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Green Synthesis of Chitosan Nanoparticles Using *Cinnamomum Cassia* L. Hexane Bark Extract and Its Antibacterial Activity Against MDR *Klebsiella Pneumoniae*

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Abstract

The biosynthesis of nanoparticle is suggested as an eco-friendly and economical alternative to both physical and chemical approaches. This study sought to assess the antibacterial with cytotoxic effects of *Cinnamomum cassia* L. oil extract (COE) and cinnamon oil extract/chitosan nanoparticles (COE/ChNPs) against MDR *Klebsiella Pneumoniae* and Mouse embryonic fibroblast cell line. Cinnamon active components such as alkaloids, glycosides, phenols, steroids, terpenes, saponins, tannins, and flavonoids were detected in hexane extract. Green synthesis of COE/ChNPs was achieved using the sol-gel technique, resulting in uniform, spherical, crystalline nanoparticles ranging from 40 to 96 nm. COE/ChNPs were characterized using UV-visible spectrophotometry, Transmission Electron Microscopic (TEM), and X-ray Diffraction (XRD) analyses. Results of the antibacterial activity of COE/ChNPs showed a significant effect against 12 MDR *K. pneumoniae* isolates from urinary tract infections compared with COE. The minimal inhibitory concentrations (MIC) of COE/ChNPs and COE were ranged from 0.18 to 0.71 µg/ml, and 750 to 2810µg/ml, respectively. The *in vitro* cytotoxic effect of biologically synthesized COE/ChNPs against mouse embryonic fibroblast cell lines was assessed. Results showed that there is a cytotoxic effect of COE/ChNPs inhibition of embryonic fibroblast cell lines growth in a concentration-dependent manner as determined using MTT assay.

Key words: *Cinnamomum cassia* , Chitosan nanoparticles, *Klebsiella Pneumoniae*

البناء الأخضر لدقائق الكيتوسان النانوية باستخدام مستخلص الهكسان لقلف الدارسين وفعاليته ضد بكتريا كلبسيلا الرئوية المقاومة للمضادات الحياتية.

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الخلاصه

يعد البناء الحيوي للجسيمات النانوية صديقا للبيئة وبديل فعّال من حيث التكلفة للطرائق الكيميائية والفيزيائية المختلفة. هدفت الدراسة الحالية الى تقييم التأثير المضاد للبكتيريا والسام خلويا لمستخلص الهكسان الزيتي للقرفة (COE) ودقائق الكايتوسان النانوية/مستخلص زيت القرفة (COE/ChNPs) ضد بكتيريا *Klebsiella pneumoniae* ذات المقاومة المتعددة لمضادات الحياة, وخط خلايا الفأر الليفية الجنينية (Mouse embryonic fibroblast cell line). تم الكشف عن وجود المركبات الفعالة في مستخلص زيت القرفة وكانت كل من القلويدات والجليكوسيدات والفينولات والستيرويدات والتربين والسابونين والعفص والفلافونويد. أجريت عملية البناء الأخضر لدقائق COE/ChNPs باستخدام تقنية السول–جل، وقد كانت الجسيمات النانوية الناتجة موحدة ومنتظمة وكروية وبلورية تتراوح أطوالها من 40 إلى 96 نانومتر. تم تحديد خصائص جسيمات COE/ChNPs باستخدام مطياف الأشعة فوق البنفسجية والمرئية، والمجهر الإلكتروني النافذ (TEM) ، وتحليل حيود الأشعة السينية (XRD) , أظهرت نتائج أن للجسيمات النانوية تأثيرا معنويا ضد 12 عزلة من بكتريا *K. pneumoniae* ذات المقاومة المتعددة المعزولة من التهابات المسالك البولية مقارنةً بمستخلص الهكسان الزيتي للقرفة, اذ تراوحت التركيزات المثبطة الدنيا (MIC) لكل من COE/ChNPs و COE بين 0.18 إلى 0.71 ميكروجرام/مل، و750 إلى 2810 ميكروجرام/مل على التوالي. تم تقييم التأثير السام خلوياً COE/ChNPs المصنع بايولوجيًا ضد خط الخلايا الليفية الجنينية للفأر, وقد اظهرت النتائج أن للجسيمات النانوية COE/ChNPs لها تأثيرا ساما للخط الخلايا الليفية الجنينية و معتمدة على التركيز من خلال اختبار MTT .

Introduction

Cinnamomum cassia (*C. cassia*) is a tropical medicinal and aromatic plant from the Lauraceae family, commonly employed as a natural spice in culinary uses and traditional medicine for respiratory and digestive disorders [1,2]. *C. cassia* has a longstanding history in traditional holistic medicine due to its therapeutic characteristics, serving as antioxidants, antimicrobial, anti-inflammation, as well as antitumor agents. C. cassia contains bioactive compounds, including cinnamaldehyde, cinnamic alcohol, cinnamic acid, and cinnamate, which exhibit antimicrobial effects, in addition to pharmacologically significant phytochemicals including flavonoids, glycosides, phenols, and tannins [1,4]. Extended chemotherapy application leads to drug resistance in bacterial pathogens; additionally, the absence of novel medication research and formulations may exacerbate the difficulty of controlling bacterial infections [5]. Plant-mediated nanoparticle synthesis offers numerous advantages, including the generation of more durable and sensitive nanoparticles for biological applications [6]. Exploring novel plant-derived active chemicals may serve as an excellent alternative. Furthermore, the phytochemicals in the plant extract may facilitate the formation of therapeutic nanoparticles with medicinal properties [7]. Nanoparticles (NPs), owing to their dimensions, occupy a pivotal role in nanotechnology as a range of solutions in medical. Nanoparticles enhance the capacity of active compounds to permeate many pathways: transdermal, as well as gastrointestinal (by active endocytosis), respiratory, and injectable routes [8]. In several applications, chitosan has been shown to be the ideal material for creating nanoparticles [9]. Compared to other forms, organic chitosan-based nanoparticles (ChsNPs) have a number of beneficial qualities, including nontoxicity, biocompatibility, high permeability, biodegradability, and environmental friendliness. The matrix of polymer nanoparticles typically comprises natural polymers (such as chitosan, gelatin, and alginate), synthetic polymers (including PLA, PCL, PLGA, and cyclodextrin), and their combinations [10]. The aim of this study was to investigate the antibacterial effectiveness of cinnamon oil/chitosan nanoparticles on multidrug- resistant *Klebsiella pneumoniae* in addition to their anticytotoxic effects in *vitro*.

Materials and Methods

Plant material and extract preparation.

The bark of cinnamon (*C. cassia*) was obtained from local markets and classified by Dr. Sukaina Abbas Ealaywi / Department of Biology, College of Science/University of Baghdad. According to the plant classification certificate No. 1181 on 1/6/2023. Subsequently, the material was processed through cleaning, drying, and grinding to obtain the bark powder. A Soxhlet apparatus was employed to make the plant extract using one liter of n-hexane and 100 grams of powdered cinnamon bark. After the extraction, the mixture was filtrated using Whatman filter paper No.1. The filtrate was subsequently dried at 4 °C for analysis and screening of phytochemical constituents. The filtrate was evaporated at decreased pressure using a rotary evaporator [11]. The subsequent formula was employed to calculate the percent yield of each extract:

Yield% =Weight of the dry extract x 100/ Weight of the dry plant

Plant extracts were then dissolved in 1% (v/v) dimethylsulphoxide (DMSO) for further analysis.

Detection of active compounds in cinnamon oil extract

Phytochemicals (alkaloids, glycosides, phenols, steroids, terpenes, saponins, tannins, and flavonoids) were detected in the cinnamon oil extract- COE using standard methods [12].

Green Synthesis of Cinnamon Oil /Chitosan Nanoparticles (COE/CHNPs)

Ten milliliters of 1% organic chitosan (Sigma Chemicals) dissolved in acetic acid (v/v) were combined with 10 milliliters of cinnamon hexane extract (CHE) and incubating about 60 minutes at 50 °C in an orbital shaker rotating at 180-200 rpm. The reaction mixture was then centrifuged at 11,000 rpm for 20 minutes. The supernatant was removed after centrifugation of the solution, and the pellets were rinsed with acetic acid to remove the unreacted part. The COE/ChNPs underwent freeze-drying after additional centrifugation [13].

Characterization of Cinnamon Oil /Chitosan Nanoparticles (COE/ChNPs)

The characterization of COE/ChNPs by examining their morphology and structure using the following techniques:

1- *Fourier Transform Infrared Spectroscopy Analysis*

The FT-IR was utilized to analyze the functional groups on the surface of COE/ChNPs, with spectra recorded at a resolution of 4 cm-1 over the range of 4000 to 400 cm-1. Samples were produced by putting COE/ChNPs on a microscope slide, subsequently forming discs with potassium bromide (KBr) at a ratio of 1:99 (samples:KBr). The specimen was later analyzed[14].

2- *Atomic Force Microscope (AFM)*

Under typical air circumstances, the surface morphology of the COE/ChNPs was analyzed using an Atomic Force Microscopic (AFM) in Contact mode. Nanoparticle solution droplets were deposited on a 1x2 cm glass slide and permitted to dry. Subsequently, the slide was positioned on the AFM sample stage, and analysis was conducted according to normal methodology [15].

3- *Scanning Electron Microscopy (SEM) Analysis*

The shape of COE/ChNPs was examined using a Brucker scanning electron microscope (SEM) to determine their morphology. Seven drops of CHE-CsNPs were spread on a glass slide and examined using a conventional procedure [16].

4- *Energy Dispersive X-ray (EDX)*

The EDX Oxford instruments INCA 350 with Si detector 10 mm2 areas and resolution of Mn 133eV was used to achieve EDX analysis [17].

5- *X-Ray Diffraction*

For XRD examination, the filtrate of COE/ChNPs was put on a glass grid with silicon substrate to investigate the structural characterization of COE/ChNPs in order to gather data

on surface morphology, crystal structure, and particle dimensions. The pattern of CS and CSNPs loaded SAE samples was analyzed using an X-ray diffractometer with Cu-Kα radiation as the anode, operating at a wavelength of 0.154060 nm, and a detector set at 40 kV and 30 mA. The XRD pattern was seen in the 2θ range of 10° to 80° at ambient temperature using a fixed time mode. [18].

Antibacterial activity of cinnamon oil extracts against MDR K. pneumoniae isolates.

The antibacterial activity of different concentrations of COE against *K. pneumoniae* isolates obtained by a previous study conducted by Ma *et al*. [19]. The antibacterial activity was performed using agar well diffusion susceptibility method [20]. Briefly, nutrient agar plates were inoculated with fresh cultures of bacterial isolates. Five wells (6 mm diameter) were done by sterilized cork borer into agar media and adding 100 µl of the extracts in a concentration (100, 50, 25, and 12.5) mg/ ml in triplicates. 100 µl DMSO was added into positive control wells. Then inoculated plates were incubated for 24 h at 37Cº. After the incubation period, inhibition zones diameters in mm were measured.

Antibacterial activity of Chitosan/cinnamon oil extracts nanoparticles

The resazurin microtiter assay method was used to investigate the MICs for the antibacterial activity of serial dilution COE concentrations (50, 25, 12 5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195 and 0.0975) mg/ ml. While the COE/ChNPs concentrations (45, 22.5, 11.25, 5.625, 2.813, 1.41, 0.71, 0.36, 0.18 and 0.09) µg/ ml against 12 MDR *K. pneumoniae* isolates [21]. The MIC was established as the lowest concentration at which the color turned blue. Three duplicates of each concentration were utilized.

Evaluation of cellular viability

A mouse embryonic fibroblast cell line was used to evaluate the anticytoxicity of COE/CHNPs. The mouse embryonic fibroblast cell line (passage 25) was kindly provided by (Biotechnology Research center). Mouse embryonic fibroblast cells were grown in DMEM supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin at 37°C in a 5% CO2 atmosphere [22]. The MTT assay was utilized to evaluate the relative safety of COE/CHNPs. In this experiment, viable cells transform the yellow, water-soluble tetrazolium salt into an insoluble purple formazan precipitate via the action of succinate dehydrogenase, an enzyme of the mitochondrial respiratory chain that functions solely in viable cells. Cells were cultivated in a 96-well microplate and subjected to different concentrations of methanol (187–1500 µg/mL) and water (4–7 mg/mL) tea extracts. A 1% concentration of DMSO was employed as the solvent control. Untreated cells or those exposed to phosphate-buffered saline (PBS) served as negative growth controls. After a 24-hour exposure, 25 µL of 5 mg/mL MTT solution was added into every well and incubated for an additional three hours. The supernatants were then discarded, and 100 µL of DMSO was added to each well, followed by shaking on an orbital shaker for 5 minutes at 150 rpm. The optical density was assessed at 590 nm, using a reference wavelength of 670 nm, with a microplate spectrophotometers. The concentration of the material which inhibits 50% from the activity of the enzyme (IC50 value) was ascertained utilizing the subsequent formula [22]:

$$\text{Inhibition } (\%) = 100 - \frac{\text{corrected mean OD sample} * 100)}{\text{corrected mean OD solvent control}}$$

Statistical Analysis

Values are expressed as mean ± SEM and analyzed utilizing 2-way ANOVA, followed by the least significance difference (LSD) test with GraphPad Prism; a chi-square test was employed to compare percentages. All extracts were evaluated against the standards.
$P < 0.05$ is deemed significant, while $P < 0.01$ is regarded as extremely significant[23].

Results and Discussion

Extraction Yield of Cinnamomum cassia

The extraction of phytochemicals from *C. cassia* bark using hexane showed a good yield (1.7%) from cinnamon bark.

Active compounds of cinnamon bark oil extract

Active compounds screening the oil extraction of *C. cassia* bark to determine the presence of the active compounds, the saponins and terpenoids showed higher percent than other groups, as shown in Table 1.

Table 1: Active compounds screening of hexane extraction of *C. cassia* bark

Active Component	n-Hexane extract of bark
Tannins	+
Resins	+
Coumarins	+
Saponins	++
Alkaloids	+
Phenols	+
Terpenoids	++

(+): low amount after added the reagent 10 min; (++): high amount immediately after added the reagent

"Active groups" are secondary metabolites that are crucial to plants' defense against insects, bacteria, and other living organisms. Active organizations have also benefited humans in a variety of fields, such as food and medicine. The existence of each active constituent in the oily extract of C. cassia extracts was demonstrated by the study's active groups in the cinnamon bark.

Several medicinal herbs and plants have active compounds; the bulk of these chemicals, in good proportions, are what give aromatic and medicinal plants their biological action, according to the results of the active compound identification in the *C. cassia* oil extract. The role of these compounds suggests that it has good medicinal capabilities. Different types of secondary metabolites have at least one or more possible explanations for the antimicrobial and antibacterial effects of the plant extracts, which is the presence of these metabolites [24].

Activity of cinnamon oil extract against MDR K. pneumonia.

The antibacterial activity of cinnamon oil extract in different concentrations against MDR *K. pneumoniae* isolates was determined using well diffusion agar. The antibacterial assays showed that the oil extract in the (10, 5, and 2.5) % concentrations was able to inhibit the growth of all MDR *K. pneumoniae* isolates under study, whereas the 1.25% concentration inhibits half of the Kp2, Kp6, and Kp11 isolates (10 mm inhibition zone) was indicated in Table 2. The inhibition zone measures more than 8 mm, which means the bacteria were sensitive to the extract. These findings are similar to those obtained by Erfan and Marouf [25], who found that the inhibition zones of more than 8 mm indicate that MDR *K. pneumoniae* isolates were sensitive to the antibiotics.

Table 2: Inhibitory effect of *Cinnamon cassia* oil extract against MDR *K. pneumoniae* isolates.

Isolate	oil extract				LSD value
	Means of inhibition zone (mm)				
	10%	5%	2.5%	1.25%	
Kp1	30	20	15	13	1.29*
Kp2	23	20	17	10	1.24*
Kp3	25	18	14	0	1.42*
Kp4	20	15	13	0	1.33*
Kp5	25	18	15	0	1.47*
Kp6	30	20	15	10	1.41*
Kp7	26	17	10	0	1.38*
Kp8	25	20	15	0	1.35*
Kp9	30	20	15	15	1.26*
Kp10	27	15	15	0	1.40*
Kp11	25	20	15	10	1.35*
Kp12	26	18	15	0	1.39*
LSD Value	1.14 NS	1.05 NS	0.83 NS	1.33*	

*: Significant (P≤0.05); Results are in triplicate.

Cinnamon oil extract/Chitosan nanoparticles Synthesis

Cinnamon oil bark extract showed a significant potential for producing chitosan nanoparticles (ChsNPs). During preparation, the molecular structure of chitosan will be modified, leading to alterations in solubility in an acidic solution, as well as the formation of a gel-like or liquid state [26].

Fourier transformation infrared spectroscopy (FTIR)

Figure 1 describes the morphological appearance and size distribution pattern of COE/CHNPs. There are numerous differences in the peaks between the spectra of COE/ChNPs compared with COE; on the other hand, the prepared nanoparticles match the extracted cinnamon extract's spectrum, indicating a high degree of success in extract preparation and encapsulation. Although the primary peaks' intensity was lower for COE/ChNPs than for ChsNPs, this was because TPP cross-linking reduced the intensity of hydrogen bonding. This suggests that the big molecules were divided into smaller ones and that the bond's surface area and area both increase when Cs binds to TPP, as shown in Figure 1. These results are consistent with Zhou *et al*. [27]. The findings indicated that the chemical groups of displaced locations demonstrated the effective loading of the extract in the chitosan/TPP matrix and the creation of nanoencapsulation of the extract. Supplementary evidence indicates that the displaced peaks correspond to the formation of a novel chemical [28,29].Alterations in the functional groups of active biomolecules suggest a relationship to the synthesis of COE/CHNPs.

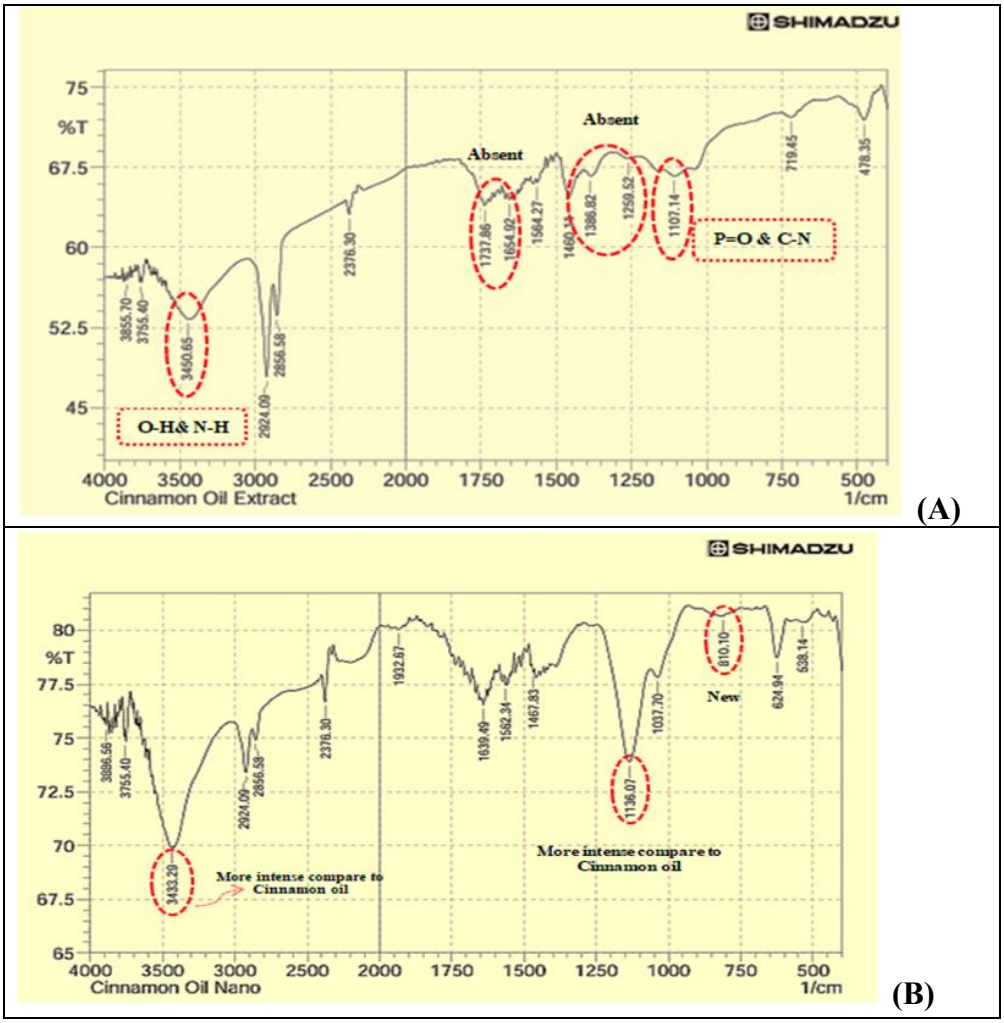

Figure 1: FTIR analysis diagrams of *Cinnamomum cassia* oil extract (A) and *Cinnamomum cassia* oil extract /chitosan nanoparticles (B).

X- Ray diffraction (XRD)

The pattern of COE/ChNPs exhibits attenuated peaks, indicating a reduction in intensity. The X-ray diffraction peaks arise from the reflection of X-rays by crystal planes. Figure 2 displays the X-ray diffraction patterns of natural chitosan and COE/CHNPs. The XRD pattern of chitosan nanoparticles (ChsNPs) exhibits two distinct peaks at 2θ= 10º and 20º, which suggest the presence of an organized crystalline arrangement in the chitosan material. Nevertheless, the intensity of these peaks diminishes in our investigation as a result of the emergence of additional peaks, which were observed at 2θ values of 18.58º and 22.57º. These findings are similar to those obtained by Shaghati [30], who mentioned that the values of 2θ were 18.7°, 16.4°, 18.41°, and 22.3°.

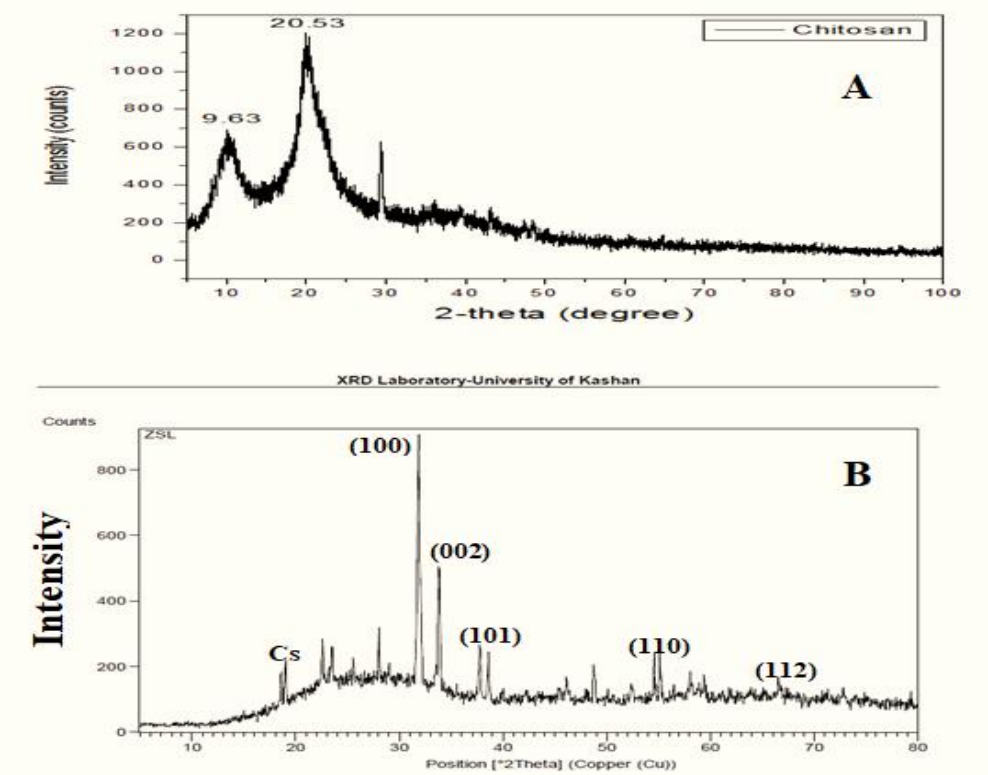

Figure 2: Diffractogram of *Cinnamomum cassia* oil extracts (A), and *Cinnamomum cassia* oil extract /chitosan nanoparticles (B).

Scanning Electron Microscope of Cinnamomum cassia oil extract / chitosan nanoparticles.

The morphology of COE/ChNPs was examined utilizing SEM. They had a smother spherical appearance with a diameter of (33.49– 76.05) nm, and their conformation with the results of a transmission electron microscope was referred to as lower than 100 nm (Figure 3). They also had a generally homogenous morphology. The unintended aggregation that takes place during the drying process could be the cause of the bigger particles. As soon as the liquid evaporates, agglomeration occurs, increasing the particle concentration. Agglomeration may be aided by the decrease in the electrostatic repulsive force brought on by the rise in dissolved ion concentration brought on by liquid evaporation [30,31].

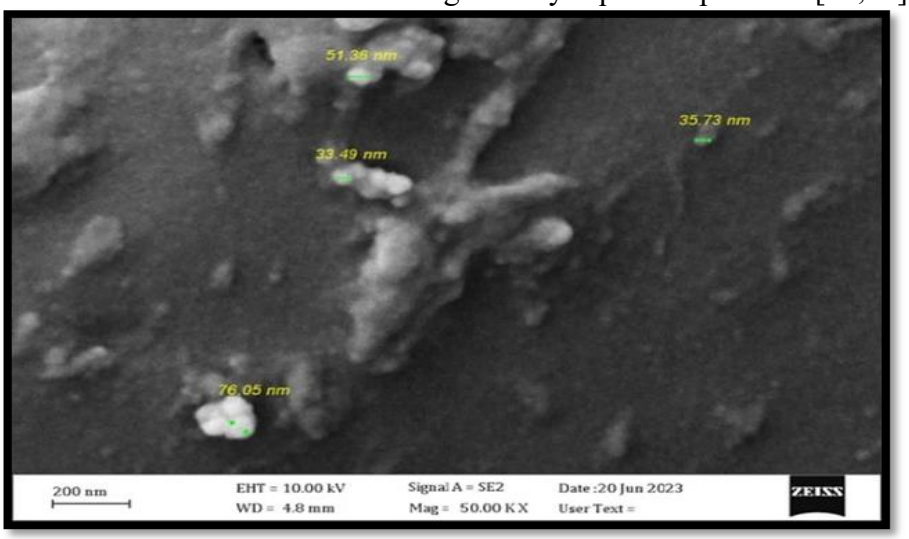

Figure 3: Scanning Electron Microscopy image of *Cinnamomum cassia* oil extract /chitosan nanoparticles.

Energy Dispersive X-ray (EDX).

The elemental analysis of COE/ChNPs by EDX demonstrates that C, O, and N are present in Table 3. The presence of chitosan is confirmed by EDX analysis, which finds a substantial signal at (1.5 and 2.3) keV owing to the presence of carbon and oxygen, respectively. The existence of COE components is the cause of the other elements, such as N. These findings are similar to those obtained by Yang *et al*.[32], who previously prepared ChsNPs, and examined their characteristics utilizing SEM outfitted with EDX. Furthermore, the elements present in the EDX spectra of Cs10 include C, O, N, and Br. ChsNPs contain the elements C, O, N, P, and Br as well [33].

Table 3: Elemental analysis of *Cinnamomum cassia* oil extract /chitosan nanoparticles using Energy Dispersive X-ray

No.	Element	Wt. (%)
1	C	73.3
2	O	24.5
3	N	2.3

Atomic Force Microscopy (AFM).

The three dimensions imaged by atomic force microscopy photographs showed a population of uniform COE/CHNPs with a normal surface shape, and the highest frequency of particles varied in size from 18.62 to 60.0 nm, as shown in Figure 4. AFM images show intelligent interactions between COE/ChNPs that result in the formation of well-defined aggregates, which were calculated. Raesi *et al*. [34] verified these findings, that the morphologically mediated chitosan produced uniform particles with an average size of 40–96 nm.

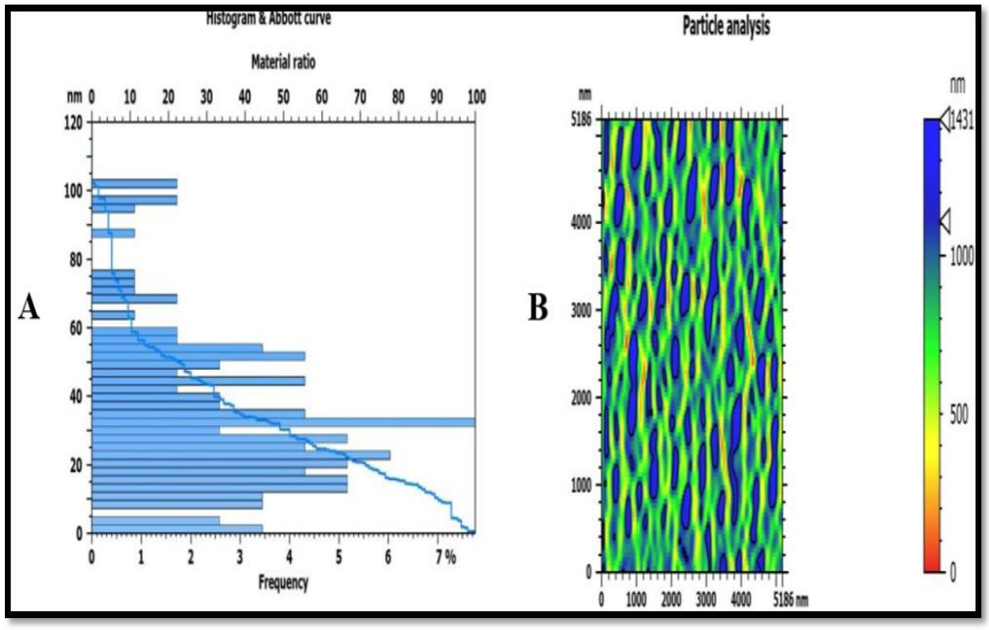

Figure 4 : Distribution of *Cinnamomum cassia* oil extract /chitosan nanoparticles according to particles size (A): Abbott-Firestone curve ; (B): particle analysis.

Antibacterial activity of Cinnamomum cassia oil extracts and Cinnamomum cassia oil extract/chitosan nanoparticles

The antibacterial activity of COE, and COE/ChNPs against MDR *K. pneumoniae* isolates was examined. The MIC range of COE was 780-2810 µg/ml, and sub-MIC was 390-1600

µg/ml, while the MIC range of COE/ChNPs was 0.81-0.71 µg/ ml and the sub-MIC range was 0.18- 0.71 µg/ml respectively, against *K. pneumoniae* isolates as shown in Table 4.

The disparities in the effectiveness of antibacterials against *K. pneumoniae* clinical isolates can be ascribed to multiple factors, including discrepancies in extraction methodologies, environmental influences (such as soil, temperatures, humidity, and climate), physiological variations of the plant (including evolutionary cycle, phase of growth, and stress conditions), and genetic determinants. Nonetheless, the results demonstrated that the hexane extract of C. cassia includes a small amount of pharmaceutically significant bioactive chemicals that have the potential to act as antibacterial agents. Similar outcomes with oil extract preventing the growth of clinical isolates of *K. pneumoniae* with various resistance profiles and MICs ranges [26]. The breakpoint concentrations for resistance to *K. pneumoniae* were 1.23 mg/ ml and 5.525 µg/ml for COE and COE/ChNPs, respectively. On the other hand, this result was agreed with Zhang *et al*. [35,] who found that the *Laurus nobilis* leaves water extract 50 mg/ ml- revealed a concentration of 50 mg/ml of high antibacterial activity against *K. pneumonia* . On the other hand, the results of Saleh *et al*. [36] who examined the effects of chitosan and alginate NPs loaded with Doxycycline Antibiotic Against MDR *Proteus Mirabilis, Escherichia coli and Enterococcus Faecalis* were increased comparison with antibiotic alone. Another study carried out by Kadhum and Zaidan [37] showed a synergistic effect between the silver NPs when loaded (biosynthesis) with antibiotics against MDR bacteria isolated were isolated from children wich diarrhea under 5 years performances than antibiotic alone. Tawfeeq *et al*.[38] referred that nano chitosan encapsulated (loaded) plant extract showed improving effectiveness in spite of the nano chitosan encapsulated plant extract showing enhanced efficacy despite the interaction with nano chitosan loaded NPK fertilizer. Additionally, the combination of nettle leaf and green tea extracts substantially impacted all vegetative development characteristics.

Table 4: Antibacterial activity of *Cinnamomum cassia* oil extracts and *Cinnamomum cassia* oil extract/chitosan nanoparticles against MDR *K. pneumoniae* isolates

Isolate No.	COE		COE/ChNPs	
	MIC	Sub- MIC	MIC	Sub- MIC
	µg/ ml		µg/ml	
4	1560	780	0.36	0.18
5	780	390	0.71	0.36
6	1560	780	0.71	0.36
7	1560	780	0.71	0.36
8	1560	780	1.41	0.71
43	1560	780	0.71	0.36
61	780	390	0.18	0.09
62	780	390	0.71	0.36
70	1560	780	0.71	0.36
71	1560	780	0.71	0.36
72	1560	780	0.36	0.18
73	2810	1600	0.71	0.36

The components of COE and COE/ChNPs primarily act on the cytoplasmic membrane. Tannin-rich plants exhibit antimicrobial properties as a result of their alkaline nature, which allows them to interact with polypeptides and create potent water-soluble compounds. Consequently, these compounds efficiently disrupt the cellular membranes of bacteria,

leading to their demise [39]. Flavonoids, a subclass of polyphenols, possess proven antibacterial and spasmolytic properties [40]. Plant alkaloids are commonly shown to possess antibacterial properties [41].

Evaluation of cellular viability

MTT assay was used to determine the cytotoxic effect of COE and COE/ChNPs on mouse embryonic fibroblast cell line passage 25 by determining cell viability percentage. Results indicated in Table 5 showed that the cytotoxic effect of COE/ChNPs was significantly ($P<0.05$) higher than the cytotoxic effect of COE on cell viability of the mouse embryonic fibroblast cell line. On the other hand, cell viability was increased significantly ($P<0.05$) with the increase of COE/ChNPs concentration as the percentage of cell death was increased to 5.7% and 33.9% after treatment of Mouse embryonic fibroblast cell line with COE/ChNPs at the concentrations of 12.5 and 25 µg/ml respectively. In contrast, COE has a less cytotoxic effect on cell viability of the mouse embryonic fibroblast cell line as the percentage of cell death reached 2.8%, 28%, and 46% when the cell line was incubated with COE at higher concentrations of 12.5, 25 and 50 mg/ml respectively. The data indicated that COE/ChNPs demonstrated dose-dependent cytotoxicity, suggesting that COE/ChNPs are promising nanoparticle with potential as treatments for cancer.

Table 5: Cytotoxic effect of *Cinnamomum cassia* oil extracts and *Cinnamomum cassia* oil extract/chitosan nanoparticles on Mouse embryonic fibroblast cell line

Concentration (µg/ml)	OD (670 nm)	Cell death (%)
COE/ChNPs		
25	0.140	33.9
12.5	0.200	5.7
6.25	0.262	0
COE		
50	0.113	46
25	0.153	28
12.5	0.206	2.8
Control	0.212	0
LSD value	---	4.811*

*: Significant ($P<0.05$)

Results obtained in this study were contrary to those reported by Willems *et al.* [42], as The MTT assay revealed non-significant variations in the viability of the treated cells (Cinnamon bark extract and its AgNPs) relative to the untreated control group.

Conclusion

The COE/ChNPs synthesized using green methods exhibited significant antibacterial activity against MDR *K. pneumoniae*. The antibacterial activity was found to be dependent on COE/ChNPs dosage. COE/ChNPs also had a higher cytotoxic effect than COE against mouse embryonic fibroblast cell lines, and the cytotoxicity effect of COE/ChNPs was dose-dependent. Remarkably, the results strongly indicated that the COE/ChNPs were highly promising candidates with medicinal value for future medical applications.

Conflicts of interest

There are no Conflicts of interest.

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