EFFECT OF GREEN SYNTHESIZED FENUGREEK METAL-ION NANOPARTICLES ON RESISTANT MICROORGANISM

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ABSTRACT
The present research work is based on Characterization and Evaluation of green synthesized Fenugreek metal ion complexes. The metal ion complexes such as Fenugreek-palladium, Fenugreek-cadmium, Fenugreek-Zinc, Fenugreek-Nickel, Fenugreek-sliver were synthesized and characterized by melting point, UV, SEM, EDS, FT-IR Spectrophotometry. The antimicrobial activities of all complexes using the Disc diffusion method were screened against normal and resistant strains of Escherichia coli and Klebsiella pneumoniae. The minimum inhibitory concentration of the metal ion complexes was determined by the broth dilution method. The antibacterial activity of 1:1 ratio of Fenugreek Palladium Chloride was carried out to study the effect of green synthesized complexes. The melting point of complexes was above 300°c. Scanning Electron Microscope showed that the 1:1 ratio of Fenugreek-Palladium complexes has a different shape in the range from 369nm to 914nm. UV spectra showed a change in absorbance which confirm the formation of complexes. The result of energy dispersive spectrophotometry confirmed the presence of elements Pd, Cl, O, C, N, Pt in the Fenugreek-Palladium complex. The FTIR spectra of all the compounds were scanned in the region of 4000-700 cm. The data showed a change in wavenumber as compared to the standard drug due to the formation of a metal ion complex. Antimicrobial activity studies revealed that complexes showed better activity against resistant organisms than normal organisms. The minimum inhibitory concentration result showed strong antimicrobial activity for all complexes at low concentrations. The present study provides that the combination of Fenugreek-metal ion would be having better antimicrobial activity against resistance strain of bacteria.

KEYWORDS: Fenugreek-metal ion, Nanoparticles, Antimicrobial activity, Resistant Microorganism

1. INTRODUCTION
Despite the diversity of antimicrobial agents, including antibiotics, multidrug resistance has been steadily emerging in past few years. However, because of the unacceptable side effects they cause, as well as adaptation and new resistance mechanisms of microorganisms that emerged and spread globally, researchers have turned to bioactive compounds in the form of plant extracts with antimicrobial properties for treatment. Fenugreek, Trigonella foenum-graecum L. is an annual herb that is widely used as a food, feed additive, and traditional remedy in herbal medicine around the world. Fenugreek contains a high concentration of phytochemicals such as flavonoids, steroids, and alkaloids, which have been found and isolated by pharmaceutical companies or industries for use in the manufacture of hormonal and therapeutic drugs. (Basch et al., 2003) Depending on the concentration of extract,

fenugreek seeds powder extract is effective against bacteria like Bacillus cereus, Staphylococcus aureus, methicillin-resistant E. coli, Staphylococcus aureus, and Pseudomonas aeruginosa, as well as fungi like Candida albicans, Trichophyton rubrum, and Aspergillus flavus.(Benyagoub et al., 2021) Researchers have been concentrating their efforts in recent years on developing efficient green chemistry methods for the synthesis of metal nanoparticles. Metallic nanoparticles such as silver, gold, platinum, zirconium, palladium, iron, cadmium, and metal oxides such as titanium oxide, zinc oxide, and others are synthesised by a variety of microorganisms, including prokaryotes and eukaryotes.(Rizwana et al., 2021) Plants appear to be the best choices among these organisms, as they are well-suited to large-scale nanoparticle biosynthesis. Plant-produced nanoparticles are more stable and have a higher rate of synthesis than microorganism-produced nanoparticles. Plant extracts are favoured for the synthesis of nanoparticles because of their environmental friendliness. The plants serve as a source of both reducing and stabilising agents for nanoparticles, which would otherwise have to be added externally through other means. Recent studies have shown that the therapeutic effects of the plants from which the nanoparticles are derived can also be infused upon the particles, providing us with ideal vehicles for therapeutic materials to act upon the site of action and nullifying the need to develop a drug for that specific ailment artificially. Taking this into account, the present work aimed to study the effect of green synthesized fenugreek metal-ion nanoparticles on resistant microorganism.

2. LITERATURE REVIEW

Fatima A. I. Younis et al., (2018) reported a green synthesis and characterization of Gold Nanoparticles using Fenugreek seeds extract. In present research, cost effective and environmentally friendly gold nanoparticles were synthesized. Biosynthesis of gold nano particles was carried out using the aqueous extract of fenugreek seeds. (Fatima A. I. Younis, 2018)

Nabila H. Hussein et al., reported a green synthesis of silver nano particles using Fenugreek seeds extract. The results confirmed that, the morphology of the prepared nano silver were of spherical shape with smooth surface and average diameter of 17nm. Produced nano silver was tested as antibacterial agent and it is successful against E-coli and staphylococcus aureus due to the increase of the inhibition zone than using 1 molar silver nitrate alone. (Hussein et al., 2018)

Sharma V et al., (2015) reported the antimicrobial activity of trigonella foenum-graceum L. (Fenugreek). It was concluded that out of three solvent extract (methanol, acetone and aqueous) the order of antibacterial activities of solvent extract of fenugreek is methanol>Acetone>Aqueous.

3. OBJECTIVES

The objective of present work was to synthesize and characterized fenugreek seed extract metal ion complexes and to study the antibacterial activity of synthesized product and its comparative effect on antibiotic resistant micro-organisms.

4. EXPERIMENTAL WORK

4.1 Fenugreek seed extract

The seeds were pulverized and the he powder was further utilized to make an aqueous extract with a concentration of 10g/L. This extract was filtered and stored at 40°C until it was needed for the current study. (Deshmukh et al., 2019)

4.2 Synthesis of Fenugreek-metal ion complex

4.2.1 Synthesis of silver chloride fenugreek complex

At 40°C, 50ml of 1mM Agcl2 solution was stirred continuously with 50ml of aqueous extract. After 30 minutes, the reaction mixture's color changed from transparent yellow to light green, indicating the formation of AgNPs. After multiple washes with distilled water, the product sample was collected and dried in an oven at 60^{o}c. The silver nanoparticles made with fenugreek extract were dried and ground before being stored in an airtight container for further analysis.

4.2.2 Synthesis of nickel chloride fenugreek complex

In a 250 ml Erlenmeyer flask, 10 ml of fenugreek seed extract was added to 100 ml of 1 mM aqueous nickel chloride solution. After adding fenugreek seed extract and stirring the resulting solution for homogenous mixing, the colour of the solution changed from green to pale yellow. Ni nanoparticles separated out and settled at the bottom of a mixed solution of nickel chloride and fenugreek seed extract after being maintained at room temperature overnight. Using a Buchner funnel, the extract was filtered. Ni nanoparticles were then dried at 80°C in an oven.

4.2.3 Synthesis of Palladium chloride fenugreek complex

At 40°C, 50ml of 1mM pdcl2 solution was stirred continuously with 50ml of aqueous extract. After 30 minutes, the reaction mixture's colour changed from yellowish green to dark brown, indicating the formation of pdNPs. After multiple washes with distilled water, the product sample was collected and dried in an oven at 60°C. Palladium nanoparticles made with fenugreek extract were dried and ground before being stored in an airtight container for future analysis.

4.2.4 Synthesis of zinc chloride fenugreek complex

At 40°C, 50ml of 1mM Zncl2 solution was stirred continuously with 50ml of the aqueous extract. After 30 minutes, the reaction mixture's colour changed from yellow to dark green, indicating the formation of ZnNPs. After multiple washes with distilled water, the product sample was collected and dried in an oven at 60°C. Zinc nanoparticles made with fenugreek extract were dried and ground into powder before being stored in an airtight container for further analysis.

4.2.5 Synthesis of cadmium chloride fenugreek complex

In a 250 mL Erlenmeyer flask, 10 ml fenugreek seed extract was added to 100 ml 1 mm aqueous cadmium chloride solution. After adding fenugreek seed extract and stirring the resulting solution for homogenous mixing, the colour of the solution changed from green to light brown. Cd nanoparticles separated out and settled at the bottom of a mixed solution of cadmium chloride and fenugreek seed extract after being maintained at room temperature overnight. Using a Buchner funnel, the extract was filtered. The Cd nanoparticles were then dried at 80°C in an oven.

4.3 Characterization Fenugreek metal ion complexes

Characterization of Fenugreek metal ion complexes was done using UV, SEM and EDS

4.4 Antimicrobial activity

The antibacterial activity of the complexes was assayed using the following bacterial species E. coli, Resistance E. coli, K. Pneumonia, Resistance K. Pneumonia. This was done using disc diffusion method in distilled water.

5. RESULT AND DISSCUCION

5.1 Organoleptic properties of fenugreek-metal ion complexes:

5.2 UV visible studies:

The Visual Study of metal complexes production from Fenugreek seed extract in methanol was confirmed by UV Visible spectrophotometer by recording the absorbance from 200-500 nm.

5.3 Antimicrobial activity of Fen-M against bacterial species K. Pneumoniae and Resistance K. Pneumoniae

The activity Fen-M complex against Resistance *K. Pneumoniae* was higher than in K. Pneumoniae.

5.4 Antimicrobial activity of Fen-M against bacterial species E. coli and Resistance E. coli

The activity Fen-M complex against Resistance K. Pneumoniae was higher than in K. Pneumoniae.

5.5 SEM Diagram and Elemental Detection (EDS) of Palladium chloride Complex

SEM confirmed the shape and size of Synthesized complex. SEM image of palladium complex clearly indicates that synthesized palladium complex has average size less than 914 nm with irregular, polygonal, cylindrical and crystalline in nature The elemental composition of various particles observed in the colloidal samples were analysed by Energy Dispersive X-Ray spectroscopy (EDS) and it confirmed the presence of Pd, Cl, O, C, N and less amount platinum.

6. CONCLUSION

The study provides a simple, cost effective and efficient route for synthesis of fenugreek-metal ion complexes. The synthesized complexes were confirmed by UV, SEM, EDS characterization techniques. Antimicrobial studies were done on different bacterial species which included *E. coli*, *Resistance E. coli*, *K. Pneumonia*, *Resistance K. Pneumonia*. The antimicrobial effect of fenugreek-metal ion complexes was observed more in resistant bacteria when compared to Fenugreek. Fen -Cd Complex showed maximum antimicrobial effect.

Table 1: Organoleptic properties of fenugreek-metal ion complexes

Sr. No.	Compounds	Colour	Melting Point	Percentage Yield
1.	Fen-Ag Complex	Yellowish	271-275^{0}C	70.46%
2.	Fen -Ni Complex	Light brown	260-263^{0}C	69.15%
3.	Fen -Cd Complex	Yellow	275-280^{0}C	76.21%
4.	Fen -Pd Complex	Brownish	286-290^{0}C	81.31%
5.	Fen -Zn Complex	Green	282-285^{0}C	80.01%

Table 2: Antimicrobial activity of Fen-M against bacterial species K. Pneumoniae and Resistance K. Pneumoniae

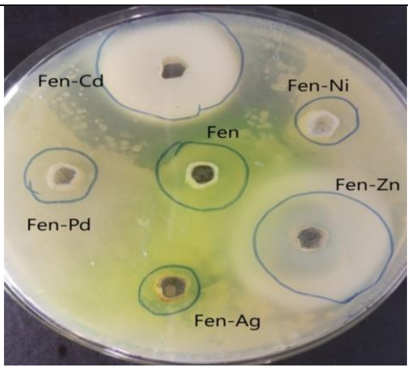
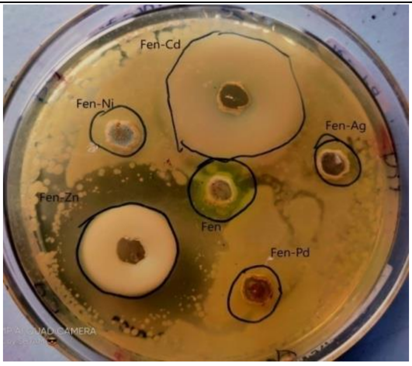
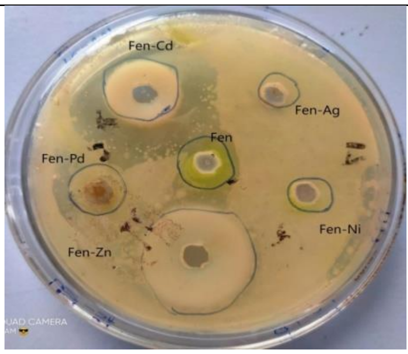
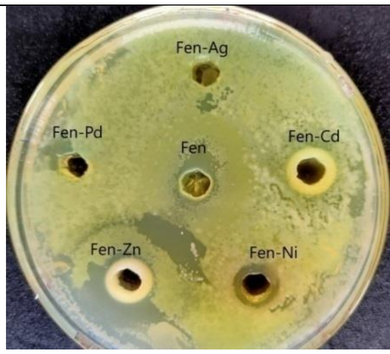
Complex	*K. Pneumoniae*	Resistance *K. Pneumoniae*
Fen-M		

Table 3. Antimicrobial activity of Fen-M against bacterial species E. coli and Resistance E. coli

Complex	*E. coli*	Resistance *E. coli*
Fen-M		

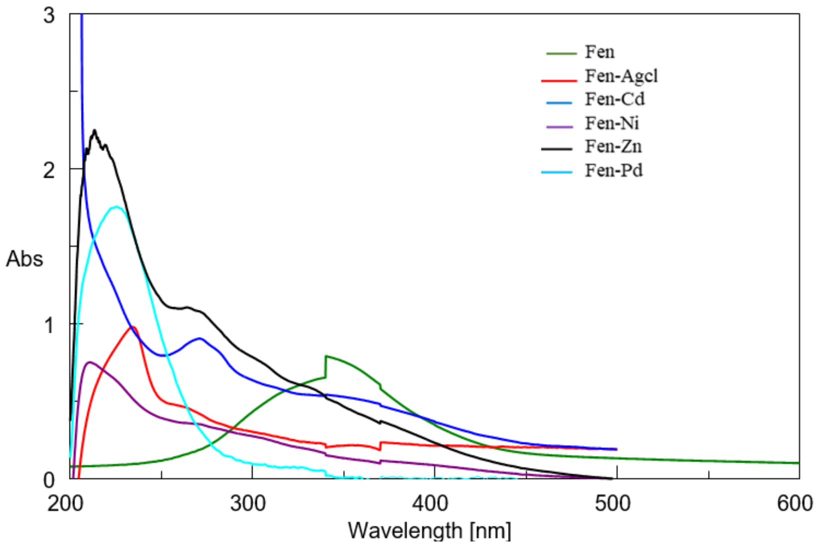


Figure 1: UV-Visible spectra of Fen, Fen-Ag, Fen-Cd, Fen-Ni, Fen-Pd, Fen-Zn complexes in Methanol

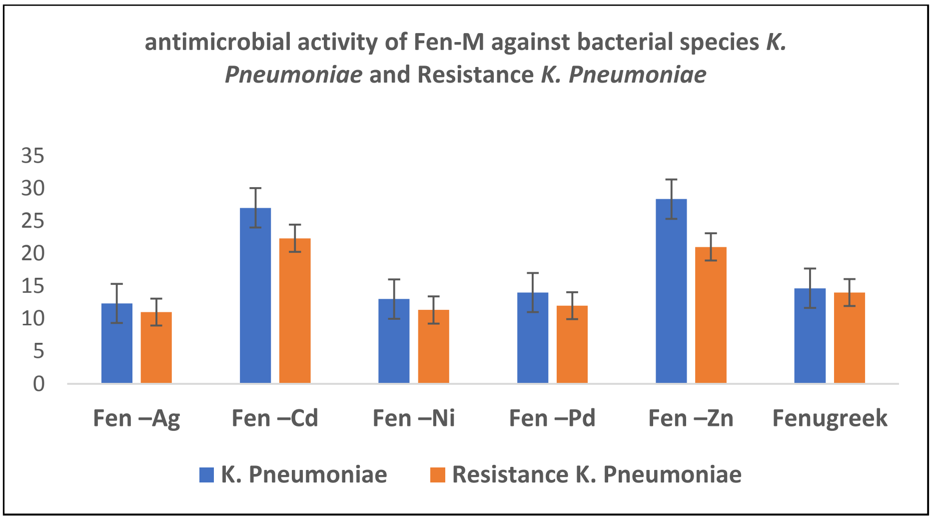


Figure 2: Antimicrobial activity of Fen-M against bacterial species K. Pneumoniae and Resistance K. Pneumoniae

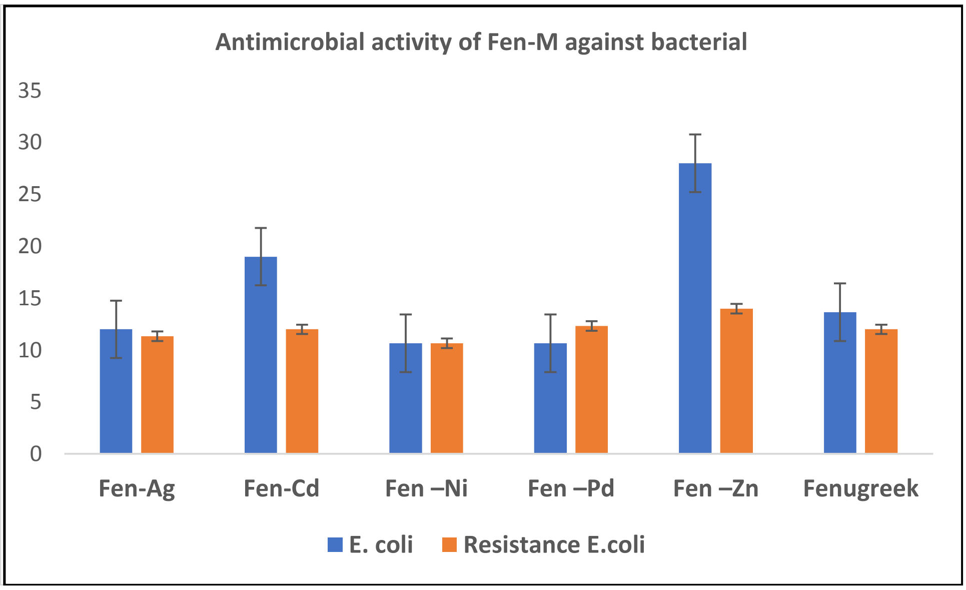


Figure 3: Antimicrobial activity of Fen-M against bacterial species E. coli and Resistance E. coli

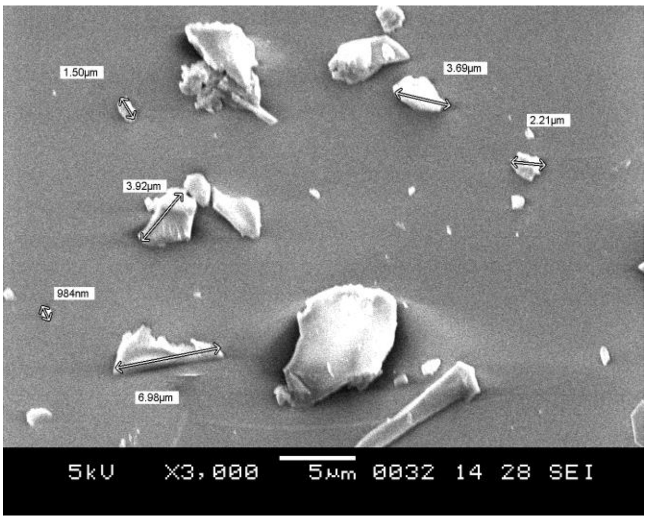


Figure 4: SEM Diagram of Palladium chloride Complex

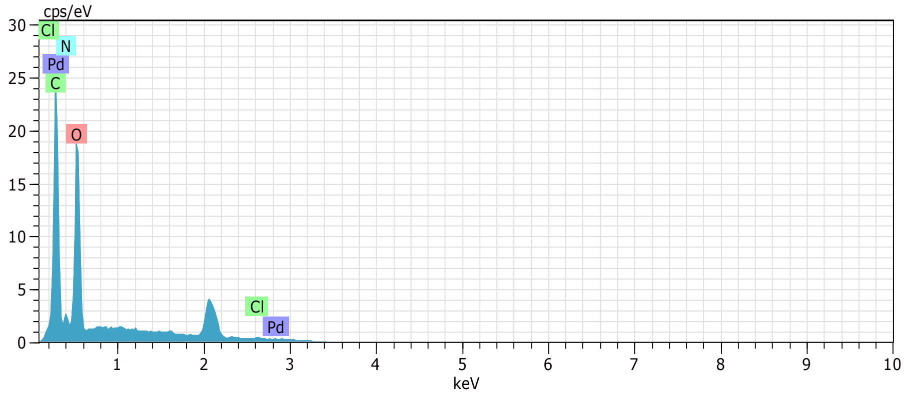


Figure 5: Elemental Detection (EDS) of Palladium chloride Complexes

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