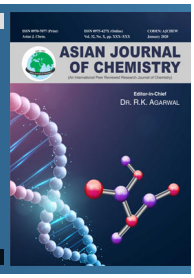
Asian Journal of Chemistry; Vol. 32, No. 7 (2020), 1660-1666

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22649



Green Synthesis and Evaluation of *Dimocarpus longan* Leaves Extract Based Chitosan Nanoparticles against Periodontitis Triggering Bacteria

N.K. Fuloria[1], M.Y. Ko[1], C.S. Rui[1], C.Z. Hang[1], S. Karupiah[1], N. Paliwal[1], U. Kumari[2], K. Gupta[3], K. Sathasivam[4] and S. Fuloria[1,*]

[1]Faculty of Pharmacy, AIMST University, Kedah 08100, Malaysia
[2]Faculty of Medicine, AIMST University, Kedah 08100, Malaysia
[3]Faculty of Dentistry, AIMST University, Kedah 08100, Malaysia
[4]Faculty of Applied Sciences, AIMST University, Kedah 08100, Malaysia

*Corresponding author: Fax: +60 44 298007; Tel: +60 14 3034057; E-mail: shivani2jaju@gmail.com

Received: 23 January 2020; *Accepted*: 26 March 2020; *Published online*: 27 June 2020; AJC-19929

Present study was intended to synthesize and evaluate the *Dimocarpus longan* leaves aqueous extract (DLLAE) based chitosan nanoparticles (CSNPs) against periodontitis triggering bacteria. In present study the DLLAE was prepared by extracting *D. Longan* leaves in distilled water using decoction method. The prepared DLLAE was subjected to phytochemical investigations, total phenolic content, total flavonoid content study and CSNPs synthesis. The CSNPs were characterized (using UV-visible, FT-IR, SEM, FESEM, XRD and EDX) and evaluated for their potential against periodontitis triggering bacteria (using agar well diffusion method). The phytochemical investigations over DLLAE exhibited presence of monosaccharides, reducing sugars, proteins, steroids, anthraquinones and coumarins glycosides, flavonoids, alkaloids, organic acid, tannic and phenolic compounds. The CSNPs were formed using DLLAE when the colour changed from white to pale brown. Absorbance of the CSNPs was in the desired range (201-310 nm). The optimization study revealed 1% acetic acid, 1% sodium alginate, 2 mg/mL chitosan, pH 5.8 and 1 mL DLLAE as the standard requirement for CSNPs synthesis using DLLAE. The CSNPs characterization data of FT-IR exhibited shifted bands. The FESEM revealed that the synthesized CSNPs were spherical in shape and their size ranged from 74.66 to 94.07 nm. The XRD data showed peaks at 2θ values of 22.28º, 25.19º, 34.72º, 40.0º, 71.16º and 73.0º. The average size for CSNPs was between 30.11-62.98 nm. The EDX spectrum revealed signal for carbon (76.17%), oxygen (22.14%) and sodium (1.53%) which confirmed formation of CSNPs. The antibacterial activity revealed high inhibitory potential of green CSNPs against *S. aureus*. Present studies establish that CSNPs biosynthesis using *D. longan* leaves aqueous extract is an efficient method and the biosynthesized CSNPs possess high antibacterial potential against *S. aureus* (bacteria that triggers periodontitis).

Keywords: Chitosan nanoparticles, *Dimocarpus longan* leaves, Antibacterial activity, Periodontitis.

INTRODUCTION

Last few decades witness for an individual microbiota to comprise 1:1 ratio of cells and bacteria. A minute disturbance in the microbiota causes several infections [1]. Periodontitis that is considered as the most complex polymicrobial disease is reported to result in dental biofilm dysbiosis, chronic inflammation of periodontal lining soft tissues, and destruction of tooth/alveolar bone [2]. The chronic periodontitis is reported to contribute in the other systemic diseases like respiratory disease, cancer, cardiovascular diseases, diabetes, chronic renal disease, rheumatoid arthritis and obesity [3]. The fact suggests bacteria *S. aureus* to trigger periodontitis. The prolonged conventional antibiotics treatment associated multiple drug resistance (MDR) and high mortality risk have raised the demand for research over antibiotics from natural source [4]. Investigations suggest chitosan a natural agent that offer high antimicrobial activity [5], mucoadhesive property [6], biodegradability [7], high permeability [7], non-toxicity [7] and cost effectiveness [7]. Based on the environmental issues, in last few years there is an emerging demand for efficient green chemistry methods to synthesize nanoparticles. The effective development of green nanoparticles offers the benefit of reduced pollution. The researchers aim to minimize the use of unsafe and expensive products

This is an open access journal, and articles are distributed under the terms of the Attribution 4.0 International (CC BY 4.0) License. This license lets others distribute, remix, tweak, and build upon your work, even commercially, as long as they credit the author for the original creation. You must give appropriate credit, provide a link to the license, and indicate if changes were made.

[8]. The extremely small size and large surface to volume ratio of nanoparticles causes improved biological, chemical and physical properties. The nano particles play an important role in drug delivery and medical imaging [9]. The nanoparticles are classified into various categories such as metallic, carbon based, ceramics, semiconductor, lipid based and polymeric nanoparticles. The carbon based nanoparticles are generally used as fillers and support medium for different inorganic and organic catalysts. The elements commonly used for metallic nanoparticles include gold, silver and copper. Ceramic nanoparticles are generally used in catalysis, photocatalysis, imaging and dyes photodegradation. Semiconductor nano particles are applied in photo-optics, photocatalysis and electronic device. Polymeric nanoparticles increase the bioavailability and solubility [10]. The metallic nanoparticles are often reported to offer thermal unstability and cellular toxicity. During synthesis of nanoparticles, the nitrides and oxides formation increases the chances of impurities. The polymeric nanoparticles possess wide variety of biomedical applications [10]. The chitosan nanoparticles are the subclass of polymeric nanoparticles that are smaller in size. Chitosan is known to enhance the drug′s efficacy based on its ability to pass blood brain barrier and efficient drug delivery [11]. Chitosan is obtained by partial deacetylation of chitin. It is a polysaccharide that comprises copolymers of glucosamine and N-acetyl glucosamine linked by 1→4 linkages. The primary amino groups are important for high antimicrobial activity [5,6] that is affected by various factors, such as degree of chitosan polymerization, molecular weight and solvent used. Under acidic conditions, the free amino groups are protonated and causes polymer solvation. Chitosan from biological sources is relatively cheap and less toxic compared to metallic nanoparticles [7]. As per the antibiotics demand from natural source and nanoparticles property to overcome drug resistance problem [9], there is a high demand for research to explore highly efficient methods for biosynthesis of nanoparticles in current decade [4]. Investigations suggest high antimicrobial potential of *Dimocarpus longan* also called as Longan or Mata Kuching or Dragon′s eye belonging to Sapindaceae family [12,13]. Longan possess vast medicinal attributes and mineral salt properties needed for our body. The longan plant is reported to contain gallic acid, corilagin, ellagic acid, quercetin, kaempferol, soyacerebrosides I and II (lipids), momor-cerebroside I and phytolacca cerebroside [14,15]. Yet formulation of *D. longan* based chitosan nanoparticles is not studied so far. Hence based on facts over periodontitis, MDR, demand for natural source antibiotics, potential of *D. longan* and chitosan, nanoparticles benefits; present study was designed to perform preparation of *D. longan* leaves aqueous extract (DLLAE), synthesis of chitosan nanoparticles (CSNPs) using *Dimocarpus longan* leaves aqueous extract, followed by the characterization, optimization and antimicrobial evaluation against periodontitis triggering bacteria.

EXPERIMENTAL

The chemicals and solvents were procured from Fisher chemicals, Sigma-Aldrich, S.D. Fine and Hi-Media. The chitosan nanoparticles (CSNPs) were synthesized using *Dimocarpus longan* leaves aqueous extract (DLLAE). The glasswares were cleaned, washed using deionized water, dried for 2 h at 160 ºC. The plastic ware was autoclaved prior to antimicrobial study.

Preparation of *D. longan* leaves extract: The *D. longan* leaves were collected from the premise of Kampung Baru, Bedong, Kedah, Malaysia. The leaves were washed with tap water, air dried and powdered. The *D. longan* leaves powder was extracted with distilled water using decoction method. The obtained crude was filtered using muslin cloth and Whatmann filter paper to offer the *D. longan* leaves aqueous extract (DLLAE).

Phytochemical study: The phytoconstituents of *D. longan* leaves aqueous extract (DLLAE) were determined by performing the tests for carbohydrate, proteins, amino acids, fats and oils, steroid, cardiac, anthraquinone, saponin and coumarin glycosides, flavonoids, alkaloids, tannins and phenolic compounds and organic acid was carried out [16].

Determination of total phenolic content: The total phenolic content of *Dimocarpus longan* leaves aqueous extract (DLLAE) was determined using Folin-Ciocalteau reagent (FCR) as per the standard protocol of Singleton with minor modifications [17]. A standard calibration curve was plotted using a mixture of 1 mL aliquots of gallic acid solutions (1.5, 2.5, 5, 10 and 15 µg/mL), 0.1 mL of Folin-Ciocalteu reagent (10-fold dilution) and 2.0 mL of 2.5 % Na_2CO_3 solution (1.25 g/50 mL). The absorbance was measured after 1 h at 765 nm using UV-visible spectrophotometer. In similar conditions, absorbance for 1 mL DLLAE (25 mg/100 mL) was recorded using the same reagents. The absorbances were recorded in triplicate. The total phenolic content was expressed as gallic acid equivalents (GAE). To estimate the total phenolic content of *D. longan* leaves aqueous extract (DLLAE) as mg gallic acid equivalent/100 g, the following formula was used:

$$C = \frac{C_1 \times V}{m}$$

where C = total phenolic content in mg/g, in GAE (gallic acid equivalent), C_1 = concentration of gallic acid established from the calibration curve in mg/mL, V = volume of DLLAE in mL, and m = the weight of DLLAE in mg.

Green synthesis of CSNPs: Into the 2 mg/mL of chitosan nanoparticles solution (prepared by dissolving 0.2 g of chitosan in 100 mL of 1% acetic acid solution), the 1% sodium alginate solution was added drop by drop until white suspension was formed. The mixture was adjusted to pH 5.8 by adding 1 M NaOH. To a mixture 1 mL of DLLAE was added under constant stirring at 115 rpm for 30 min. The obtained nanoparticle solution when centrifuged (for 1 h at 5000 rpm) resulted in CSNPs pellet, which was collected and washed with deionized water and dried. The dried powder was dissolved in 5 mL of deionized water and then subjected to UV-visible analysis at range of 200-800 nm [18]. This procedure was confirmed after optimization of the method.

Optimization of biosynthesis of CSNPs: The chitosan nanoparticles (CSNPs) biosynthesis was optimized based on UV-visible spectrometric studies maintained under different parametric conditions, such as volumetric ratio of extract to zinc acetate, concentration of acetic acid, concentration of

chitosan, concentration of sodium alginate and pH required for green synthesis of CSNPs. Optimization was conducted as per reported protocols with minor modifications [18].

Optimization of CSNPs for concentration of acetic acid: For optimization of biosynthesis of CSNPs using DLLAE the three solution mixtures maintained in same conditions were prepared except for acetic acid concentration that was taken in 0.5, 1.0 and 1.5% concentration.

Optimization of CSNPs for concentration of chitosan: For optimization of biosynthesis of CSNPs using DLLAE, three solution mixtures maintained in same conditions were prepared except for chitosan that was taken in 1, 2 and 3 mg/mL concentration.

Optimization of CSNPs for concentration of sodium alginate: For optimization of biosynthesis of CSNPs using DLLAE, three solution mixtures maintained in same conditions were prepared except for sodium alginate that was taken in 0.5, 1.0 and 1.5% concentration.

Optimization of CSNPs for concentration of extract: For optimization of biosynthesis of CSNPs using DLLAE, three solution mixtures maintained in same conditions were prepared except for DLLAE that was taken in 1.0, 2.5, 5.0 and 7.5 mL concentration.

Optimization of CSNPs for pH of mixture: For optimization of biosynthesis of CSNPs using DLLAE, three solution mixtures were maintained in same conditions except for pH 3.8, 5.8 and 8.5.

Characterization of CSNPs: Once the optimization of biogenic CSNPs was completed, pure chitosan nanoparticles (CSNPs) were subjected to characterization studies as mentioned in other research studies [3]. Prior to characterization, the CSNPs were repeatedly washed and centrifuged using deionized water. The formation of green CSNPs was determined based on a change in colour of the solution, UV-Visible spectrometer (Shimadzu U-2800) and FT-IR (Perkin-Elmer SLE/MSC4/29) spectral data. The FESEM measurement was performed to understand the morphology of CSNPs using FEI Nova NanoSEM 450. The CSNPs crystal nature was determined by observing their XRD pattern using PANalytical X′Pert PRO MRD PW 3040/60 X-Ray diffractometer. The XRD instrument was operated at 40 kV and 40 mA and spectrum was recorded by Cu$K\alpha\beta$ radiation with wavelength of Å in the 1.54060 2θ ranged from 10-80º. The EDX analysis was performed using EDX unit attached with FEI Nova NanoSEM 450.

Antimicrobial assay: The experimental protocol was based on reported method with slight modifications [4]. Briefly, fresh bacterial strain culture (10^5-10^6 CFU/mL) was sub-cultured over Muller-Hinton broth at 37 ºC (before hand shaken using rotary shaker at 200 rpm). Using sterile cotton swab, the bacterial strain of *S. aureus* was uniformly swabbed over Muller-Hinton agar plates. In each Muller-Hinton plate, 8 mm diameter wells were created using sterile metallic borer. To individual wells of each agar plate were added DLLAE (100, 80, 60, 40, 20 and 10 mg/mL), CSNPs (100, 80, 60, 40, 20 and 10 mg/mL). Finally, after incubation of plates at 37 ºC for 24 h, the zone of inhibition was measured in mm.

RESULTS AND DISCUSSION

Phytochemical studies: The prepared *D. longan* leaves aqueous extract (DLLAE) when subjected to phytochemical investigations, it offered the data given in Table-1. The data clearly shows that DLLAE possess reducing sugars, hexose

TABLE-1
PHYTOCHEMICAL SCREENING OF DLLAE

Specific test	Result	Constituents	Specific test	Result	Constituents
Fehling's test	+	Reducing sugars	Test for coumarin glycosides	+	Coumarin glycosides
Benedict's test	+		Lead acetate	+	Flavanoids
Bial's Orcinol test	–	Pentose sugars	Addition of NaOH	+	
HCl + Phloroglucinol	–		Mayer's test	+	Alkaloids
Tollen's Phloroglucinol test for galactose	+	Hexose sugars	Dragendorff's test	+	
Iodine test	–	Non reducing sugars	Wagner's test	+	
Tannic test for Starch	–		Addition of lead acetate solution	+	
Ruthenium red	–	Mucilage	Addition of gelatin solution	+	Tannins and phenolic compounds
Xanthoprotein test	+	Tyrosine / tryptophan	Addition of potassium dichromate	+	
Proteins containing sulphur	+		Addition of dilute iodine solution	+	
Precipitation test	+		General test	+	Organic acids
Millon's test	+	Proteins	5 % Lead acetate	+	Oxalic acid
Ninhydrin / NaOH and lead acetate	+	Amino acids	$KMnO_4$ + Dil. H_2SO_4	+	
Staining of filter paper	–	Fats or oils	NH_4OH + 5 % $AgNO_3$	+	
Salkowski reaction	+	Steroids	2 % Resorcinol solution + Conc. H_2SO_4	+	Tartaric acid
Legal's test & Keller – Killiani test	+	Cardiac glycosides (Cardenoloids & Deoxysugars)	Dil. NH_4OH + $AgNO_3$	+	Citric acid
Borntrager's test	+	Anthraquinone glycosides	5 % $FeCl_3$ solution	–	Malic acid
Foam test	+	Saponin glycosides			

(+) presence of constituents, (–) absence of constituents

sugar, proteins (tyrosine or tryptophan), steroids, cardiac glycosides (cardenoloids and deoxysugars), anthraquinone, saponin and coumarin glycosides, flavonoids, alkaloids, tannins, phenolic compounds and organic acids. The results were in agreement with other recent researches [12,19].

Total phenolic content (TPC): Recording of total phenolic content using UV-Visible spectrophotometry offered a calibration curve (Fig. 1). Total phenolic content was correlated to the antioxidant capacity of *Dimocarpus longan* leaves aqueous extract (DLLAE) by which gallic acid was used as standard. The absorbance was recorded at 765 nm for different concentration of gallic acid and DLLAE. The standard curve given in Fig. 1 presented the equation: $y = 0.0045x + 0.0051$, where $R^2 = 0.9838$. Phenolic compounds are believed to have redox properties, which make them as antioxidants. Total phenolic contents value of DLLAE of 14.088 ± 0.844 mg GAE/g dry sample indicates its high antioxidant property [20].

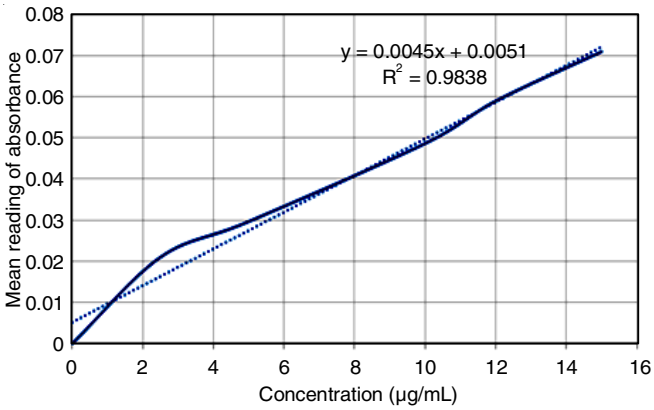


Fig. 1. Standard curve of total phenolic content

Green synthesis of CSNPs: The formation of CSNPs was confirmed by the colour change from white to pale brown. The biosynthesized CSNPs revealed their UV-visible absorbance signal in the desired range (201-310 nm) [21].

Optimization of CSNPs: In this study, four key parameters were selected for the biosynthesis of CSNPs using DLLAE, namely concentration of acetic acid, chitosan, sodium alginate, DLLAE and pH. In present study, the optimization results were validated based on presence of SPR peak within 201-310 nm claimed by other standard investigations [21].

Optimization of CSNPs for concentration of acetic acid: The optimization of CSNPs using three different concentrations of acetic acid (0.5, 1.0 and 1.5%) under UV-visible analysis (Fig. 2). The spectrum displayed three curves. The first curve (0.5% acetic acid) exhibited a broad peak at 277 nm, the second curve (1% acetic acid) at 278 nm, and the third curve (1.5% acetic acid) at 275 nm. Since the second curve showed most significant peak in the desired range, so 1% acetic acid was selected as optimum acetic acid concentration requirement for CSNPS synthesis using DLLAE.

Optimization of CSNPs for concentration of chitosan: The optimization of CSNPs using three different concentrations of chitosan (1, 2 and 5 mg/mL) under UV-visible analysis generated a spectrum shown in Fig. 3. The spectrum displayed

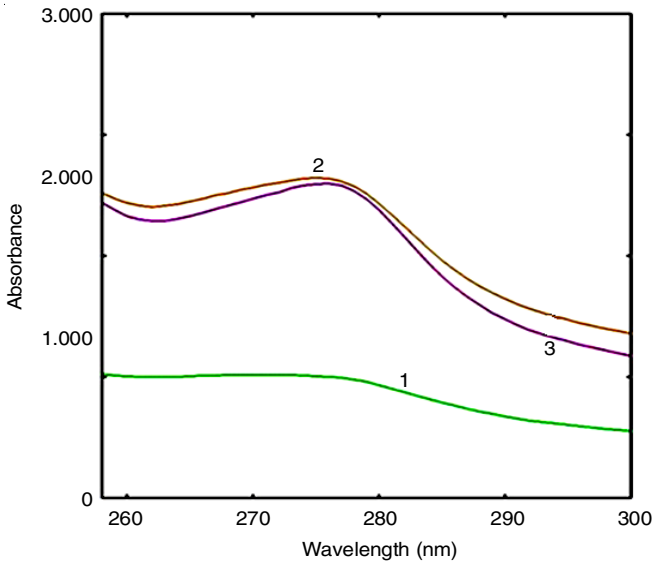


Fig. 2. Optimization of concentration of acetic acid

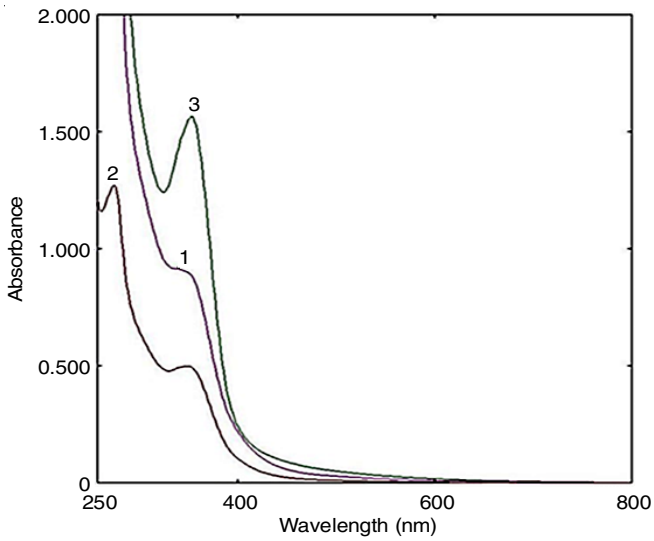


Fig. 3. Optimization of concentration of chitosan

three curves. The first curve (1 mg/mL chitosan) exhibited a broad peak at 390 nm, the second curve (2 mg/mL chitosan) at 263 nm and the third curve (1 mg/mL chitosan) at 401 nm. As only second curve exhibited the signal in desired range, so 2 mg/mL chitosan was selected as optimum chitosan concentration requirement for CSNPS synthesis using DLLAE.

Optimization of CSNPs for concentration of sodium alginate: The optimization of CSNPs using three different concentrations of sodium alginate (0.5, 1.0 and 1.5%) under UV-visible analysis generated a spectrum shown in Fig. 4. There is no sharp peak for curve 1 (0.5 % sodium alginate) and curve 3 (1.5 % sodium alginate), but only curve 2 (1% sodium alginate) exhibits a signal at 278 nm. As only second curve exhibited the signal in desired range, so 1% sodium alginate was selected as optimum sodium alginate concentration requirement for CSNPS synthesis using DLLAE.

Optimization of CSNPs for concentration of DLLAE: The optimization of CSNPs using four different concentrations

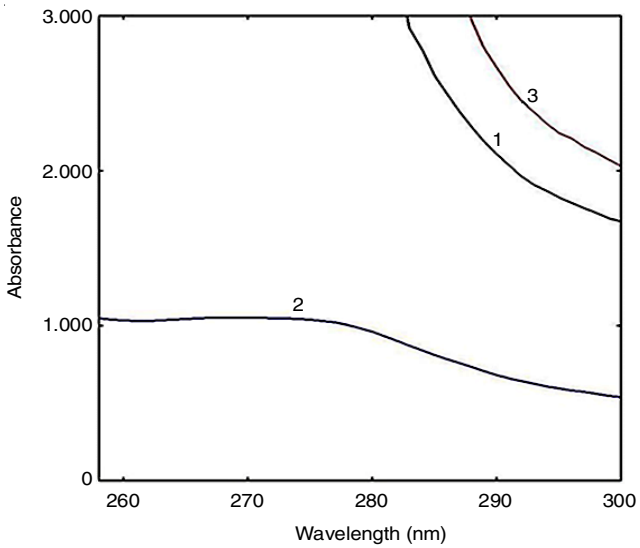


Fig. 4. Optimization of concentration of sodium alginate

of DLLAE (1, 2.5, 5 and 7.5 mL) under UV-visible analysis generated a spectrum shown in Fig. 5. All four curves exhibited absence of sharp peak at the desired range of 201-310 nm, except for curve 1 (1 mL) which exhibited a sharp peak at 263 nm. Hence, 1 mg/mL of DLLAE was selected as optimum DLLAE concentration requirement for CSNPS synthesis using DLLAE.

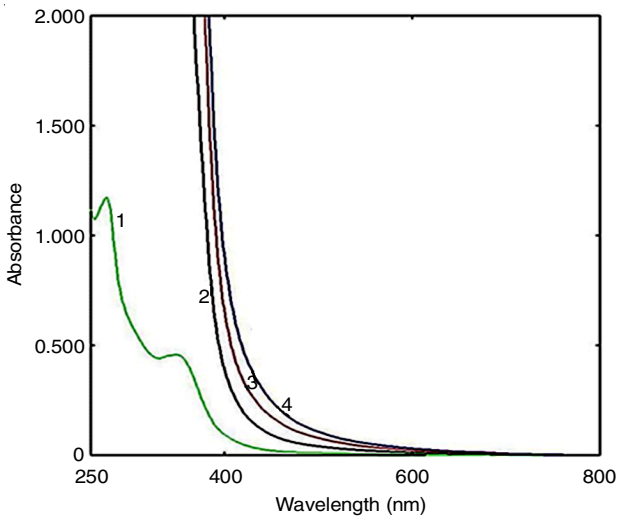


Fig. 5. Optimization of concentration of DLLAE

Optimization of CSNPs for pH of mixture: The optimization of CSNPs using three different pH (3.8, 5.8 and 8.5) under UV-visible analysis generated a spectrum shown in Fig. 6. As observed, curve 3 (pH 8.5) did not exhibited any signal. But the curve 1 (pH 3.8) exhibited a signal at 271 nm and curve 2 (pH 5.8) exhibited a signal at 279 nm. As curve 2 exhibited better result compared to curve 1, so pH 5.8 was selected as the optimum pH requirement for CSNPS synthesis using DLLAE.

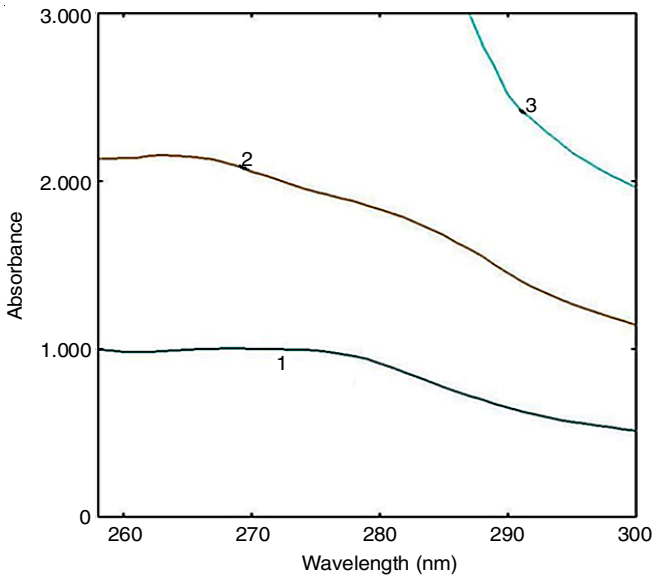


Fig. 6. Optimization of pH

Characterization of CSNPs: Before characterization, the CSNPs were subjected to purification to prevent the absorbance of aqueous extract of plant which could interfere with results accuracy. The repeated washing and centrifugation process were done to avoid interference of unbound residual biochemical entities of aqueous leaves extract of *Dimocarpus longan* with characterization data of biogenic CSNPs [14].

FT-IR analysis: The FTIR characterization study aided in determination of ionic gelation induced by phytochemicals groups present in the DLLAE and formation of CSNPs. The FT-IR spectrum of DLLAE given in Fig. 7a, displayed characteristic IR bands at 3466 cm^{-1} (O-H vibrations), 2891 cm^{-1} (C-H vibrations) and 1695 cm^{-1} (>C=O vibrations). The FTIR spectrum for CSNPs given in Fig. 7b, displayed shifted bands 3467 cm^{-1}

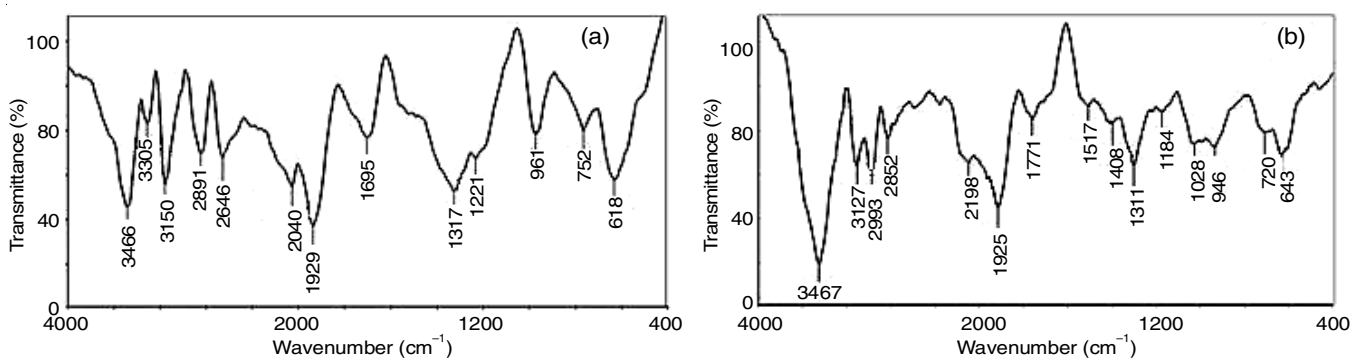


Fig. 7. FT-IR spectrum of DLLAE (a) and CSNPs (b)

(O-H vibrations), 2993 and 2852 cm^{-1} (C-H vibrations), 1771 cm^{-1} (C=O vibrations), 1517 cm^{-1} (C=N vibrations). The formation of CSNPs and the ionic gelation of chitosan by phytochemical moieties present in the DLLAE were also supported by other studies [15]. The DLLAE was recognized as a dual capping (stabilizing) and ionic gelating agent based on the comparison of FTIR spectrum of DLLAE and biosynthesized CSNPs. The FTIR spectrum of CSNPs (Fig. 7b) was similar to FTIR spectrum of DLLAE (Fig. 7a), as it retained the majority of signals with marginal shifting and broadening. For example, 3466 cm^{-1} (O-H vibrations) narrow band in FTIR spectrum of DLLAE (Fig. 7a) was shifted to 3467 cm^{-1} as broad band in FTIR spectrum of CSNPs (Fig. 7b).

FESEM analysis: The surface morphology of synthesized CSNPs was further analyzed using the FESEM technique. The FESEM image of CSNPs at higher and lower magnification scale of 400 nm is shown in Fig. 8.

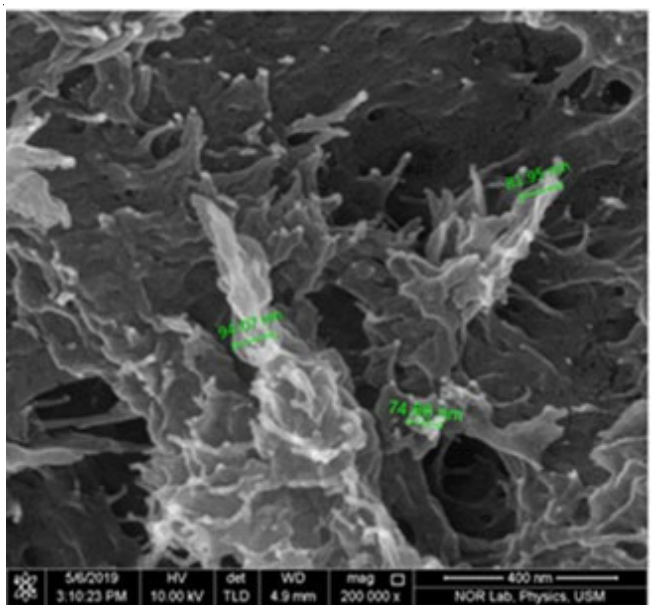


Fig. 8. FESEM of CSNPs

Fig. 9 indicates that CSNPs were spherical in shape. The size ranged from 74.66-94.07 nm and were found in agreement with the other standard research work [12]. The FESEM image revealed a spherical-like highly porus agglomerated state of CSNPs. Agglomeration has been considered as the primary phenomenon for synthesis for novel CSNPs for biomedical applications and nanomedicines [21].

X-ray diffraction analysis: Biosynthesized CSNPs were characterized using powder XRD analysis to confirm the size of chitosan nanoparticles and to understand the structural information. Crystal nature of biogenic CSNPs was confirmed based on analysis of XRD patterns. The XRD spectrum (Fig. 9) of the CSNPs shows sharp peaks at various 2θ values such as 22.28º, 25.19º, 34.72º, 40.0º, 71.16º and 73.83º.

The average crystallite size for CSNPs was found to be 30.11-62.98 nm. The diffraction peak of pure chitosan usually observed at 22.04º was slightly shifted to higher values (22.28º, 25.19º). These peaks indicate that more crystalline forms were

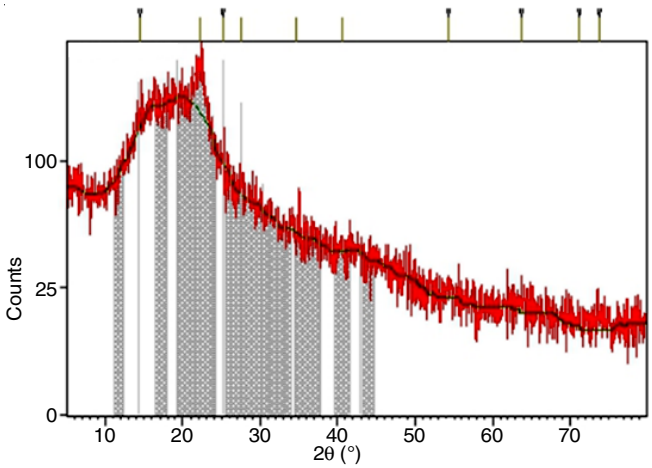


Fig. 9. CSNPs XRD spectrum

introduced in the bead formation. The chitosan molecule easily formed the crystalline regions, and this may be due to the presence of plenty of -OH and -NH_2 groups in the chitosan structure. The CSNPs size was determined in nm using Debye Scherrer formula [21].

EDX analysis: The EDX spectrum of CSNPs (Fig. 10) revealed a strong signal in the carbon region (wt. 76.17%), oxygen (wt. 22.14%) and sodium (wt. 1.53%), thereby confirmed the formation of CSNPs.

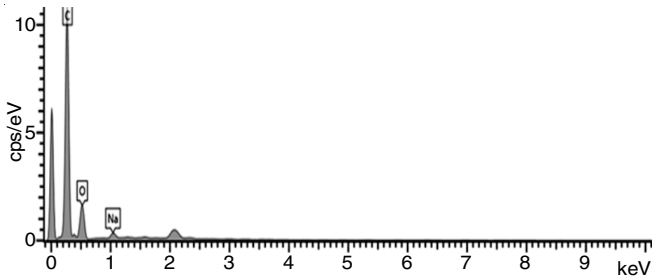


Fig. 10. EDX spectrum of CSNPs

Antibacterial assay: The biogenic CSNPs and DLLAE were tested for their inhibition potential against *S. aureus* the periodontitis triggering pathogen and the resultant data is given in Table-2. The *Dimocarpus longan* leaves aqueous extract (DLLAE) exhibited no inhibition activity against *S. aureus*. The results clearly indicate the inhibition zone of green synthesized CSNPs in comparison to DLLAE. Interestingly there was an increase in CSNPs antibacterial activity, when the concentration of CSNPs was increased from lower to higher concentration. This pattern of increment in the antibacterial activity of CSNPs was in agreement with the data of other standard investigation also. The results of present study were supported by other investigations which also claims that CSNPs in small size and high dose exhibits higher antibacterial potential [5]. Many studies [22-24] reported biosynthesis of chitosan nanoparticles using different plant extracts. Present study offers a benefit of low-cost production of chitosan nanoparticles using DLLAE, a commonly available plant in Malaysia. The plant material with high TPC are commonly used in food industry

TABLE-2
ZONE OF INHIBITION (mm) OF DLLAE AND CSNPs

Microorganism	DLLAE						CSNPs					
Conc. (mg/mL)	10	20	40	60	80	100	10	20	40	60	80	100
S. aureus	NA	NA	NA	NA	NA	NA	22	23	28	33	36	39

NA = No activity

as they reduce the oxidative degradation of lipids and possess high scavenging potential. The high total phenolic content of DLLAE supports its potential as natural antioxidant agent. After comparison of the resultant data of present study over DLLAE phytochemical investigation, TPC analysis, green synthesis of CSNPs (using DLLAE) and their antimicrobial potential, it is recommended that green synthesis of CSNPs using DLLAE is an efficient method. Yet further toxicity analysis and clinical investigations over chitosan nanoparticles (CSNPs) synthesized using *Dimocarpus longan* leaves aqueous extract (DLLAE) should be done to establish the CSNPs as effective antimicrobial agents in periodontitis.

Conclusion

The high total phenolic content value of *Dimocarpus longan* leaves aqueous extract (DLLAE) suggests to its antioxidant potential. The visual examination of colour change from white to pale brownish, UV-visible spectrometric and FT-IR data of the present study confirmed the success of green synthesis of chitosan nanoparticles using DLLAE. The FESEM, EDX, and XRD data established the morphology of green CSNPs size (smaller than 94.07 nm), spherical shape, agglomerated and crystalline nature. Present study concludes that chitosan nanoparticles (CSNPs) obtained using *Dimocarpus longan* leaves aqueous extract exhibits no antimicrobial response against *S. aureus* and recommends *Dimocarpus longan* leaves aqueous extract as a potential source for green production of potent antimicrobial chitosan nanoparticles. Further study on the toxicity of chitosan nanoparticles is needed to establish it as an antimicrobial agent in the treatment of periodontitis.

ACKNOWLEDGEMENTS

The authors are thankful to AIMST University for providing the necessary facilities to successfully accomplish this study. The authors are also highly thankful to Nano-Optoelectronic Research and Technology (NOR) Lab, School of Physics, Universiti Sains Malaysia for assisting in spectral analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. R. Sender, S. Fuchs and R. Milo, *Cell*, **164**, 337 (2016); https://doi.org/10.1016/j.cell.2016.01.013
2. T.G.B. Lourenco, S.J. Spencer, E.J. Alm and A.P.V. Colombo, *J. Oral Microbiol.*, **10**, 1487741 (2018); https://doi.org/10.1080/20002297.2018.1487741
3. N.K. Fuloria, S. Fuloria, K.Y. Chia, S. Karupiah and K. Sathasivam, *J. Appl. Biol. Biotechnol.*, **7**, 46 (2019); https://doi.org/10.7324/JABB.2019.70408
4. S. Fuloria, N.K. Fuloria, C.J. Yi, T.M. Khei, T.A. Joe, L.T. Wei, S. Karupiah, N. Paliwal and K. Sathasivam, *Bull. Environ. Pharmacol. Life Sci.*, **8**, 112 (2019).
5. L. Qi, Z. Xu, X. Jiang, C. Hu and X. Zou, *Carbohydr. Res.*, **339**, 2693 (2004); https://doi.org/10.1016/j.carres.2004.09.007
6. T. Banerjee, S. Mitra, A.K. Singh, R.K. Sharma and A. Maitra, *Int. J. Pharm.*, **243**, 93 (2002); https://doi.org/10.1016/S0378-5173(02)00267-3
7. J.J. Wang, Z.W. Zeng, R.Z. Xiao, T. Xie, G.L. Zhou, X.R. Zhan and S.L. Wang, *Int. J. Nanomedicine*, **6**, 765 (2011); https://doi.org/10.2147/IJN.S17296
8. V.K. Sharma, R.A. Yngard and Y. Lin, *Adv. Colloid Interface Sci.*, **145**, 83 (2009); https://doi.org/10.1016/j.cis.2008.09.002
9. S. Iravani, *Green Chem.*, **13**, 2638 (2011); https://doi.org/10.1039/c1gc15386b
10. I. Khan, K. Saeed and I. Khan, *Arab. J. Chem.*, **12**, 908 (2019); https://doi.org/10.1016/j.arabjc.2017.05.011
11. B.A. Aderibigbe and T. Naki, *Appl. Sci.*, **9**, 2219 (2019). https://doi.org/10.3390/app9112219
12. F.A. Ripa, M. Haque and I.J. Bulbul, *Pak. J. Biol. Sci.*, **13**, 22 (2010); https://doi.org/10.3923/pjbs.2010.22.27
13. Y. Jiang, Z. Zhang, D.C. Joyce and S. Ketsa, *Postharvest Biol. Technol.*, **26**, 241 (2002); https://doi.org/10.1016/S0925-5214(02)00047-9
14. Y.Y. Tang, X.M. He, J. Sun, C.B. Li, L. Li, J.F. Sheng, M. Xin, Z.-C. Li, F.-J. Zheng, G.-M. Liu, J.-M. Li and D.-N. Ling, *Molecules*, **24**, 1186 (2019); https://doi.org/10.3390/molecules24061186
15. R. Jiyoung, K. Ju Sun and K. Sam Sik, *Arch. Pharm. Res.*, **26**, 138 (2003); https://doi.org/10.1007/BF02976659
16. K.R. Khandelwal, Practical Pharmacognosy: Techniques and Experiments, Nirali Prakashan, New Delhi, India (2004).
17. N. Siddiqui, A. Rauf, A. Latif and Z. Mahmood, *J. Taibah Univ. Med. Sci.*, **12**, 360 (2014); https://doi.org/10.1016/j.jtumed.2016.11.006
18. D. Nagaonkar, S. Gaikwad and M. Rai, *Colloid Polym. Sci.*, **293**, 1465 (2015); https://doi.org/10.1007/s00396-015-3538-3
19. F. Mujeeb, P. Bajpai and N. Pathak, *BioMed Res. Int.*, **2014**, 497606 (2014); https://doi.org/10.1155/2014/497606
20. M.A. Johari and H.Y. Khong, *Adv. Pharmacol. Sci.*, **2019**, 7428593 (2019); https://doi.org/10.1155/2019/7428593
21. M. Agarwal, M.K. Agarwal, N. Shrivastav, S. Pandey, R. Das and P. Gaur, *Int. J. Life Sci. Scient. Res.*, **4**, 1713 (2018); https://doi.org/10.21276/ijlssr.2018.4.2.17
22. M. Bilal, Y. Zhao, T. Rasheed, I. Ahmed, S.T.S. Hassan, M.Z. Nawaz and H.M.N. Iqbal, *Int. J. Environ. Res. Public Health*, **16**, 598 (2019); https://doi.org/10.3390/ijerph16040598
23. N. Zafar, B. Uzair, M.B. Niazi, S. Sajjad, G. Samin, M.J. Arshed and S. Rafiq, *Adv. Polym.Technol.*, **2020**, 8456024 (2020); https://doi.org/10.1155/2020/8456024
24. L.M. Ali, H.E. Hassan, A.E. El-Raie, A.E.A. Ahmed and S.S. Saleh, *Adv. Nat. Sci.: Nanosci. Nanotech.*, **10**, 045005 (2019); https://doi.org/10.1088/2043-6254/ab4804