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Synthesis, Characterization And Applications Of Chitosan Linked Nanopolymer Using *Jasminum sambac* Leaf Extract.

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ABSTRACT

Nanotechnology makes an impact in all spheres of human life and creates increasing excitement especially in the field of biomedicine and bio-nanotechnology. The main synergism between nanotechnology and green chemistry is the use of bioactive chemicals for the synthesis of nanoparticles. Chitosan is a natural, biocompatible, biodegradable, nontoxic and easily available polymer that can be used to prepare nanoparticles. Chitosan nanoparticles can be widely used in pharmaceutical industries as drug delivery vehicle. The present research was focused on fabrication of biomolecules loaded chitosan nanoparticles using *Jasminum sambac* leaf extract. The nanopolymer was synthesised using ionic gelation technique and was characterized using UV spectrophotometer and FTIR. The optical density of the broad peak of Chitosan-jasmine nanopolymer was found at 3.5242. The FTIR results recorded the absorption peaks at 2112 cm^{-1}, 1636 cm^{-1} and 3327 cm^{-1} which represented the C-H stretch, C=C stretch and O-H bond stretching respectively. Thus, there was a stretching from higher wavelength region to lower wavelength region demonstrating the interaction of tripolyphosphate with chitosan and biomolecules by reduced hydrogen bonding. The present study strongly suggested that the fabricated phytochemicals cross-linked with chitosan nanoparticles from *Jasminum sambac* leaf extract exhibited various activities such as enhanced cytoprotective effects and 75% DNA damage inhibition effects. Thus, the results of our study lend pharmacological credence to the bio-medical applications and ethno medical use of this plant in traditional system of medicine in the near future.

Keywords: Chitosan, *Jasminum sambac,* Cytoprotection, DNA damage.

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INTRODUCTION

Nanotechnology has created potential impact in various fields of medicine and its highly utilized in specialized areas like drug delivery, brain targeting, tumor targeting and gene delivery [1]. As nanopolymers are greatly increasing and playing an important role in a broad spectrum of areas, it has been widely studied as particulate carriers in the pharmaceutical and medical fields because they exhibit promising drug delivery systems as a result of their subcellular size, controlled and sustained release properties, biocompatibility with cells and tissue [2].

Chitosan is the most commonly used natural nanopolymer. It's a cationic polysaccharide obtained by partial deacetylation of chitin. Natural chitosan has captivated greater attention in pharmaceutical and biomedical fields due to its beneficial functional properties [3].

There is also a need to investigate the potential phytochemical based nanoparticle that has been prompted due to the dramatic expansion of the nanotechnology. Thus, 'green chemistry' that uses plant biomass or plant extracts have become an alternative for the production of nanomaterials in a clean, non-toxic, eco-friendly and sustainable manner [4]. *Jasminum sambac*, the queen of flowers is a popular fragrant flower from the "Oleaceae" family that are annual plants which are classified as shrubs or vine. In India, Jasmines are cultivated throughout the country [5].

Reactive oxygen species (ROS) are generated spontaneously in living organisms during metabolism [6,7]. An imbalance between the generation of ROS and cellular antioxidant capacity can lead to oxidative stress which is implicated in the etiology of several degenerative diseases such as stroke, rheumatoid arthritis, diabetes mellitus, peptic ulcer and cancer [8,9]. Therefore in recent years, research in phytomedicine has increased with interest in natural products that possess antioxidant and DNA protecting properties.

In spite of possessing various bioactive compounds and the wide pharmacological credence of the leaves of *J.sambac*, little is known about its DNA-protecting abilities. So, in the continuous effort to evaluate its potential medicinal values, the current research was focussed on the fabrication and characterisation of bio-molecules cross-linked with chitosan nanoparticles and to investigate its DNA protective property against induced oxidative stress by ferrous sulphate and further assess its repairing potential against DNA oxidative damage.

MATERIALS AND METHODS

Chemicals

Methanol, Hydrochloric acid, Sodium hydroxide, Chloroform, Sulphuric acid , acetic acid, ethyl acetate, Boric acid, oxalic acid, Alluminium chloride, Phosphoric acid, Sodium sulphate, Folin Ciocalteau's reagent, Ethanol, n-butanol, Methyl red indicator were of analytical grade obtained from Fischer Scientific. TLC Silica gel plate was of silica gel $GF_{254,}$ Merck. Ferric chloride, Dragandroff's reagent was obtained from Laboratory reagents. Quercitin, Gallic acid, Chitosan, Sodium-tri-phosphate (TPP), Salmon milt DNA were analytical grade from HiMedia, Karnataka. Acetonitrile was obtained from Lab scan, Analytical Science, Bangalore. Ferrous sulphate, L-Ascorbic acid AR was obtained from SD Fine Chem Ltd.

Plant Source

The tender leaves of Jasmine (*Jasminum sambac*) were collected from Lalbagh Botanical Garden Bangalore, Karnataka, India by convenience sampling technique as it's simple, economical, readily available and an uncomplicated approach.

Preparation of plant extract

The plant extract was prepared from the leaves of *Jasminum sambac*. The leaves were dried and made into fine powder. The coarse powder of *J.sambac* was extracted with water and methanol (30:70) using soxhlet apparatus for 12h at 64°C not exceeding the boiling point of the solvents. The extract was then concentrated to dryness and used for further experimental analysis.

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Phytochemical analysis

Qualitative analysis

Test for Alkaloids, Flavonoids, Phenolics and Tannins using 1 mL extract was carried out allowing it to react with the specific reagents as described by the standard protocols [10].

Quantitative analysis of Phenolics, Flavonoids and Alkaloids

Quantification of Total Phenols

A 4% plant extract was prepared using chilled 80% ethanol. The slurry obtained centrifuged at 6000 rpm for 10 min. The supernatant was collected and made up to 5mL with 80% ethanol. The resulting solution was used for quantification of total phenol content using Folin-Ciocalteau's method [11]. The method based on oxidation reduction reaction measuring the absorbance at 560 nm against the blank using colorimeter of ComSys CT-212M [12].

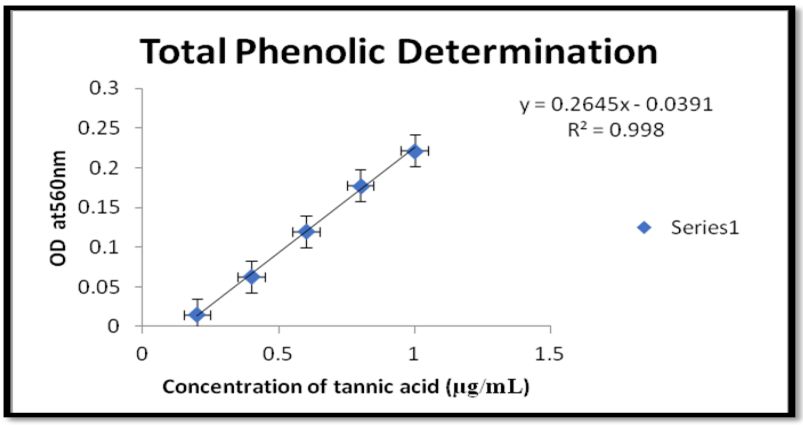

Figure 1: Total phenolic determination of methanolic extract of jasmine.

Quantification of flavanoids

A 10% plant extract was prepared in 70% chilled methanol. The slurry was transferred into a reagent bottle and was incubated overnight at room temperature. Then the slurry was centrifuged at 6000 rpm for 15 min and supernatant was used for determination of total flavanoids using 10% $AlCl_3$ and 1M Potassium acetate. The tubes were incubated for 30mins at room temperature and absorbance was read at 415 nm using colorimeter against suitable blank [13].

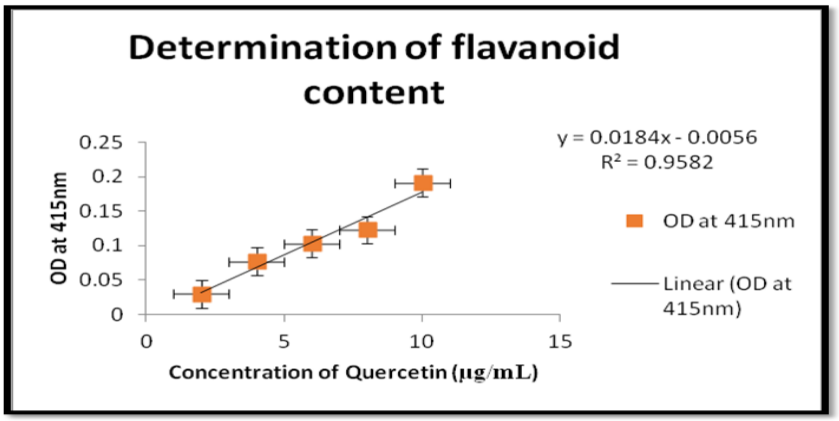

Figure 2: Determination of flavanoid content in the methanolic extract of jasmine

Quantification of Alkaloids

A 25% extract was prepared using n-butanol and vigorously stirred. The content was transferred into a reagent bottle and incubated at room temperature overnight. Then it was centrifuged (REMI C-30BL, R-236, 6 x 200ml. AH) at 6000 rpm for 10 min and the supernatant was made up to 50mL with n-butanol. Obtained supernatant of the plant sample was used for the determination of total alkaloids by titrimetric methods. The total amount of alkaloids was calculated by considering the following equivalent: 1mL 0.1N HCl ≡ 0.0162 g alkaloid.

Analysis by Thin Layer Chromatography

TLC was performed on a silica gel plate (silica gel GF_{254}, Merck) 2- 5 µL of jasmine leaves extract was deposited to the origins of TLC plates from the 2cm of the origin with the help of capillary tubes. The plates were kept in TLC glass chamber (solvent saturated) then mobile phase was allowed to move through adsorbent phase up to ¾ th of the plate. The different solvent systems used were: Alkaloids: Ethyl acetate: Chloroform: water (5:3:1); Flavonoids: n-Butanol: ethyl acetate: water (5:10:15); Phenolics: methanol: water (6:3); Tannins: chloroform: water (6:4).

Purification by HPLC

The HPLC analysis was performed using an Agilent 1200 series HPLC system (Agilent Technologies Inc., Chemetrix, South Africa) which was equipped with a binary pump and fitted with an AscentisTM C18 column (Supelco; 25 cm x 4.6.mm x 5 µm particle size). 5 µL of *J.sambac* leaves extract was analysed throughout. Diode array detector was then used for quantification at 200 nm. The mobile phase consisted of 85% aqueous phosphoric acid (Solvent A; pH 1.76) and 10% aqueous acetonitrile (Solvent B, Lab scan, Analytical Science, Poland) at a flow rate of 1.5 mL/min. A linear gradient was applied from 0-70% Solvent B in 20 min.

Synthesis of Nanopolymer

Preparation of plant extract:

The plant extract was pre pared as explained in the previous section. The extract was then concentrated to dryness and used for further analysis.

Synthesis of chitosan nanoparticles

The ionic-gelation method was employed for the synthesis of chitosan nanoparticle using Sodium tripolyphosphate (TPP) as cross-linking agent. About 1% (w/v) of the *J.sambac* extract was mixed with 0.5% (w/v) of TPP and the solution was added drop wise into the chitosan solution containing 0.5% (w/v) chitosan and 1% (v/v) acetic acid under gentle magnetic stirring of REMI 2-MLH. The solution was incubated for 20 mins and used for characterization.

Characterisation of nanopolymers

UV spectrophotometer

The chitosan and the target compound interactions were monitored by measuring the UV-Vis spectrum of the nanopolymer suspension. To assess the stability of nanopolymer, the absorption spectrum was recorded for pure chitosan, Jasmine extract, TPP, TPP-Jasmine extract, Chitosan-TPP, Chitosan-Jasmine extract and Chitosan-TPP- Jasmine extract. The spectrophotometer used was a Chemito UV Scan 2600 (Thermo Fisher) and the software was Spectrum™ Version 6.87. Absorbance spectra were recorded over the range of 220 – 400 nm. Wavelength of peak absorbance and λmax was calculated.

FTIR spectral analysis

The biomolecules loaded chitosan nanoparticles were freeze dried, powdered and used for FTIR spectroscopy studies. The dried experimental sample was directly placed on the KBr crystals and was

compressed to form pellets. Then, FTIR analysis of chitosan nanoparticles sample was performed with a2 technologies portable attenuated total reflectance (ATR) Fourier transform infrared spectroscopy (ATR-FTIR). Sample spectra were recorded in the middle infrared range from 4000 cm^{-1} to 400 cm^{-1} with a resolution of 4cm in the absorbance mode for 10 scans at room temperature. FTIR spectra of chitosan nanoparticles were obtained by placing 1mg of sample on the sensor of the instrument and spectrum was then compared with the spectrum of Chitosan standard.

Detection of DNA protection by Chitosan nanoplomer using UV-Spectrophotometer

The level of Salmon milt DNA protection by Chitosan nanopolymer was compared to the standard antioxidant (Ascorbic acid) using UV Spectroscopy. For the standard reaction mixtures Salmon milt DNA (200µg/mL), the standard antioxidant Ascorbic acid (10mM) and standard oxidant ferrous sulphate (10mM) were taken. The absorbance was read at 220-400nm, TAE buffer was used as reference standard. Different reaction mixtures were prepared as follows and absorbance was noted down.

DNA inhibition assay using Agarose gel electrophoresis

The ability of Chitosan nanopolymer to protect Salmon milt double stranded DNA from devastating effects of free radicals generated was assessed by the DNA damage inhibition assay. The reaction mixtures contained 2mL DNA (200µg /mL), 1mL 10mM ferrous sulphate and 1mL 10mM ascorbic acid or 1mL of 1% nanopolymer Jasmine extract. 20µL of each reaction mixture were analyzed on 1% agarose gel (prepared by dissolving 0.5 g of agarose in 50mL of 1X TAE Buffer). Electrophoresis was carried out at 100V and 120A for 1 hour. The gel was then visualised under Gel Documentation for the appearance of bands.

RESULTS AND DISCUSSION

Phytochemical analysis

Methanol being a universal solvent has the ability to attract almost all bioactive compounds from plants because of the presence of polar group (OH) and non-polar group (CH) [14]. Thus, *J.sambac* could be used in vast applications due to the presence of numerous bioactive compounds. The result of phytochemical screening of plant extract has been tabulated in table-1.

Table 1: Qualitative analysis of phytochemicals in the methanolic extracts of Jasmine.

PHYTOCHEMICAL	COLOUR	JASMINE
Alkaloids	Reddish brown	+++
Tannins and phenolics	Violet	-
Flavanoids	Violet (Anthocyanins)	-
	Yellow (Flavones)	+
	Orange(Flavanones)	-
Terpenoids	Reddish brown	++

Note: '+' indicated a positive result and '-' indicated a negative result.

The findings illustrated that the total phenolic content of crude ethanolic extact of jasmine was found to be 2.567 mg/g of tannic acid equivalents. Phenolic compounds have redox properties that enable them to act as antioxidants. The total phenolic content in plant extract depends on the polarity of the solvent used for extraction. High solubility of phenols in polar solvents provides a high concentration of phenolic compounds in the extract [15]. The content of phenolics was expressed in terms of tannic acid equivalents (the standard curve equation: $y=0.2645x-0.0391$, $R^2=0.998$), mg of TA/g of extract.

Determination of Total Flavonoid Content

The total flavonoid content of crude ethanolic extact of jasmine was found to be 1.103 mg/g of Quercetin equivalents. Flavonoids, including flavones, flavanols and condensed tannins, show antioxidant activity which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants *in vivo* [16]. The content of flavonoids was expressed in terms of quercetin equivalents (the standard curve equation: y=0.0184x-0.0056, R^2=0.9582), mg of QE/g of extract.

Quantitative Analysis of Total Alkaloids

The analytical method followed for determination is a rapid means of assay for total "alkaloids," using a back titration with acid employing a potentiometric determination of endpoint. The concentration of HCl neutralised by amino groups present in alkaloids helps in calculating the concentration of alkaloids. The range of total alkaloids content was found to be 0.704±1.3g%w/v dry weights in butanolic extract of jasmine.

Analysis by Thin Layer Chromatography

TLC profiling of the methanolic extracts of Jasmine plant denoted an imposing result that guided towards the incidence of a number of phytochemicals. Different solvent systems confirmed the presence of diverse potent bio molecules in the plant. These potent biomolecules of plant were further used for development of the different drugs in future or in miscellaneous applications.

As illustrated in the table-2, Jasmine confirmed the presence of alkaloids, flavonoids and phenolics by the appearance of reddish brown, greyish and green fluoroscence spots on spraying Dragendroff's reagent, $FeCl_3$ spray and 3% boric acid+10% oxalic acid solution respectively under the view of UV trans-illuminator. TLC profiling of *J.sambac* has revealed several phytochemicals such as Alkaloids, anthraquinones, coumarins, essential oils, flavanoids, anti-oxidants, cardiac glycosides, phenolics, saponins and tannins [17].

Table 2: Qualititative confirmatory test for the phytochemicals in the extract of Jasmine by TLC.

Phytochemicals	Solvent System	Confirmatory	Jasmine
Alkaloids	Ethyl acetate: $CHCl_3$: H_2O (5:3:1)	Dragendroff's Reagent	+
Flavonoids	n-Butanol: Ethyl acetate: H_2O (5:10:15)	3% boric acid + 10% oxalic acid	+
Phenolics	CH_3OH : H_2O (6: 3)	1% $FeCl_3$	+
Tannins	$CHCl_3$: H_2O (6:4)	1% $FeCl_3$	-

TLC profiling of *J.sambac*

Under short UV light of 254nm and long UV of 365nm, Jasmine confirmed the presence of phenolics, alkaloids, flavanoids and tannins with Rf values of 0.81, 0.95, 0.90 and 0.72 by the appearance of grey colour spots, reddish brownish spots, green fluorescence bands and brownish grey spots respectively on TLC plates. Thus, the extract of Jasmine leaves was observed to be more promising in terms of the bioactivities and hence was subsequently considered in our further investigation.

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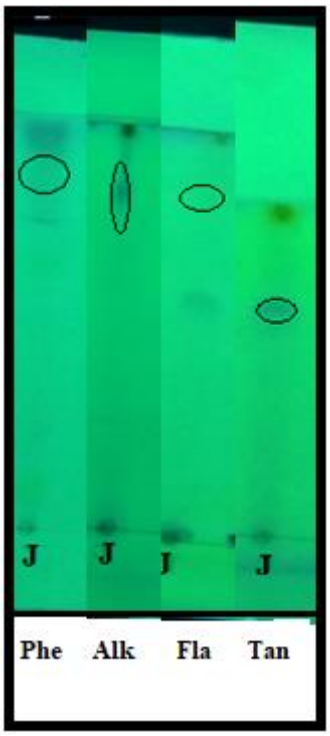

Figure 3(a): At short UV (254nm)

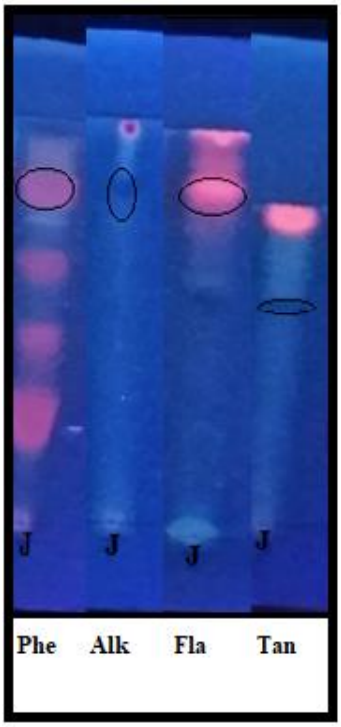

Figure 3(b): At long UV (365nm)

Purification by HPLC

The fig-4(a) illustrated two peaks at a time interval of 2.103 min and 3.240 min respectively. Resolution of the baseline of analytes and IS was achieved with a C18 column, without any amine modifiers in the mobile phase. The standard retention time and other factors pertained to our studies has been tabulated in the table-3.

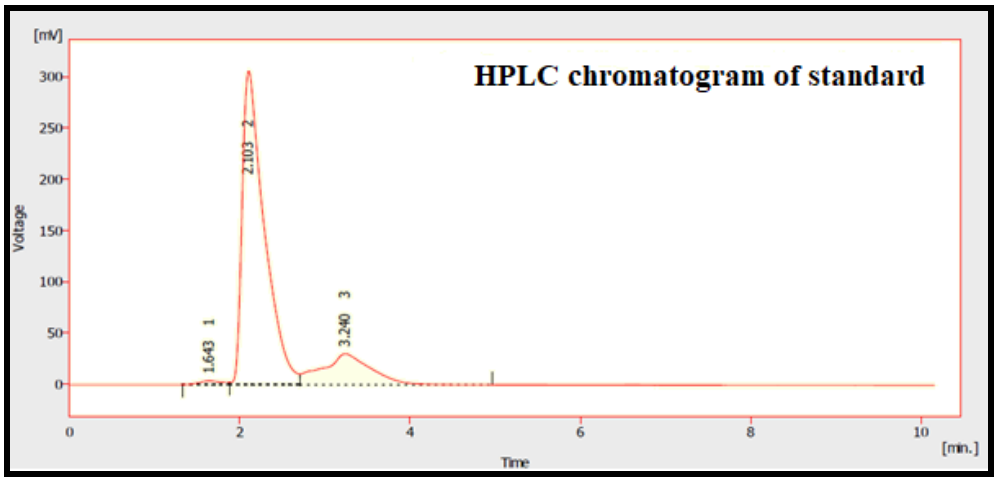

Figure 4(a): HPLC chromatogram of the caffeic acid (standard)

Table-3: Retention time of standard Caffeic acid by HPLC.

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	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.643	69.185	3.651	1.0	1.1	0.37
2	2.103	5538.941	306.087	81.3	90.0	0.27
3	3.240	1201.542	30.183	17.6	8.9	0.64
	Total	6809.668	339.921	100.0	100.0	

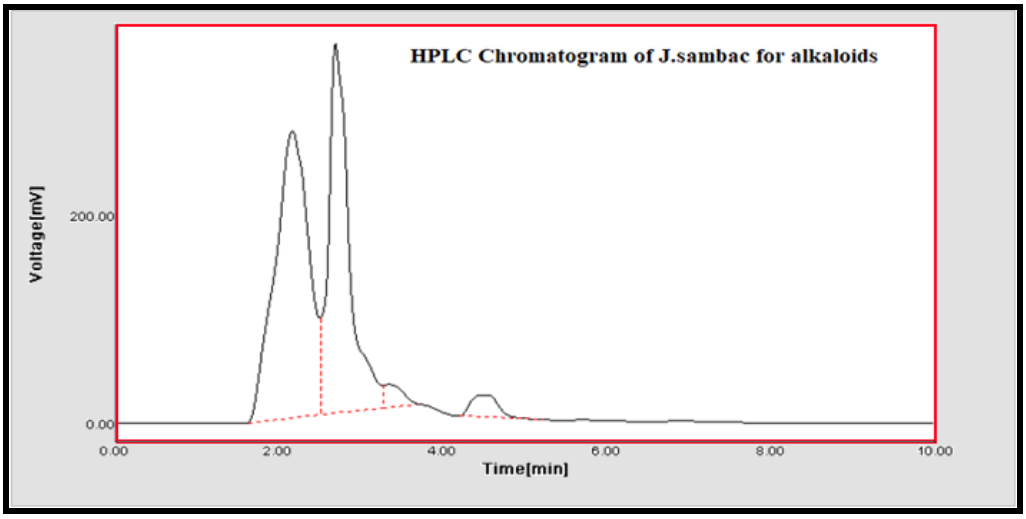

Figure 4(b): Chromatogram of the purification of methanolic extract of Jasmine by HPLC

The illustrated HPLC chromatogram of the purification of methanolic extract of Jasmine determined the quantification of alkaloids at different time intervals. The sample's chromatogram revealed two major peaks at 2.1667 min and 2.6833 min, and two minor peaks was also revealed at 3.3333min and 4.4667 min respectively. On correlating the RP-HPLC chromatograms of the sample with the standard, it was revealed that there was a similarity in two peaks of 2.103 min of standard with 2.1667 min of sample and 3.240 min of standard with 3.3333 min of the sample, which determined quantitatively the presence of alkaloids in the methanolic extract of *J.sambac* leaves. The amount of alkaloids present in the *J.sambac* methanolic extract of leaves was found to be 142µg/10mg . It was assumed that the two peaks with retention time 2.6833 min and 3.3333 min were codeine and caffeine respectively.

Characterisation of nanopolymers.

The preliminary characterisation of Chitosan-jasmine nanopolymer opalescent solution was determined in a UV-Vis spectrophotometer to verify the validity of prepared chitosan nanopolymer. Fig-5 illustrated that all the optical density coincided at a specific wavelength of 290nm which revealed the formation of a nanopolymer on a preliminary aspect. The maximum peak absorbance of the Chitosan-jasmine nanopolymer was found at 3.5242 OD. Our current study correlated with his revelation as the broad peak of pure chitosan was also in the range of 280-322nm [18].

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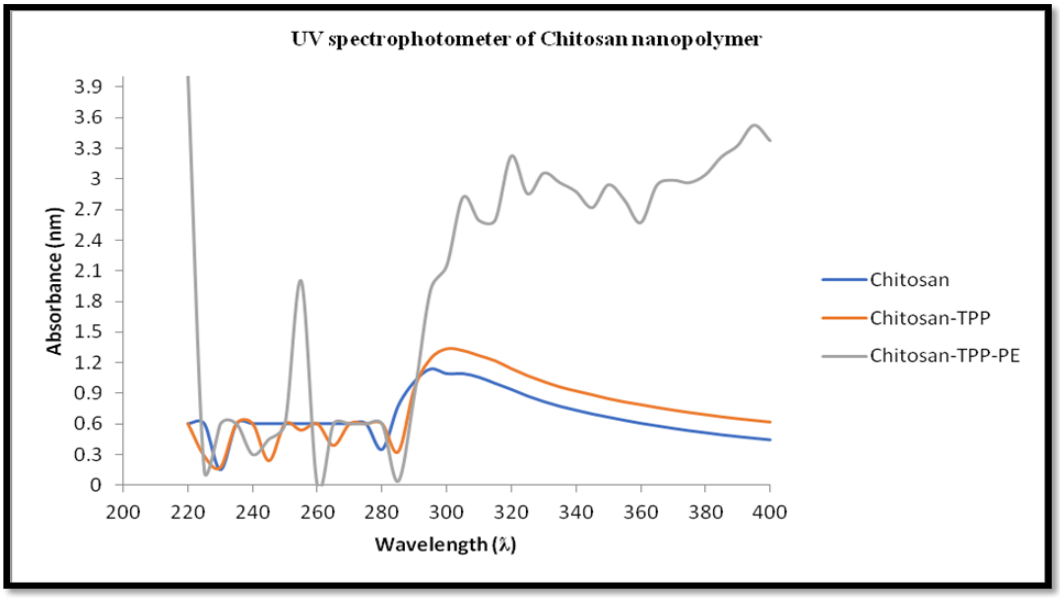

Figure 5: UV-Vis spectrum of pure chitosan(Chi), solution of 0.5% chitosan and 1% TPP (Chi-TPP), and solution of 0.5% chitosan, 1% TPP and 1% P.E(Chi-TPP-P.E).

The capability of the ionic gelation process to form biomolecules loaded chitosan nanoparticles was assessed by FTIR for the determination of plant-chitosan interactions. The spectral analysis of chitosan nanoparticles and bioactive molecules loaded chitosan nanoparticles were depicted in the figure 6(a) and figure 6(b). The IR studies of chitosan nanoparticles (CNPs) revealed the presence of peaks at 3333 cm^{-1}, 2111 cm^{-1}, 1637 cm^{-1}, 1391 cm^{-1}, 1278 cm^{-1}, 528 $cm^{-1}$and 514 cm^{-1} while the FTIR spectra of *J.sambac* plant extract derived chitosan nanoparticles (JSCNPs) revealed strong absorption peaks at 3327 cm^{-1}, 2112 cm^{-1}, 1636 cm^{-1}, 1279 cm^{-1}, 1017 cm^{-1} and 521 cm^{-1}. The spectra observed at 3327 cm^{-1} for the synthesized nanoparticle indicated the presence of O-H bonds. The strong band noticed at 1636 cm^{-1} indicated the presence of phosphorous groups confirming TPP with ammonium group of chitosan. The isolated alkene groups of the C=C stretch was found at 1636 cm^{-1} for the biomolecules loaded chitosan nanoparticle. The C-N stretching of aliphatic amines was observed at the peaks of 1017 cm^{-1} and the aromatic groups were presented with intense peaks at 521 cm^{-1} for the synthesized nanoparticles. It was found that inter and intra-molecular actions were enhanced in chitosan nanoparticles due to polyphosphoric groups of sodium polyphosphate interaction with the ammonium groups of chitosan [19].

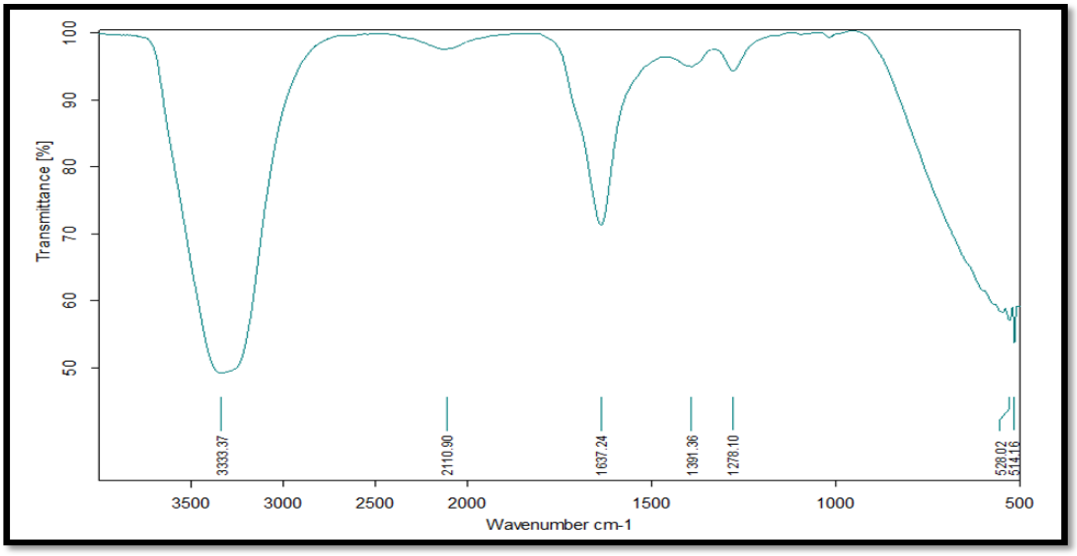

Figure 6(a): FTIR spectral analysis of chitosan nanoparticles

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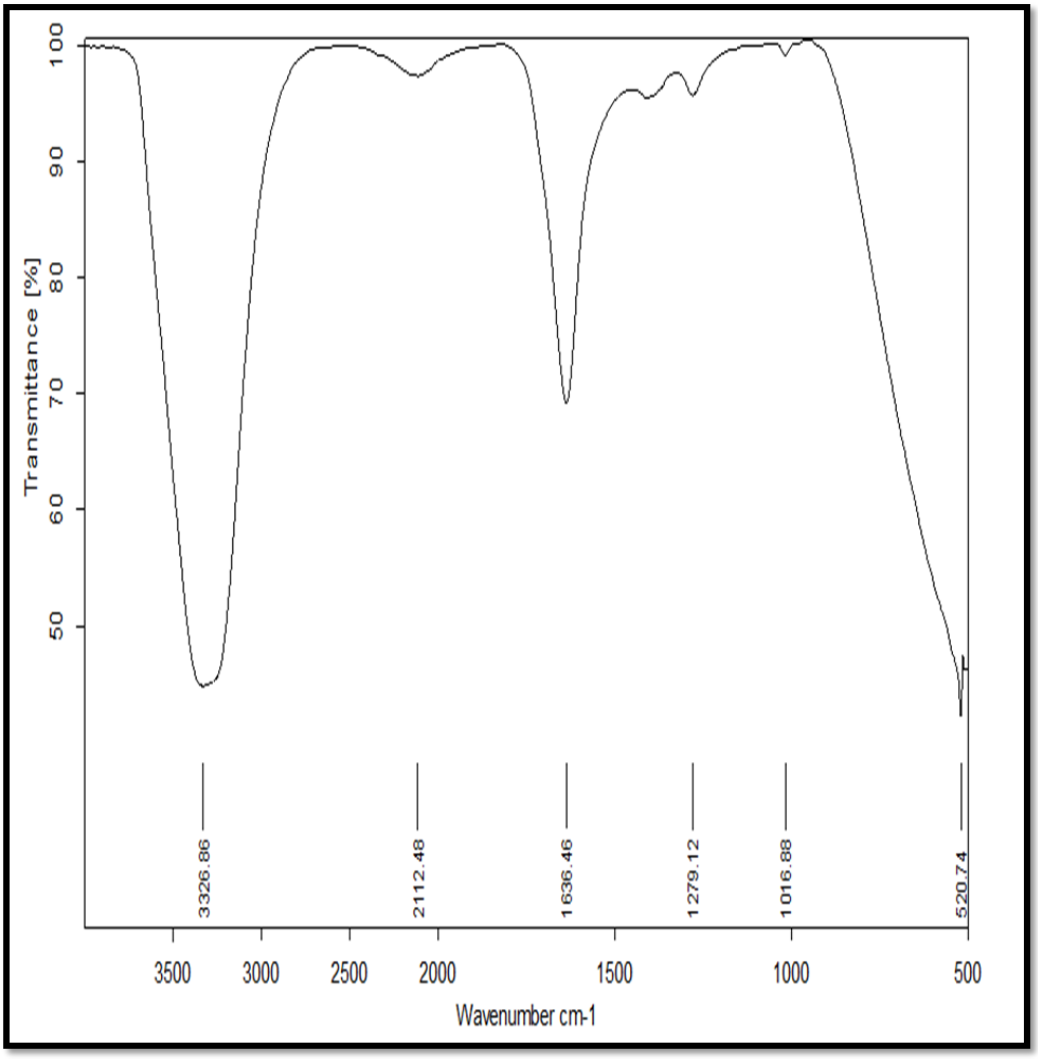

Figure 6(b): FTIR spectral analysis of *J.sambac* leaf extracts derived phytochemicals Cross-linked chitosan nanopolymer

Detection of DNA protection by Chitosan nanopolymer using UV-Spectrophotometer

Free radicals can trigger as well as aggravate damage to cellular DNA in humans. Even a slight partial damage to DNA can make a cell cancerous. In principle, the level of oxidative DNA damage in an organ or cell was studied by the measurement of strand breakage in extracted DNA using UV Spectrophotometer [20]. An optical density (absorbance) value higher than that of standard DNA implied either denaturation or strand breakage. It was noticed that the addition of oxidant to the standard DNA, resulted in increased absorbance at 290nm. On addition of Chitosan-Jasmine nanopolymer, relatively low increase in the absorbance was noted which indicated that the extracts did not have DNA damaging activity.

In figure 7, treatment-1 was the pre-treatment with oxidant followed by treatment with the chitosan-jasmine nanopolymer and treatment-2 was the pre-treatment with chitosan-jasmine nanopolymer followed by treatment with an oxidant. Treatment 1 is an assay of DNA protection provided by the chitosan-jasmine nanopolymer and treatment 2 was performed to evaluate their DNA repair potential. It was noticed that the chitosan-jasmine nanopolymer exhibited a significant DNA protective activity in treatment 1 and moderate-low DNA repair in treatment 2. The results, however indicated that the chitosan-jasmine nanopolymer contemplated highest DNA protective and repair potential in both the treatments followed by the effect of anti-oxidant alone and anti-oxidant treatment with oxidant respectively ,as detected by their optical density values. Conversely, it was observed that oxidant effect on DNA and oxidant treatment by the anti-oxidant exhibited a moderate DNA protective function. This assay was based on the ability of the chitosan-jasmine

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nanopolymer to protect the Salmon DNA against oxidative damage. Salmon milt DNA has a molecular mass of 1.3 x 106 Da (~2,000 bp) and is double stranded [21]. Oxidative damage to DNA caused the denaturation of double strands or fragmentation of strands, both of which gave a higher optical density value at the same concentration as that of dsDNA. Thus, an elevation in optical density values higher than that of standard DNA was a direct measure of DNA damage, and the reduction in these values on treatment with low concentration (1%) of chitosan-jasmine nanopolymer indicated the convincing DNA protection and repair potential.

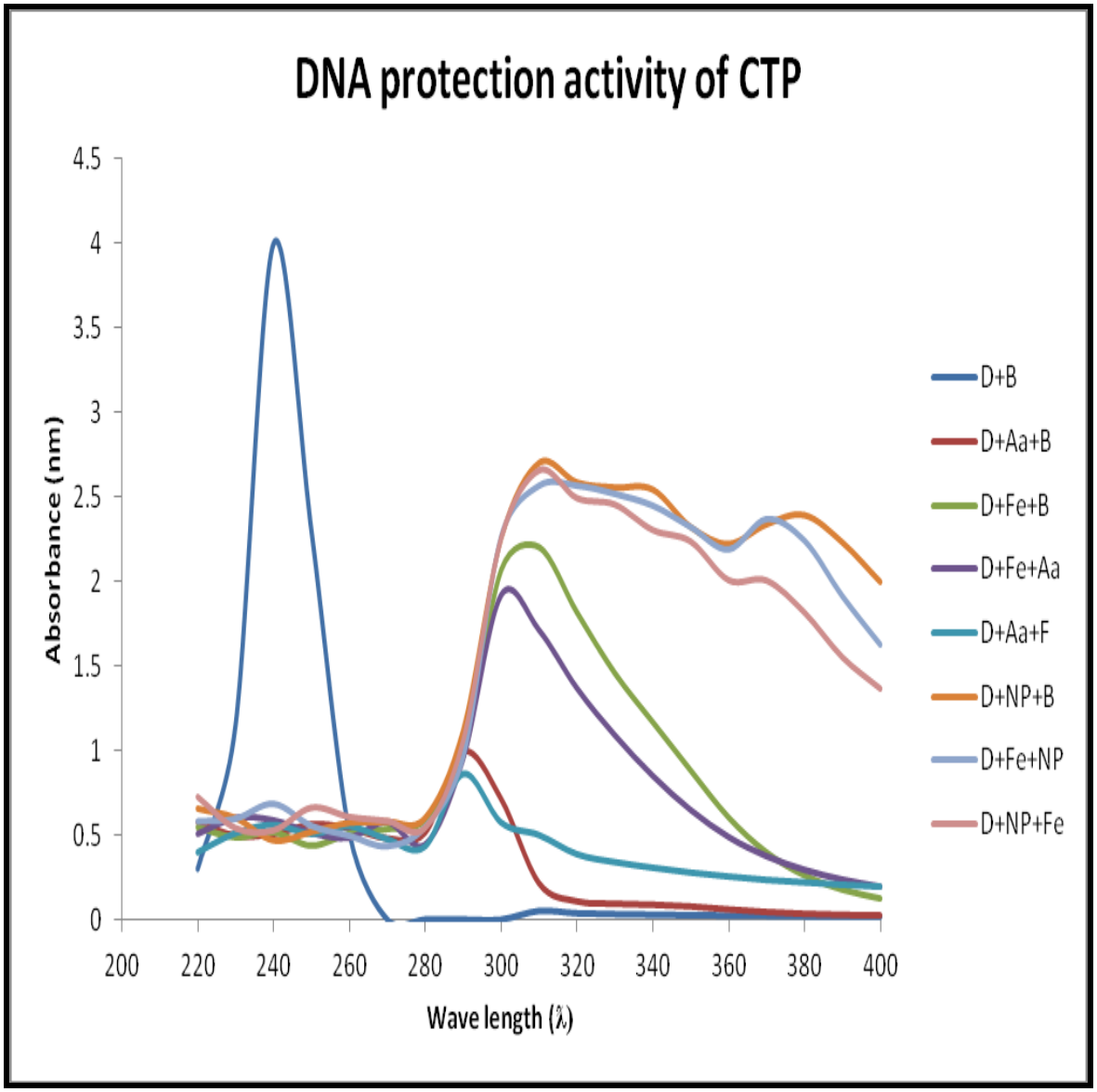

Figure 7: Spectroscopic analysis of DNA protection by Chitosan-Plant extract nanopolymer - (1) DNA+ Buffer, (2) DNA+ Ascorbic acid(anti -oxidant) + Buffer, (3) DNA+ $FeSO_4$ (oxidant) + Buffer , (4) DNA+ $FeSO_4$+ Ascorbic acid, (5) DNA+ Ascorbic acid + $FeSO_4$, (6) DNA+ nanopolymer (NP)+ buffer, (7) DNA+ $FeSO_4$+ nanopolymer (8) DNA+ nanopolymer + $FeSO_4$.

DNA inhibition assay

In the illustrated figure-8, EtBr and DNA with EtBr revealed the least optical density serving as controls for this assay. A slight increased in the optical density was observed by the treatment of oxidant with EtBr which indicated the formation of free radicals by ferrous sulphate on DNA that led to the DNA inhibition, a slight dip in the optical density was observed on treatment with anti-oxidant and EtBr indicating the release of oxidative stress on DNA, an optical density was noted in-between the anti-oxidant and oxidant treated. Furthermore, the addition of oxidant and EtBr to the pre-treated DNA with chitosan-jasmine nanopolymer

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revealed a consecutively highest curve than the silver-CTP nanopolymer optical density indicating that it relatively led to the inhibition of DNA damage. Therefore, it was concluded that chitosan-jasmine nanopolymer conferred a moderate activity of DNA damage inhibition.

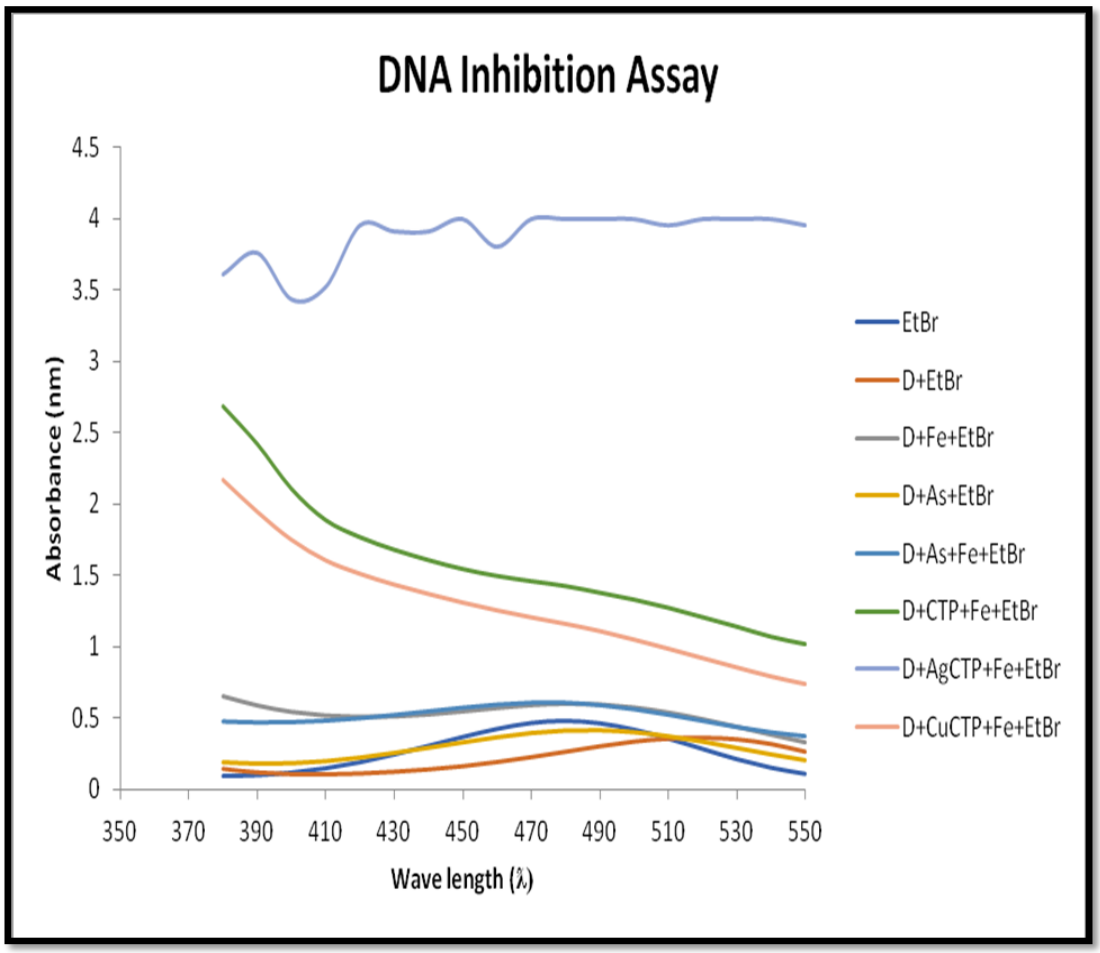

Figure 8: Spectroscopic analysis of DNA inhibition by Chitosan-Plant extract nanopolymer – (1) Ethidium Bromide (EtBr), (2) DNA+ EtBr, (3) DNA + $FeSO_4$ + EtBr, (4) DNA + ascorbic acid + EtBr, (5) DNA + ascorbic acid + $FeSO_4$ + EtBr, (6) DNA + Chitosan nano polymer + $FeSO_4$ + EtBr, (7) DNA + Chitosan- silver nano polymer + $FeSO_4$ + EtBr, (8) DNA + Chitosan-copper nano polymer + $FeSO_4$ + EtBr

DNA Inhibition assay using Agarose gel electrophoresis.

In the figure 9 - Lane 6, 7and 8 represented the results of treatment 1 (i.e, pretreatment of DNA with chitosan-jasmine nanopolymer / chitosan- silver nano polymer plant / chitosan- copper nano polymer plant followed by treatment with the oxidant).

Oxidative stress can lead to several types of DNA damage, including oxidized bases and single- and double-strand breaks [22]. DNA molecules were separated by size within an agarose gel in a pattern such that the distance travelled is inversely proportional to the log of its molecular weight [23]. In the gel picture, light band patterns were visible with the chitosan-jasmine nanopolymer and dark bands were observed in the rest of the wells. Gel electrophoresis showed the negatively supercoiled native form of DNA as the main band, nicked DNA as open circular form and the relaxed closed circular form as minor bands on the gel. Control sample (lane 2) revealed DNA almost in the supercoiled native form with an orange fluorescent band, whereas in lane 3, an oxidative stress due to free radicals formation was observed on DNA following the addition of ferrous sulphate, with a less traverse of blue fluorescent bands. Lane 4 indicated the DNA protection by the addition of ascorbic acid with orange fluorescent bands which was in same lines with the control. In lane 6, 7 and 8 of the gels- DNA was pre-treated with different chitosan nano polymer plant extracts. A potent inhibition

effect towards DNA damage was evident as shown in the figure-9, after the addition of chitosan nanopolymer plant extracts. The order of DNA damage inhibition potential was found to be: Chitosan-copper nanopolymer plant extract> chitosan-jasmine nanopolymer > Chitosan-silver nanopolymer plant extract. These findings were in accordance with those of UV spectrophotometric analysis.

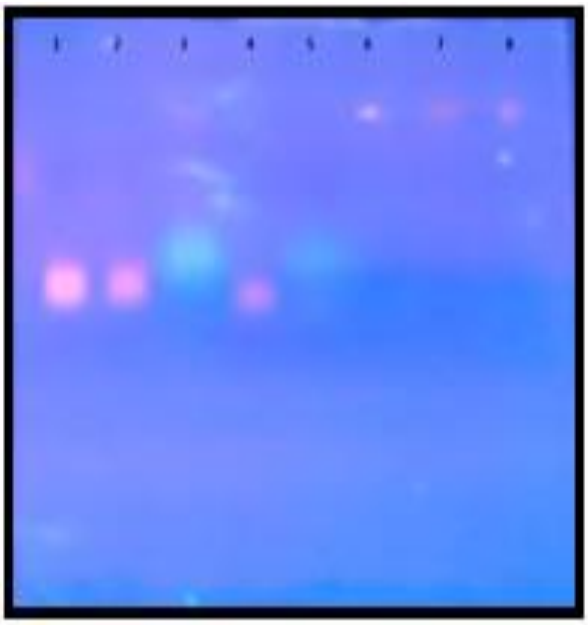

Figure 9: DNA inhibition of nanopolymer observed by agarose gel electrophoresis:
Lane 1: Ethidium Bromide (EtBr); Lane 2: DNA + EtBr; Lane 3: DNA + $FeSO_4$ + EtBr; Lane 4: DNA + ascorbic acid + EtBr; Lane 5: DNA + ascorbic acid + $FeSO_4$ + EtBr; Lane 6: DNA + chitosan-jasmine nanopolymer + $FeSO_4$ + EtBr; Lane 7: DNA + Chitosan-Silver nano polymer + $FeSO_4$ + EtBr; Lane 8: DNA + Chitosan-copper nano polymer + $FeSO_4$ + EtBr.

CONCLUSION

The present study indicated that the leaves of *J. sambac* contained significant amount of various secondary metabolites and the phytochemical studies revealed the presence of alkaloids, phenolics and flavonoids which were further confirmed by thin layer chromatography profiling. Thus, an efficient protocol using the ionic gelation technique for the synthesis of *J. sambac* cross-linked chitosan nanoparticles has been described in the present investigation. The synthesized nanoparticle was characterized by UV spectrophotometer and FTIR analysis. The crystalline nature and the functional group of the *J. sambac* cross-linked chitosan nanoparticles were also studied.

The synthesised chitosan-jasmine nanopolymer conferred DNA protection potency in the order of Chitosan-copper nanopolymer plant extract> chitosan-jasmine nanopolymer > Chitosan-silver nanopolymer plant extract. DNA damage inhibition was also revealed by different samples of nanopolymer in the the order: Chitosan-copper nanopolymer plant extract> chitosan-jasmine nanopolymer > Chitosan-silver nanopolymer plant extract.

Since the biomolecules encapsulated nanoparticles displayed potential ability, the finding of this study opens for its future applications in pharmaceuticals such as antibiotics, carrier in drug delivery, gene delivery, wound dressing, tissue engineering, and cosmetic industry as well as other biotechnological applications.

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