Green Synthesis, Characterization and Antibacterial Activity of Chromium Oxide Nanoparticles Synthesised Using *Ecbolium ligustrinum* Ethanolic Extracts

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ABSTRACT

Chromium Oxide nanoparticles (NPs) possess enormous applications in varied fields. Synthesis of chromium oxide NPs using chemical methods pollute the environment. Hence green method of synthesis of chromium oxide NPs using ethanolic extract of Ecbolium ligustrinum (E. ligustrinum) plant extract is selected for this study. E. ligustrinum plant extract possesses very high antibacterial activity. Synthesis of chromium oxide NPs using E. ligustrinum enhances the antibacterial property of the chromium oxide NPs. The NPs were characterised by UV-Vis and FT-IR The antibacterial activity was tested with bacteria such as E. Coli, Klebsiellza sp., Bacillus sp., Pseudomonas aeruginosa, Staphylococcus aureus. The results show that the antibacterial activity of E. ligustrinum is very high and it can be used as a good antibacterial agent against E. coli, Klebsiellza sp., Bacillus sp., Pseudomonas aeruginosa Staphylococcus aureus.

Keywords: *Green synthesis, antibacterial activity, Chromium oxide nanoparticles, Ecbolium ligustrinum, ethanolic extracts.*

1. Introduction

Extracts of medicinal plants play a key role in obtaining bioactive compounds for various industries. It involves the use of specialised techniques like solvent extraction, distillation and more to isolate and concentrate the beneficial components of plants. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us, a very rich health, wealth, and lot number of diverse plants grow in different parts of countries. India is one of the richest countries in the world with regard to the diversity of medicinal plants. Plants are the primary producers which are the source of many organic substance. Usually, plants contain many types of chemical constituents like alkaloids, glycoside, organic acid, resins, volatile oils, sugar, amino acids, proteins, and enzymes, tannins, plant pigments oils, water and

inorganic ingredients [1,2]. *Ecbolium ligustrinum (E. ligustrinum)* is a well-known medicinal herb, termed "green ice crossandra", green, shrimp plant and turquoise Crossandra. It's flowers maybe green or blue in colour. It is highly used for the treatment of jaundice, menorrhea, rheumatism and anti-inflammatory activity [3]. The extract of the plant *E. ligustrinum as a* reducing agent involves using sustainable solvent like ethanol or water to isolate the active compound. Once extracted the reducing potential can be determined through various assays or tests. The leaves are isolated from ethanol extract. The leaves are the abundant sources of several photo constituents, the existence of traditional claims and finding in literature reports and attempt was made to explore the anti-diabetic and antioxidant properties of leaf extracts [4]. Additionally, the plants have been studied for its potential pharmacological benefits, including its anti-diabetic and hepatoprotective effects [5,6].

Research into the pharmacological properties of *E. ligustrinum* continues to expand shedding light on its potential therapeutic applications and contributing to the exploration of natural sources for healthcare solutions [7].

1.1. Green synthesis and characterisation of plant derived NPs

During the last decade, the concept of "Green Chemistry for Sustainable Development" has been widely investigated, that Sustainable development is described as development that meets the current demands while also balancing the ability of future generations to satisfy their needs. Due to its concern with the evidence of pollution and the indiscriminate use of natural resources, sustainable development is especially important for various chemistry-based sectors. The selection of a green or environmentally friendly solvent (the most widely used are water, ethanol, and their mixtures), a suitable non-toxic reducing agent, and a safe substance for stabilisation are the three most important requirements for the green synthesis of NPs. It is essential that these NPs be precisely and thoroughly characterised in order to ensure reproducibility in their production, biological activity, and safety. For this purpose, a wide range of physicochemical methods are used to very precisely characterise the synthesised NPs including UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FT-IR), etc. [8].

1.2. Methods and Synthesis of NPs

The conventional chemical methods of NPs synthesis have been effectively replaced by NPs mediated by plants. The current study describes the environment friendly synthesis of chromium oxide NPs using *E. ligustrinum* plant extract. The synthesis of Chromium oxide NPs was characterised using UV-Vis spectroscopy [9].

1.2.1. Antibacterial activities

Research on the antibacterial activity of *E. ligustrinum*, also known as *Andrographis paniculata*, suggests that it contains bioactive compounds, particularly andrographolides, which may contribute to its potential antibacterial effects. Some studies have explored its efficacy against various bacteria, indicating a range of inhibitory actions. The antibacterial activity of *E. ligustrinum* has been investigated against both Gram- positive and Gram-negative bacteria. It is believed to interfere with bacterial cell walls and membranes, leading to the inhibition of bacterial growth [10].

2. Materials and Methods

2.1. Preparation of plant extract

The medicinal plant, namely *E. ligustrinum* was collected in the month of December from Samita police, Kanyakumari, Tamil Nadu, India. The fresh leaves of the plants were selected for the study. These leaves were washed with water and then it was dried in room temperature. The dried leaves were crushed with the mortar and pestle. After fine crushing the leaves were packed in cotton clothes. The ethanolic extract of the leaves is prepared by using Soxhlet extractor. The ethanolic extract was collected, kept in an airtight bottle and reserved under normal temperature for further use.

2.2. Synthesis of NPs using *E. ligustrinum*

20 mL of *E. ligustrinum* ethanolic extract was taken and 30 mL of distilled water was added taken in a beaker, 20 g of chromium (III) chloride was also added in the same beaker, heat the 80°C. The mixture was kept in the oven for five hours. Chromium oxide NPs was formed which was indicated by a rapid colour change. The NPs were collected and characterised by UV-Vis spectroscopy and for antibacterial activities.

A buffer solution was prepared using acetic acid and sodium acetate. 0.6 M of acetic acid and 0.82 g of sodium acetate was taken in an SMF and make up to 100 mL. Add both the solution in an iodine flask and add about 1.5 g of chitosan in the same iodine flask, kept it for overnight. The extract and the chitosan solution or mixed together. It was mixed thoroughly in a sonicator at room temperature for one hour. The antibacterial activity of the resulting chromium oxide, incorporated chitosan was also recorded.

3. Results and Discussion

3.1. UV- Visible spectrum of chitosan

The UV-Vis spectrum of chitosan is shown in Fig.1. The peak at 283nm may be due to $\pi \rightarrow \pi^*$ (or) $n \rightarrow \pi^*$ transition.

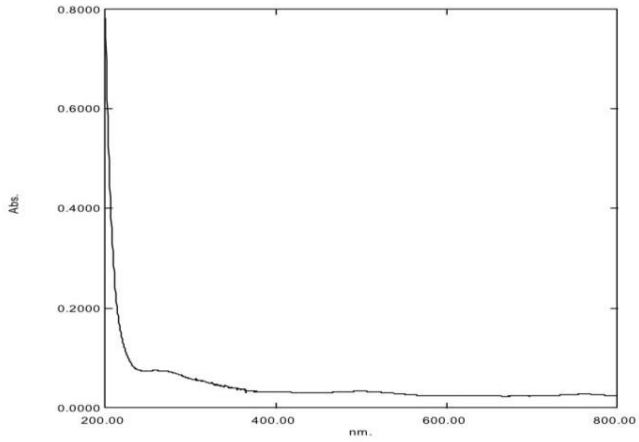

Fig. 1 UV-Visible spectrum of chitosan

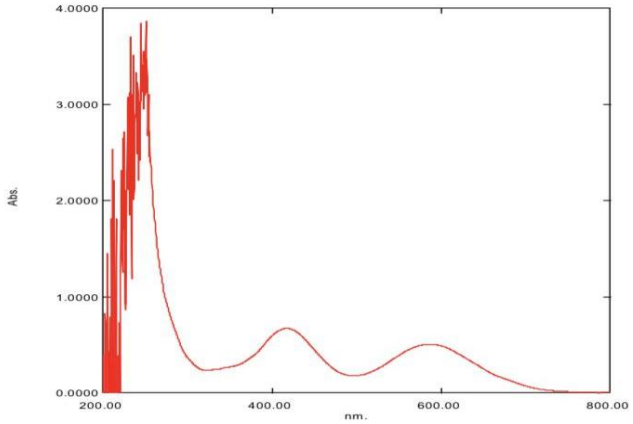

Fig. 2 UV-Visible spectrum of chromium oxide NPs

The absorption wavelength and possible transition of chromium oxide NPs synthesised using the plant extracts is shown in Fig. 2. Chromium oxide NPs showed two peaks around 410 nm and 595 nm. This may be due to $\pi \rightarrow \pi^*$ (or) $n \rightarrow \pi^*$. Similar results are observed in literature [12]. This proved the formation of chromium oxide NPs.

Table 1. Absorption wavelength and possible transition of chromium oxide NPs

Absorption Wavelength (nm)	Possible Transition
410	$\pi \rightarrow \pi^*$ (or) $n \rightarrow \pi^*$
595	$\pi \rightarrow \pi^*$ (or) $n \rightarrow \pi^*$

3.2. FT-IR spectrum of *E. ligustrinum* extract

The FT-IR spectrum of *E. ligustrinum* is shown in Fig. 3. The chacteristic peaks corresponds to the functional groups present. The FT-IR spectrum of *E. ligustrinum* ethanolic extract showed strong broad stretching at 3331 cm^{-1} which may be due to hydrogen bonded alcohol. The strong bands at 2975 cm-^{1}, 2921 cm^{-1}, 2879 cm^{-1} may be due to the C-H stretching and at 1443 cm^{-1} and 1381 cm^{-1} were due to the C-H bending of alkane. C-F bonded alkyl halide produced strong stretching bands at 1080 cm^{-1} and 1040 cm^{-1}. The C-H bond of alkene produced strong bands at 876 cm^{-1} and 616 cm^{-1}. The band at 787 cm^{-1} may be due to C- Cl stretching of alkyl halide.

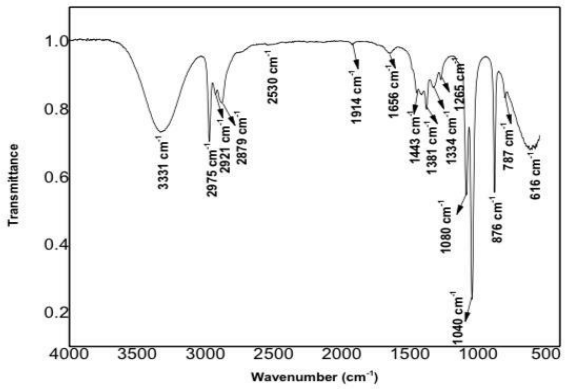

Fig. 3 FT-IR spectrum of *E. ligustrinum* methanolic extract

The absorption frequencies and hence the possible functional group or given in Table 2

Table 2. FT-IR absorption frequencies of *E. ligustrinum m*ethanolic extract

Absorption frequencies	Possible functional groups
3331	Strong, broad stretching of H- bonded –OH group
2975, 2921, 2879	Strong, stretching of C-H bonded alkane
1443, 1381	Bending of C-H bonded alkane
1080, 1040	Strong, stretching of C-F bonded alkyl halide
876, 616	Strong, bending of =C-H of alkene
787	Strong, stretching of C-Cl bond of alkyl halide

3.3. Antibacterial activities

Table 3 shows the zone of inhibition produced by *E. ligustrinum*, Chromium oxide NPs and Chitosan against *E. coli*, *Klebsiella sp.*, *Bacillus sp.*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Table 3 Zone of inhibition of extract, NPs and chitosan

S. No	Bacteria	Zone of inhibition (mm)			
		Control streptomycin	***E. ligustrinum***	**Chromium oxide NPs**	**Chitosan**
1.	*E. coli*	23	23	13	-
2.	*Klebsiella sp.*	21	26	16	-
3.	*Bacillus sp.*	20	20	14	-
4.	*Pseudomonas aeruginosa*	22	23	16	-
5.	*Staphylococcus aureus*	22	28	16	-

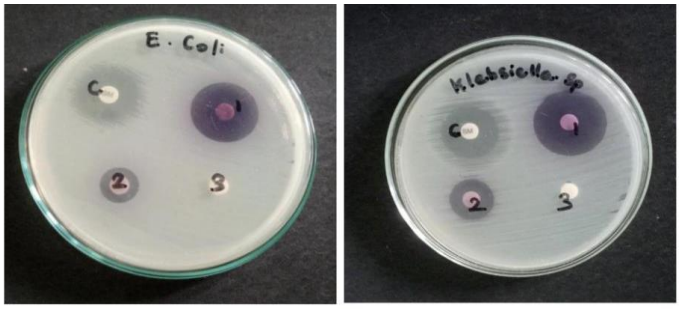

Fig. 4 **a)** **b)**

a) Antibacterial activity against *E. Coli*

b) Antibacterial activity against *Klebsiella sp.*

E. ligustrinum extract 2. Chromium oxide NPs 3. Chitosan

Fig. 4a) Shows the zone of inhibition against *E. coli* and Fig.4 b) shows the zone of inhibition produced against *Klebsiella sp.* The zone of inhibition produced by *E. ligustrinum* against E. coli is greater when compared with the control streptomycin and

chromium oxide NPs. This shows that *E. ligustrinum* acts as a good antibacterial agent than the control. The zone of inhibition produced by *E. ligustrinum* against *Klebsiella sp.* is very high when compared to the control streptomycin and chromium oxide NPs. This shows that *E. ligustrinum* acts as a good antibacterial agent than the control.

Fig. 5 Antibacterial activity against *Bacillus sp.*

Fig. 5 shows the zone of inhibition produced by control, *E. ligustrinum* ethanolic extract, chromium oxide NPs and chromium oxide incorporated chitosan NPs. It is evident that *E. ligustrinum* and show equal antibacterial activity. The antibacterial activity of chromium oxide NPs is lesser than the control and chromium oxide incorporated chitosan NPs does not show antibacterial activity.

Fig. 6 Antibacterial activity against *Pseudomonas aeruginosa*

The antibacterial activity of *E. ligustrinum* ethanolic extract is greater when compared to the control streptomycin. Chromium oxide NPs also show good antibacterial activity against *Pseudomonas aeruginosa.* The zone of inhibition is shown in Fig. 6.

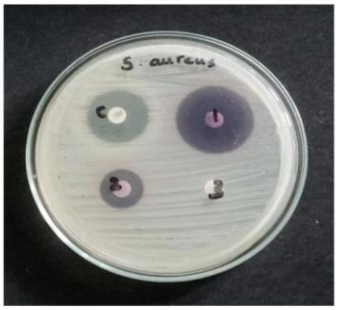

Fig. 7 Antibacterial activity against Staphylococcus aureus

The zone of inhibition produced by *Staphylococcus aureus* is shown in Fig.7. Out of the five species of bacteria chosen for the study, the antibacterial activity of *E. ligustrinum* against *Staphylococcus aureus* is very high. The zone of inhibition produced is nm. This suggest that when compared with the control streptomycin *E. ligustrinum* is very active. Hence it can be used as a good antibacterial agent.

4. Conclusion

The ethanolic extract of *E. ligustrinum* serves as a good reducing agent for the synthesis of chromium oxide NPs. The UV-Vis spectrum proved the formation of chromium oxide NPs. The FT-IR spectrum showed the functional groups present in the plant extracts. The ethanolic extract of *E. ligustrinum* serves as a good antibacterial agent. The control streptomycin shows highest antibacterial activity against *E. coli*. The control streptomycin shows lowest antibacterial activity against *Bacillus sp*. Chromium oxide incorporated chitosan nanocomposites does not show antibacterial activity against the five bacterial species selected for the study. The ethanolic extract of *E. ligustrinum* has very high antibacterial against all the five bacterial species than control streptomycin. *E. ligustrinum* can be used as a good antibacterial agent against *E. coli, Klebsiella sp*., *Bacillus sp., Pseudomonas aeruginosa, Staphylococcus aureus*.

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