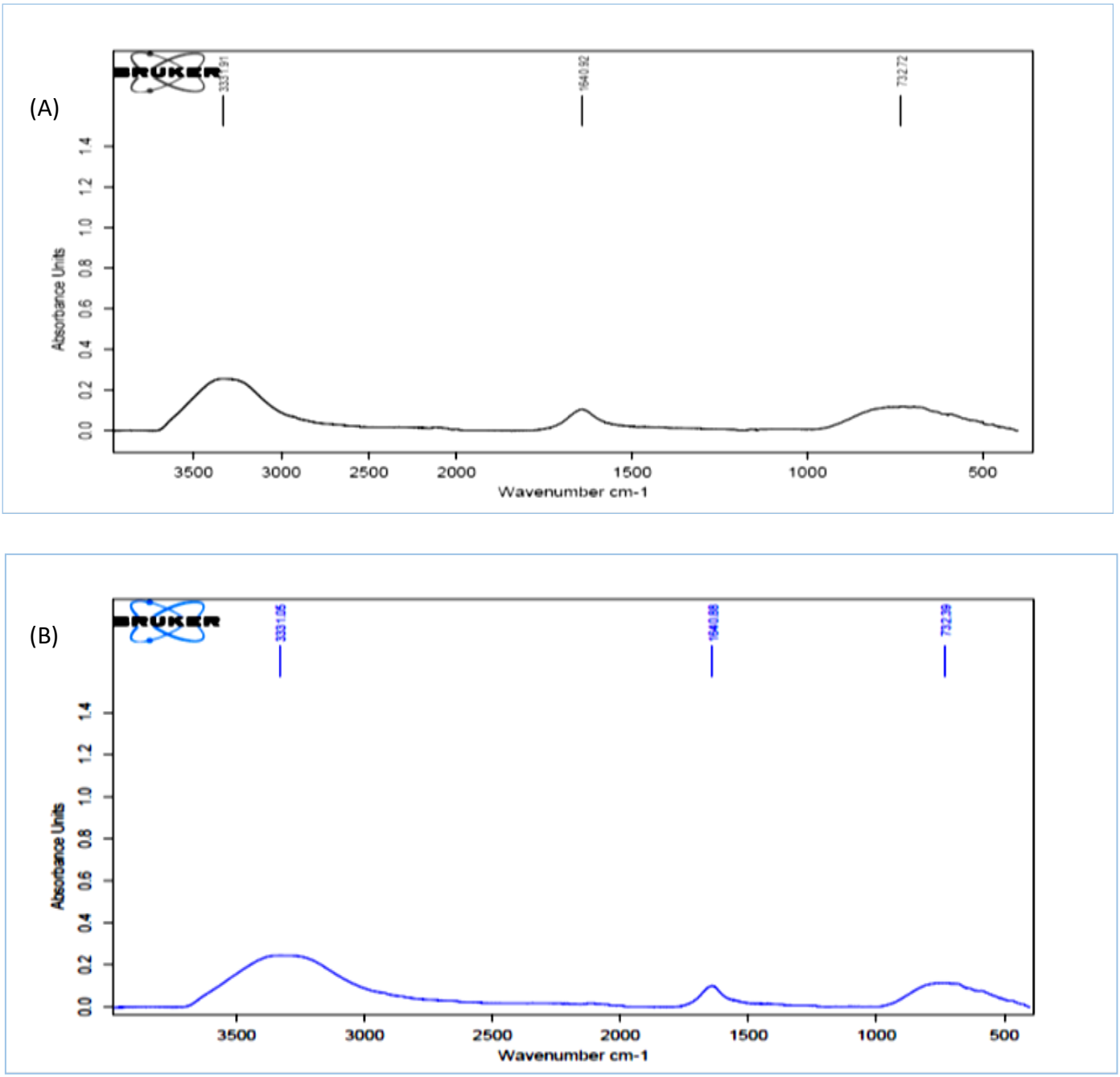

Figure 3. FT-IR spectra of (A) orange peel extract (B) chitosan nanoparticles loading of orange peel extract.

Table 3. Quantification of total phenols and flavonoids in OPE and Chitosan nanoparticales (CSNPs) loading of orange peel extract.

Samples	Total Phenols mg /g	Flavonoids mg /g
OPE	36.9 ± 1.15^{A}	12.47 ± 0.41^{A}
OPE loaded with CSNPs	27.66 ± 0.53^{B}	2.21 ± 0.15^{B}

Values were performed in triplicates and represented as mean ±SD.Mean values followed by different superscript in a column are significantly different (P<0.05).

Data presented in Table 3. showed the concentration of total phenols referring to gallic acid and flavonoids referring to quercetin as an antioxidant model. The results in Table 3 indicated that compounds in orange peel extract had significantly higher contents of total phenols. In addition, it was observed that the same behavior occurred in the quantification of the flavonoids followed by chitosan nanoparticles (CSNPs) loading of orange peel extract: 36.9, 27.66, and 12.47, 2.21 mg/g, respectively. The current study was also in agreement with the results of (Pal et al., 2017), who showed the total phenolic contents in orange peels were 35.73 mg/g dw, and the results were also in agreement with (Rabab et al., 2021), who reported that the total flavonoids in orange peels were 12.84 mg/g dry weight (dw).

Table 4. Antioxidant activity in OPE and Chitosan nanoparticles (CSNPs) loading of orange peel extract.

Samples	DPPH %
OPE	86.3 ± 0.3^{A}
OPE loaded with CSNPs	47.9 ± 0.5^{B}

Values were performed in triplicates and represented as mean ±SD. Mean values followed by different superscript in a column are significantly different (P<0.05).

The DPPH is the stable free radical. This assay has been widely used to evaluate the free radical scavenging ability of various plant extracts. As shown in Table 4. the DPPH radical scavenging activity of both of samples (orange peel extract and Chitosan nanoparticles (CSNPs) loading of orange peel extract) showed at different concentration was 87.3% in OPE while OPE loaded with CSNPs showed at the concentration was 47.9%. The current study also agreed with the result of (Pal et al., 2017) who reported the orange peel extract showed the radical scavenging activity was (OPE) 86.3 %.

Chitosan, owing to its general recognition as safe (GRAS), has some advantages, such as nontoxicity, biocompatibility, and antimicrobial properties (Keawchaoon and Yoksan, ,2011), which make it suitable for in vivo use in biomedical treatments (Dehghani et al., 2019). From the results of Table 5, OPE-loaded with CSNPs showed higher inhibitory antimicrobial activity as compared to pure orange peel extract (OPE) against, Escherichia coli (E. coli) , Salmonella cyphimurium as a model for Gram-negative bacteria; Staphylococcus aureus (S. aureus), Bacillus ceries as a model for Gram- positive bacteria and Candida albicans (C. albicans) as a model for yeast were 11.4, 11.3, 12.8, 11.9 and 11.8 (mm) , respectively, compared to OPE were 0.16, 0.00, 0.34, 0.20, and 0.10 This may indicate that the encapsulation of OPE inside CSNPs successfully limits the leakage of OPE from NPs during the time interval between the preparation and the antimicrobial assay.

Table 5. Antibacterial activity of OPE and Chitosan nanoparticles (CSNPs) loading of orange peel extract.

Samples	Diameter of inhibition zone (mm)				
	E. coli	Salmonella Cyphimurium	Staphylococcus aureus	Bacillus ceries	Candida albicans
OPE	0.16	0.00	0.34	0.20	0.10
OPE loaded with CSNPs	11.4	11.3	12.8	11.9	11.8

4. CONCLUSIONS

Results of this present study showed that a combination of the orange peel extract (OPE) with chitosan nanoparticles $CSNP_S$ (1:2) discovered that it keeps functional active aggregates present in orange peel extract. Moreover, this loading of orange peel extract with nano chitosan were significantly inhibited of Gram-negative and positive bacteria and yeast effect compared with orange peel extract only.

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