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Green Production Of Silver Chelate Utilizing Soybean Seeds Extract And Analysis Of Its Biological Activities

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ABSTRACT

A rapidly expanding area of study in the field of coordination chemistry is the environment friendly, green- mediated synthesis of inorganic chelates. This paper describes the formation of silver chelate using aqueous extract of soybean seeds without using toxic chemicals. Visual confirmation of the formation of silver chelate was obtained by observing the colour change from light yellow to dark brown. Additionally, an intense peak at 434 nm in the UV-Vis spectrophotometer was observed. FT-IR and ^{1}H NMR analysis were also performed to confirm the formation of chelate. Biological activities were also performed from which *S. aureus* and *C. albicans* shows the best antibacterial and antifungal activities.

Keywords: Aqueous soybean seeds extract, Soxhlet apparatus, Silver chelate, antibacterial, antifungal activities.

INTRODUCTION

Nobel metals like gold, platinum, silver exhibits unique properties at micro or nano-regime[1, 2] which results into many applications such as biotechnology[3], catalysis[4], drug delivery[5], magnetic recordings[6] as well as sensors[7]. Silver has long been known as one of the metal with antimicrobial properties in medical and industrial processes. It has low toxicity, has diverse in vivo and in vitro applications[8]and it is an effective antimicrobial agent. Green chelate synthesis is an environmentally benign strategy that might pave the door for researchers all over the world to investigate the potential of various herbs in order to form chelate. This type of synthesis makes use of plants or bacteria as a source. Plants are thought to be the greatest alternative since they can be easily made available for large-scale production of nanoparticles[9]. There are many methods to synthesize silver chelates may it be in nano-size. Chelates are very stable compounds; they can keep metal ions surrounded by organic molecules (chelating agent) hence precipitate should be avoided. Because of their vast variety of applications in physical and biological sciences, including agriculture and associated sectors, silver chelates have become the subject of intense study[10]. Plants are phytochemical reservoirs; their extracts have been employed in the formation of metal chelates. Keeping this in mind, a current study was

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conducted on the green production of silver chelates utilizing soybean seeds extract. Soybean (glycine max) is the most widely grown leguminous plant which belongs to the family fabaceae[11]. It contains a lot of vitamins, minerals, proteins, and important fatty acids, thus it's a good source of nourishment. It is said to be a herbal plant that helps the liver, heart, stomach, and kidneys operate properly. Legumes are an excellent source of lignins and isoflavones, which are two types of phytoestrogens found in the human food. These molecules have antioxidant property and might help to fight and prevent several pathologies[12]. Isoflavones, a type of plant-derived steroidal molecule, have a vital role in the prevention of heart disease, menopausal symptoms, osteoporosis, and cancer [13]. It can also be found in nature as aglycone and glucoside. 12 isomers of isoflavons in four distinct isoforms, three glucoside conjugates, malonyl-β-glucoside, acetyl-β-glucoside, β-glucoside, and aglycones (genistein and daidzein) have been discovered in soybeans[14, 15]. The main objective of the study was to report the soybean aqueous extract mediated synthesis of silver chelates and its biological properties.

Materials and Method

Reagents

Silver nitrate ($AgNO_3$), pure and fresh soybean was purchased from a local natural products super market and double distilled water.

Soybean seeds extraction method

Soybean seeds were dried at room temperature for ten days. These seeds were weighed, washed 3-4 times with distilled water, and dried again. Dried 50gm seeds were weighed and broken into powder with a pestle and mortar before being extracted with a soxhlet apparatus. The packed powdered soybean (50gm) was put to a thimble. The thimble was placed in the soxhlet apparatus's extraction tube. Approximately 250mL of solvent was put into the flask via the tube. The extraction tube's bottom was connected to a soxhlet flask, while its top was connected to the condenser. Vacuum filtering with Whatman filter paper separated the extracted extract from the insoluble fractions. The filtered extract was kept in the refrigerator for a while.

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Fig.1. Soxhlet apparatus

Preparation of silver nitrate stock solution

The concentration of silver nitrate employed in the synthesis process is 1mM. The $AgNO_3$ stock solution is obtained by dissolving 169.86gm of $AgNO_3$ in 1L of double distilled water.

Silver chelate synthesis

Culture tube of 30ml was taken in which 10 mL of $AgNO_3$ solution was mixed with 1 mL of soybean seeds extract. For around three hours, a culture tube containing the aforementioned solution was exposed to sunlight. While being exposed, the solution begins to turn brown, and after three hours it has transformed into a dark brown color, indicating that the reaction has been finished. Green syntheses of silver chelates were observed by exposing the reaction in sunlight and observing the colour change from colourless to dark brown. Further confirmation was done by UV-Vis, FT-IR and NMR

Fig.2. Reaction mixture (soybean seeds extract and silver nitrate solution) before (left) and after (right) solar exposure

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Results

UV- Vis Analysis

For structural characterization one of the most widely used techniques is Ultraviolet-Visible spectroscopy (UV-Vis). The absorption spectra (Fig. 3.) of the yellowish-brown silver solution generated using the suggested approach revealed an absorption band with a maximum wavelength of 434 nm, suggesting the presence of silver particles[8]. Figure 3 shows the spectrum reading of the reaction combination (silver nitrate and soybean seeds extract).

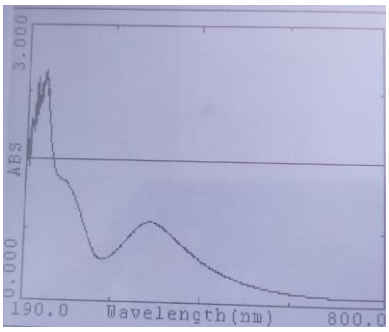

Fig. 3. UV- Vis spectrum of chelate

FT- IR Analysis

The FT-IR (Fourier transform infrared) analysis was utilized to study the interactions of metal ions with amino acid present in *Glycine max* (soybean) seeds. Fig.4 depicts active IR vibrations of newly synthesized chelate. The detected signal at 3328.50 cm-1 is attributed to O-H stretching and may be indicative of intermolecular bonding. Monosubstituted alkene has peaks at 2102.20 cm-1 and 1636.30 cm-1. All of these different peaks show that chelate was formed using soybean seeds extract.

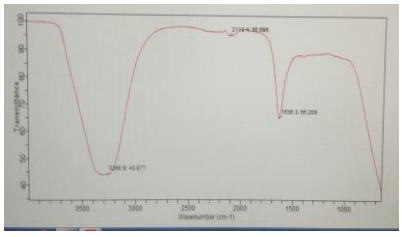

Fig. 4. FT-IR spectrum

^{1}H NMR Analysis

^{1}H NMR (Nuclear magnetic resonance) was used to identify the protonic environment of the chelate. As a solvent, D2O was utilized. The solvent achieves its maximum concentration at 4.72

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ppm. The presence of the vinylic group is shown by the peak at 4.96 ppm. The proton present in the alcoholic group linked to the aromatic ring is shown by the peak 3.86 ppm. The presence of a methanolic group is shown by the peak at 3.48 ppm. The presence of an alkylic group in the molecule is shown by the signal at 1.1 ppm.

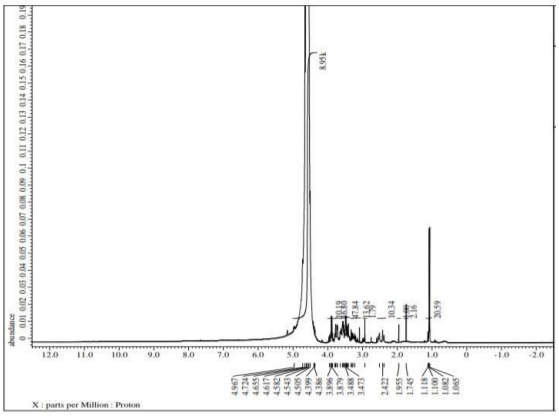

Fig.5. NMR spectrum

Biological activities of the complex

In this antimicrobial and antifungal activities were performed. The activities of the complex are observed as the diameter of the inhibition (Zone of inhibition i.e. ZOI) area growth in mm.

- Antibacterial analysis- For the analysis *Escherichia coli (E. coli.)*, *Pseudomonas aeruginosa (P. aerugiosa)* and *Staphylococcus aureus (S. aureus)* were used.

SI. No.	Pathogens	ZOI (mm)
1	*E. coli*	10.1
2	*P. aerugiosa*	8.4
3	*S. aureus*	12.1

- Antifungal Analysis- For the analysis *Microsporum canis (M. canis), Trichophyton rubrum (T. rubrum), Candida albicans (C. albicans)* and *Epidermophyton floccosum* (*E. floccosum)* were used.

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SI. No.	Pathogens	ZOI (mm)
1	*T. rubrum*	12
2	*M. canis*	11
3	*E. floccosum*	13
4	*C. albicans*	13.2

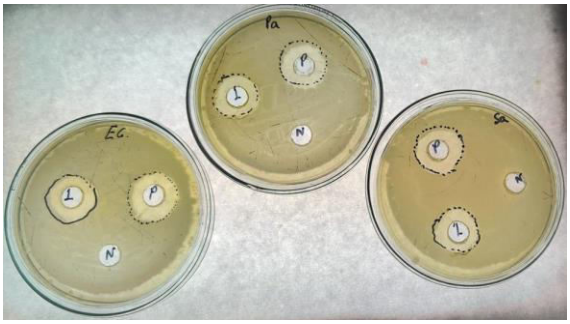

Fig. 6. Antibacterial activity of the chelate along with positive and negative control

DISCUSSION

When 1 mM silver nitrate solution was combined with soybean seeds extract and exposed to intense sunshine for three hours, silver ion decrease was detected. In the early stages of reduction, the color changes from practically colorless to dark brown, indicating the synthesis of silver chelates in the reaction mixture, as depicted (Fig.2). Within 10 minutes, the color changed from colorless to pale yellow, signaling the start of the reaction. The color intensity increased with increased exposure to sunshine over time. The yellow solution ultimately turns dark brown as time passes, which might be owing to increased concentration of complicated molecule as well as particle size decrease. When there was no change in color after three hours, the reaction was completed. UV-Vis, FT-IR, and ^{1}H NMR were used to characterize the newly produced chelate.

The protein and enzymes included in seeds extract cause the reaction mixture's color to change. It aids in the reduction of silver particles, and additional proof was obtained using a UV-Visible spectrophotometer, with a peak identified at 434 nm (Fig.3). The functional groups, mostly flavanoids, contained in the extract were identified by FT-IR analysis. The protonic environment was discovered via NMR. The chelate also shows biological activities such as antibacterial and

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antifungal, with the greatest results obtained against *S. aureus* (ZOI 12.1 mm) and *C. albicans* (ZOI 13.2 mm).

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Conflict of interest

The final text has been approved by all authors, and there are no conflicts of interest.

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