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Biosynthesis and characterization of cotton seed oil cake gold nanoparticles with reference to its anti-inflammatory activity

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Abstract--The gold nanoparticles synthesis is processed using a crude concentrate from cottonseed oil cake. Aqueous gold chloride makes stable nanoparticles of gold with crude concentrate from cottonseed oil cake. The combined gold nanoparticles were distinguished using a scanning electron microscopic study (SEM), UV-VIS spectrophotometer, Fourier transform infrared study and X-ray diffraction method. The gold nanoparticles color observed from yellow to purple to dark. In 1 mM NaCl solution, the gold nanoparticles were stable at pH 7-8, 25-35°C. The synthesized gold nanoparticles show a 568 nm plasmon reverberation. The SEM study additionally proved the gold nanoparticles with a circular morphology. The XRD survey reveals the facecentered, translucent, and cubic shape of the gold nanoparticles. Regardless, at a pH of 6-7 at a temperature of 35-55 °C gold nanoparticles are best. The typical gold nanoparticles size is showed in the 18 to 25 nm range. The presence of carboxylic acids, flavonoids and phenols was confirmed with FT-IR spectrum. With the most remarkable volume of 100 µL, biosynthesized gold nanoparticles showed an extremely strong inhibition of denaturation of 74.09%.

Keywords--Cotton seed oil cake, Gold Nanoparticles, SEM, XRD, FTIR, and denaturation inhibition.

Introduction

The presence in recent years, nanoparticles from various sources have been studied vastly used in the fields of science and innovation as well as medicine. In particular, gold nanoparticles (AuNPs) have been traced in science, medicine, and materials design because of their wide range of physicochemicals and physiochemical optical nature. The physicochemical strategies was maintained for the production of gold and various metallic based nanoparticles (H. Huang and Y. Yang 2008; R. Emmanuel et al. 2014). Especially, the compound combination of

AuNPs can't keep away from the utilization of harmful synthetic compounds (A. Farhangi et al. 2009). Along these lines, the biosynthesis of AuNPs acquired impressive consideration in the previous ten years attributable to their apparent mechanical significance and ecological advantages (D. A. Kumar et al. 2017). A few normal assets like plant crude extracts (T. Selvankumar et al. 2015), microbial secondary metabolites (A. R. Shahverdi et al. 2007), dairy by-products (M. Govarathanan et al. 2013), oilcake (K. J. Lee et al. 2016), and panchakavyam (K. Manoharan et al. 2014) have been investigated for the natural combination of AuNPs. Among the natural courses, plants and agricultural derived industrial wastes based AuNPs blend acquired consideration because of their fast development, simple taking care of, and enormous scope creation.

The cotton plant (*Gossypium hirsutum*) has a place in the Malvaceae family, a **continual** shrub that evolved as yearly. This plant primarily developed for harvesting cotton and used in fabrics and clothing (N. Kanipandian and R. Thirumurugan 2014). Aside from the skeins, cottonseed also has conservative merit. The oil from Cottonseed is an edible obtained from the cotton plant seeds. The Cottonseed Oil Cake was made because of the cottonseed oil producing industry. Because of its high protein content, it is utilized as livestock feed. Late investigations of point by point blending of AuNPs utilizing cottonseed oil cake (N. Kanipandian and R. Thirumurugan 2014). In any case, there is no report on the combination of AgNPs utilizing cottonseed oil cake.

Materials and Methodology

Cotton seed oil cake material preparation

Cottonseed oil cake was brought from local shops in Chennai, Tamil Nadu. The collected cotton seed oil cake was powdered using mixer and 100 gm of powder was extracted using ethanol as a solvent. The ethanolic crude extract was fractionated using maceration method. The ethanolic extract was stand alone for 2 to 3 days for complete extraction. From that point forward, the unrefined concentrate was sifted utilizing Whatman #1 filter paper. The gathered filtrate was aggregated at room temperature.

Method to synthesis gold nanoparticles

The gold nanoparticles were prepared according to the technique of Ahmed S et al. synthesized. (2016) with some changes.

Preparation of metal solutions for gold nanoparticle synthesis

1 mM gold chloride solution (AuCl_3 , Merck) was prepared in 500 mL deionized water and set aside in amber jars to avoid direct light and intensity. The prepared reaction mixture was utilized to prepare gold nanoparticles synthesis (G.V. et al. 2017; Ahmed S et al. 2016).

Method for gold nanoparticles production

In this procedure, 1 mL of crude cottonseed oil cake concentrate was added with 1 mM aqueous gold chloride solution at a (1:15) level with consistent mixing at

room temperature. The solution's yellowish colour quickly turned to a purple color, confirming the gold nanoparticles. The reacting chemical contents were undisturbed for 24 hours to completely reduce gold chloride to gold nanoparticles using crude concentrate from cottonseed oil cake (Ahmed S et al. 2016; G.V. et al. 2017).

Study on stability analysis of biosynthesized NPs

The stable capacity of gold nanoparticles has been tested against temperature, pH and salt (Wan Mat Khalir WKA et al. 2020; Kashan and Javed, Saleem. 2021).

Temperature stress study

The synthesized gold nanoparticles from the crude cottonseed oil cake concentrate were placed on a hot plate. A thermometer was used to check the nanoparticles for thermal expansion. A UV-VIS spectrophotometric analysis was carried out on the nanoparticles heated between 25–100 °C (Wan Mat Khalir WKA et al. 2020).

pH stress study

We used 1M HCl and 1M NaOH solutions to change the pH ranges of gold nanoparticles. This aqueous solution was dropped dropwise on the synthesized gold nanoparticles. From acidic to basic, the pH was changed (3-8) and held for 24 hours or briefly. After incubation of 12 hours, the gold nanoparticles were actually taken a look at utilizing UV-VIS spectrophotometer (Ovais et al. 2018).

Salt stress study

To check the salt stress test for combined gold nanoparticles, different molar focuses (1, 10, 100 mM and 1 M) of sodium chloride were added autonomously to gold nanoparticles in a 1:1 ratio (1 ml NPs mixed with 1 ml saline) and incubated for 12 hours for the time being. The results were then recorded the following day using a UV-VIS spectrometer (Ovais et al. 2018).

Characterization methods for biosynthesized gold nanoparticles

The biosynthesized gold nanoparticles were then read up for the characterization by the accompanying strategies referenced as follows.

UV-visible spectrophotometry Analysis

Biosynthesized gold nanoparticles were examined with an UV-Vis spectrophotometer to affirm the total decrease of gold particles to gold nanoparticles in the presence of crude cottonseed oilcake cake concentrate. The gold nanoparticles were attenuated at a ratio of 1:2 (1 mL AuNp:2 mL deionized water) and analyzed with a UV-Visible spectrophotometer (HITACHI, JAPAN, U2900 SpectroPhotometer). The UV-Vis spectra were recorded from frequencies of 300–800 nm with an interval of 2 nm (Anandalakshmi, K et al. 2016; Ashraf, J.M. et al. 2016).

Scanning electron microscopic examination

The morphology and typical size of nanoparticles were depicted by Scanning Electron Microscope instrument (JSM 5910, JEOL, Japan). A couple of drops of biosynthesized gold NPs in deionized water were poured on a detached glass slide with cover slip. The coverslip was air dried for a surprisingly long time. The items were gold-plated for 2 minutes at 40 Dc milliamps utilizing a gold coater (SPI, USA, Model No. SPI-Spatter Coater). After this, the gold cover was then dissected under SEM at 15 kV/20 kV voltage and at various additions (Banerjee et al., 2014). Acquire, voltage, and content size data was referenced on the pictures. Resulting pictures were dissected utilizing Image J programming (M. et al. 2016; Khan, M.J. 2019).

Study using Fourier Transform Infrared spectroscopy

The Fourier change infrared spectroscopy study was performed to recognize useful functional chemical groups present in gold nanoparticles. The decreased type of nanoparticles from crude concentrate. The freeze-dried crude concentrate and gold nanoparticles were freely grounded with KBR (FTIR grade) and tested with FTIR (SHIMADZU, IR Prestige 21 Model) utilizing movement mode on a 4cm1 objective. The ensuing reach was recorded from 400 to 4000 cm⁻¹ esteem range (Javan Bakht Dalir, S et al. 2020).

X-ray diffraction

The translucent property, size and nature of response combinations were really taken a look at by X-Ray diffraction technique (JEOL X-Ray diffraction, JDX-3532 Model). The combined gold nanoparticles were freeze-dried. The refrigerated nanoparticles powder was used for examination. The well in the point of convergence of the glass test holder was equally stacked with the gold nanoparticles for sporadic transport. (The external layer of the example ought not be higher or lower than the example holder). The gold NPs were then analyzed by XRD utilizing copper K-radiation (1.5404 Å) and handled at 40 kV voltage and 30 milliamps current with 0.2 mm cut, 2 dissipate cut and 1 dissimilarity cut. The XRD configuration was recorded at the 2 Braggs point going from 10 - 70 theta and the magnitude was resolved (Kero Jemal et al. 2017).

$$D = 0.94 \beta \cos \theta$$

Where D = the crystalline size of NPs

λ = the wavelength of the X-Ray (0.154nm)

β = the full width at half maximum of the diffracted peak

θ = the Bragg angle

In vitro anti-inflammatory test by following inhibition of protein denaturation process

The bovine serum albumin denaturation inhibition is one such measure to recognize inflammation action. The impact was driven with the gold nanoparticles to verify their capacity to repress BSA denaturation. A relationship was laid out between the compound quercetin and the biosynthesized gold nanoparticles (Douglas Bosco Aidoo et al. 2021).

Results and Discussion

Gold nanoparticles biosynthesis

Synthesized gold nanoparticles from crude cotton seed oil cake extract includes re-process, in which the gold particles are decreased by the bioactive phytochemicals of the cotton seed oil cake crude to gold nanoparticles. The incorporated nanoparticles have free electrons that result in surface plasmon reverberation detectable by UV-Vis spectrophotometer. The biosynthesized nanoparticles have been studied, optimized and described using different strategies (Zhang, X.F. et al. 2016).

UV-VIS Spectrometer analysis for confirmation of gold nanoparticles

The prepared nanoparticles from gold solution were first examined externally by changing the variety from yellow to purple, which is the sign of decline and the cycle of merging. The color of the reaction mixture changes and becomes darker after 24 hours of incubation. The development of gold nanoparticles has been further considered (Rajkiran Reddy Banala et al. 2015).

UV-Vis Spectrophotometry Detection

The crude concentrate solution obtained from cotton seed oil cake and 1 mM gold chloride aqueous solution was examined in UV-VIS spectrometry. The take-up of gold nanoparticles was shown to have a sharp retention band of 1.721 at 568 nm because of the surface plasmon reverberation of gold nano-particles (Figure No.1) (Rajkiran Reddy Banala et al. 2015).

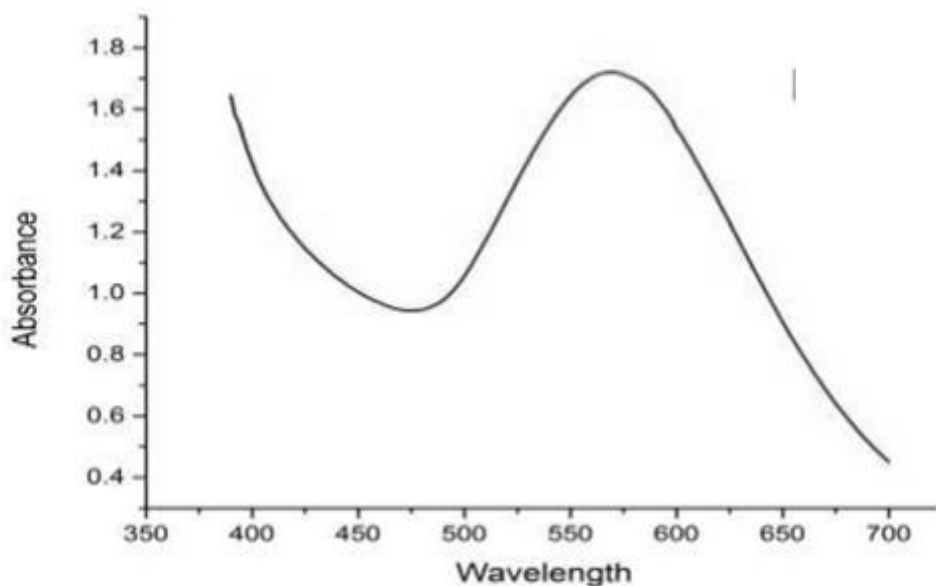


Figure No.1: Gold nanoparticle UV-VIS Spectrum by 1:15 crude concentrate gold chloride solution, showing most elevated band sharp at 568 nm.

Studying the stability of gold nanoparticles

Studying the various temperature impact

As shown in Figure No. 2, the gold nanoparticles were more stable at a lower temperature of 25-35 °C, when tested under a UV-Vis spectrophotometer. At 35-50°C and 50-70°C, gold nanoparticles' UV spectra have a peak area that is lower than the peak at 25-35°C. Similarly, the UV spectrum at 35-50°C and 50-70°C shows that their stability decreases as temperature increases. In addition, the UV range showed no discernible extinction for gold nanoparticles at 50-70°C.

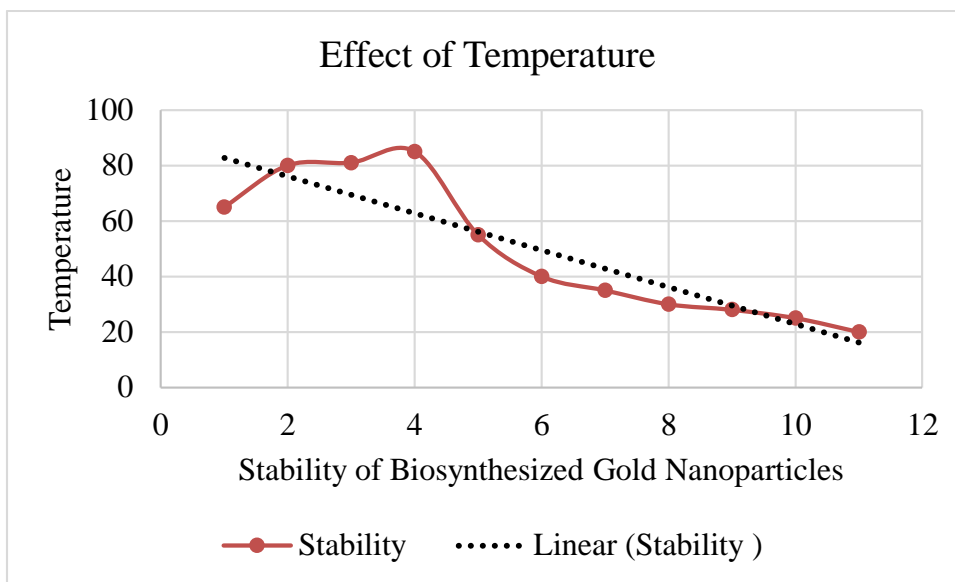


Figure No. 2: Biosynthesized gold nanoparticles are tested at different temperatures from the nanoparticles. Peak intensities are higher at 35-50 °C and lower at 50-70 °C in the UV spectra.

Influence of pH

The pH of the solution also affects the size and state of the nanoparticle. The spectra of gold nanoparticles at different pH values are shown in Figure 5. Gold nanoparticles were observed to be improved at pH 7 to 8. This suggests that the bioreduction process for the combination of gold nanoparticles should be finished at basic pH (Figure No.3), as changing the pH from acidic (3-4) to basic 7-8 also increased the strength of the absorption. Anyhow, a low pH value inhibited gold nanoparticle development, as no or an unknown absorption band was found in the studied region.

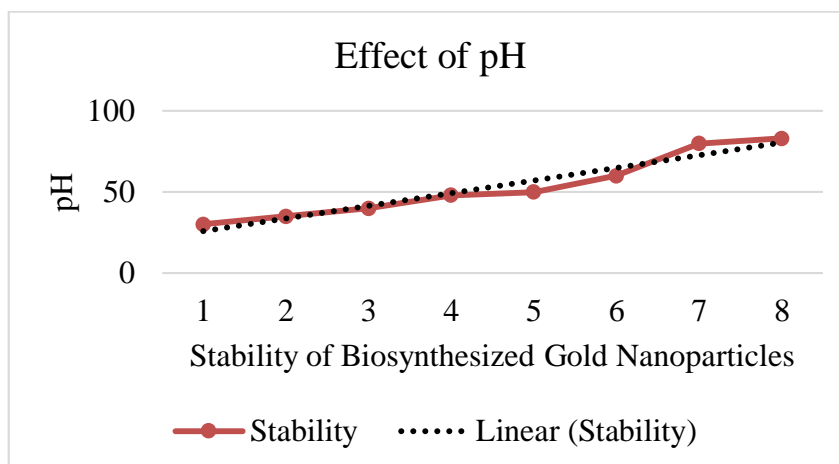


Figure No.3: Effect of pH was studied at alkaline pH of 7 to 8. The absorbance peak and intensity increased by raising pH from acidic (3-4) to basic (7-8).

Influence of NaCl Salt

Figure No.4 illustrates how salt stress affects gold nanoparticle dissolution. 1mM saline showed the best UV-Vis spectrum range. In comparison with different salt solutions, such as 10 mM, 100 mM, and 1M, gold nanoparticles were considered to be more stable with this fixation. According to spectrophotography, the highest concentrations of salt (100 mM and 1 M) inhibited the accumulation of gold nanoparticles, as no noticeable retention peak was located in the plasmon region.

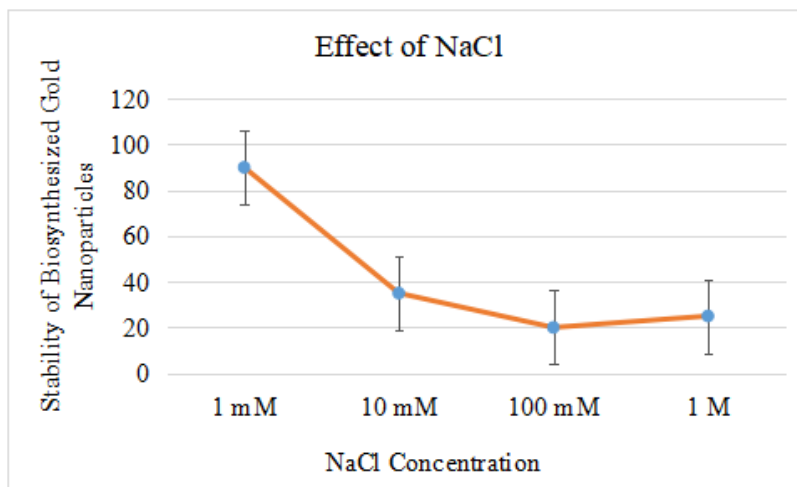


Figure No.4: The UV-Vis spectrum shows that a salt concentration of 1 mM was the best. The stability of the gold nanoparticles was higher compared to other salt concentrations of 10mM, 100mM and 1M.

Scanning electron microscopic study

In addition to providing information on the morphology and size of incorporated gold nanoparticles, scanning electron microscopy is one of the instrumental characterization analyses. Images of test gold nanoparticles at different magnifications (Figure No.5) demonstrate round morphology of the gold nanoparticles. According to image J. programming, the calculated gold nanoparticle size ranged between 18 to 24 nm.

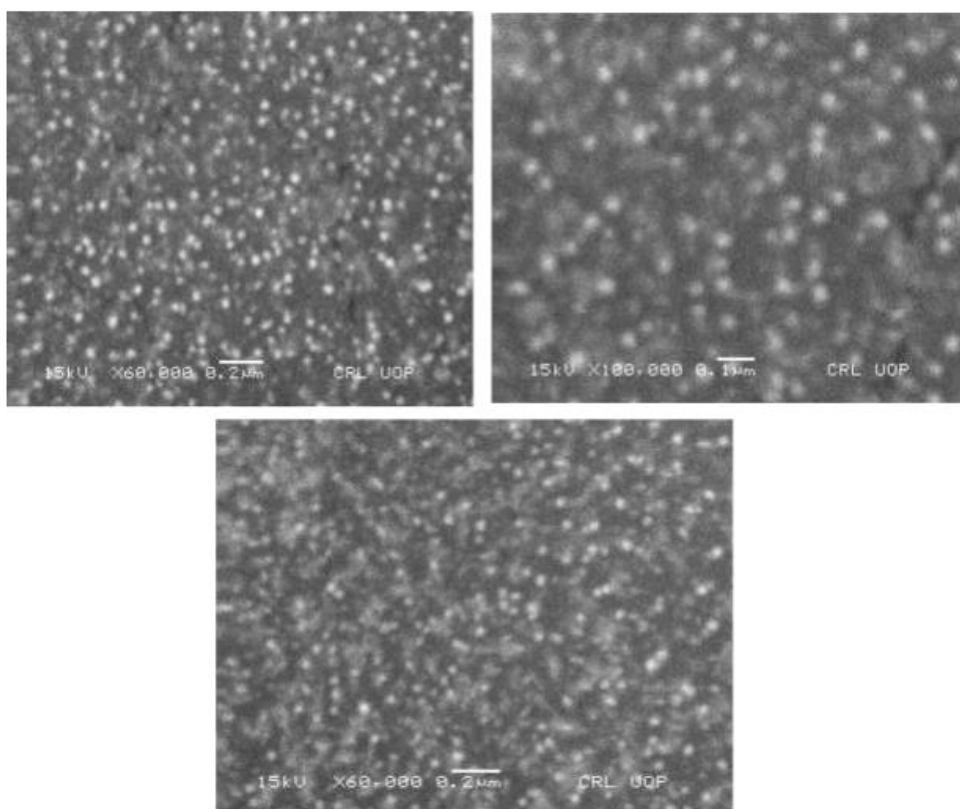


Figure No.5: Examining electron micrographs of synthesized gold nanoparticle at various amplification uncovering circular morphology and size of 18-24 nm.

FTIR spectrum summary

The presence of functional groups in the raw concentrate and its investigation as a reducing agent in the biosynthesis of nanoparticles was confirmed by FTIR investigation. The FTIR range of crude cotton seed oil cake extract addresses functional groups at 3448.7 cm^{-1} , 2677.1 cm^{-1} , 2600 cm^{-1} , 2062 cm^{-1} , 1635.6 cm^{-1} , 1249 cm^{-1} (Figure No.6). A broad band of 3448.7 cm^{-1} is due to the OH-extending vibration of alcoholic and phenolic functional groups. A peak stretch at 2677.1 cm^{-1} and 2600 cm^{-1} relates to the presence of carboxyl groups. The noted peak at 2062 cm^{-1} relates to the NH_3 stretch, showing the presence of free amino acids and their halides. The sharp upper peak at 1635.6 cm^{-1} is assigned to the attached C=C alkenyl group. Nevertheless, the band at 1249 cm^{-1} has a place with the CN stretch of an amine group. The peaks obtained have a place mainly in alkaloids, carboxylic acids and flavonoids, terpenoids, etc., which are the main phytochemicals of cottonseed oil cake, which are responsible for the plant-assisted reduction process related to the nanoparticle synthesis process.

The FTIR study of cottonseed oil cake concentrate and gold nanoparticles. The FTIR range of the example containing gold nanoparticles synthesized using

cottonseed oil cake concentrate and gold chloride solution is introduced in (Figure No.6 and Figure No.7). The FTIR obtained spectrum of the gold nanoparticles shown the potential reduction capacity of the cottonseed oil cake extricate in the bioreduction cycle of nanoparticles. In Figure No.6 uncovered that absorbance band of 2677.1 cm^{-1} initially present in cotton seed oil cake extricate totally vanished in combined gold nanoparticles. The FT-IR spectrum which addresses the carboxylic acid functional group uncovers that the carboxylate content present in cotton seed oil cake for the reduction of gold to gold particles. A significant 3525.8 cm^{-1} - 3448.7 cm^{-1} shift was noticed confirming the phenol, alcohol groups of cotton seed oil cake concentrate to be engaged with the reduction process. The most nearer absorbance and correlation of the spectra of gold nanoparticles and cotton seed oil cake removes uncovered little changes in the wavenumbers of various functional groups asserted the biosynthesis of gold nanoparticles (Figure No.7) from absolutely cotton seed oil cake crude extract.

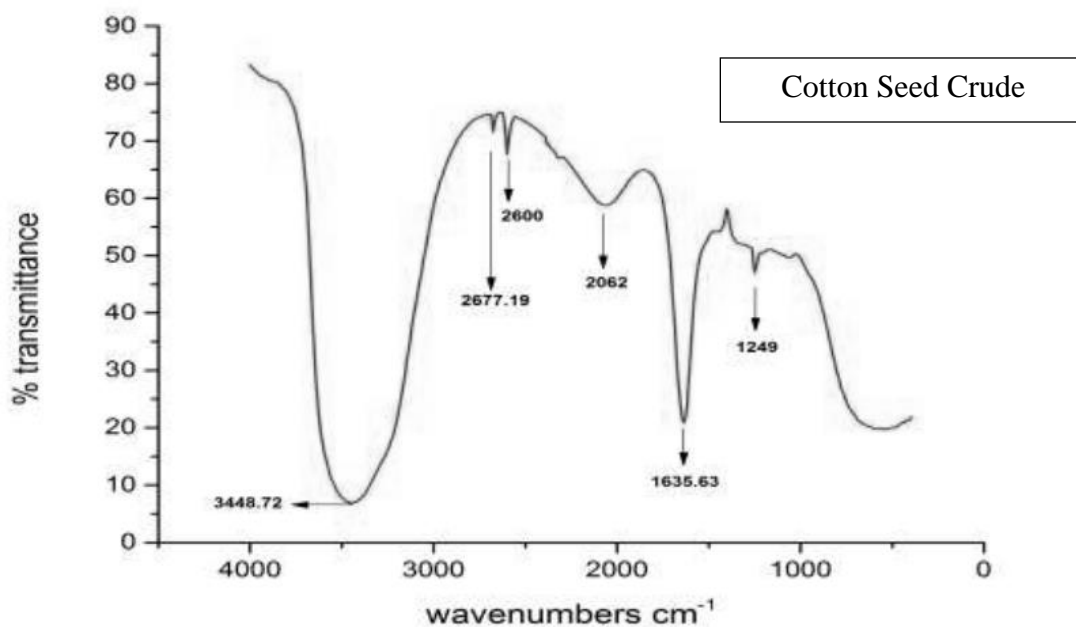


Figure No. 6: FTIR range of crude extract of cottonseed oil cake showing retention groups at wavenumbers cm^{-1} with % transmittance.

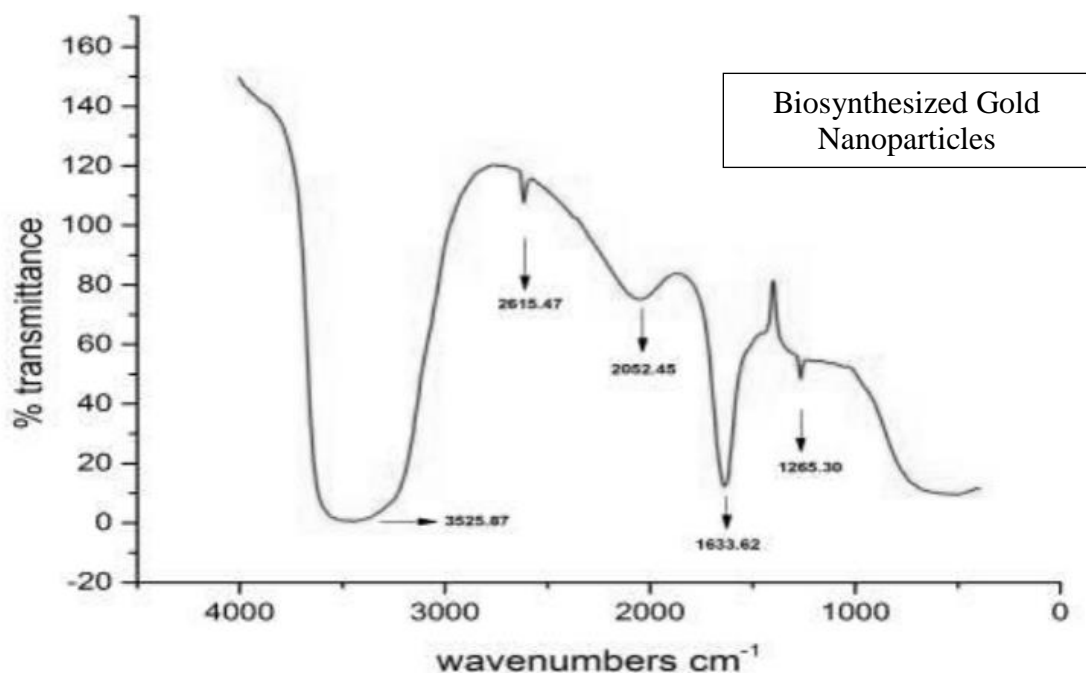


Figure 10: FTIR range of cotton seed oil cake crude incorporated gold nanoparticles showing retention groups at wavenumbers cm^{-1} with % transmittance.

Crystallinity property checking of gold nanoparticles with XRD Analysis

X Ray diffraction was utilized to affirm the crystal phase identification of biosynthesized gold nanoparticles using crude cottonseed oil cake extract. Diffraction powers were recorded at 2 points from 10 to 70 (Figure 8). These obtained diffraction patterns are comparable to (111), (200) and (220) phases in Profex software. The average size of the gold nanoparticles examined was 10.32 nm for the most extreme diffraction pattern of 38.4. The average mean size for different samples was individually obtained as 6.6 and 7.5 nm. Subsequently, the typical size emerged to be 8.1 (Table No.1). The presence of various different peaks and coordinating of XRD design with the Profex software obviously shows that the biosynthesized Gold Nanoparticles were of crystalline nature (Döbelin 2015; Döbelin 2015; Döbelin et al. 2008; McCusker, L. B et al. 1999; Toby, B. H. 2006; Döbelin, N. 2010).

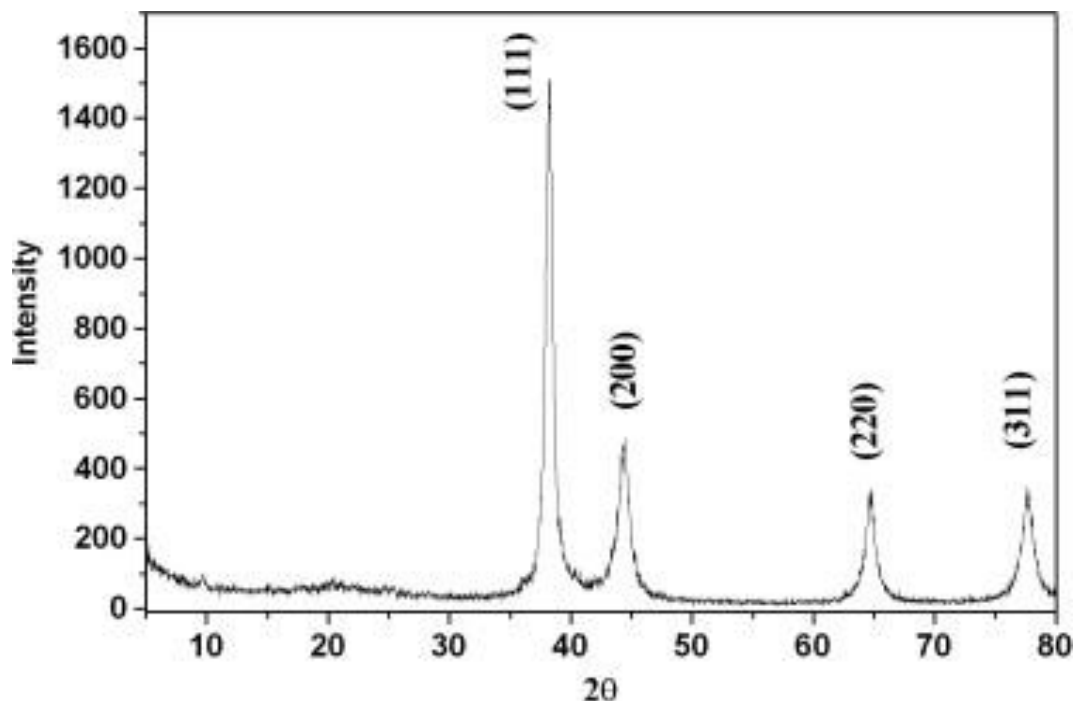


Figure No.8: X Ray diffraction was utilized to affirm the crystal phase identification of biosynthesized gold nanoparticles using crude cottonseed oil cake extract. Diffraction powers were recorded at 2 points from 10 to 70 (Figure No.8). These obtained diffraction patterns are comparable to (111), (200) and (220) phases in Profex software.

Table No.1: Size of biosynthesized Gold nanoparticles (AuNPs)

Test Sample	Peaks Obtained in XRD Study			Average Crystalline Size (nm)
	38 (111) nm	44.5 (200) nm	64.8 (220) nm	
Biosynthesized Gold Nanoparticles	10.36	6.4	7.56	8.10

Anti-inflammatory result

We assessed the anti-inflammation property of the gold nanoparticles and contrasted them with standard control quercetin. In the ongoing study, inflammation reaction was contrasted with denaturation of BSA. Protein denaturation is the essential source of inflammation. The anti-inflammation of the biosynthesized gold nanoparticles was taken into account and showed this

inhibition of protein denaturation. At the most extreme inhibition was shown in 100 μL , the biosynthesized gold nanoparticles showed the most remarkable inhibition of denaturation at 74.09%, and the IC_{50} esteem was viewed as 67.92 μL , while the quercetin showed inhibition at 68.02%. The IC_{50} value for quercetin was given as 73 l. Diclofenac sodium was utilized as a standard medication with anti-inflammation and showed a most extreme inhibition of 76.99% at 100 μL (Figure No. 9). Thus, the information indicates that the biosynthesized gold nanoparticles displayed a higher rate inhibition of protein denaturation than the compound quercetin, demonstrating the adequacy of the biosynthesized gold nanoparticles as an anti-inflammatory drug.

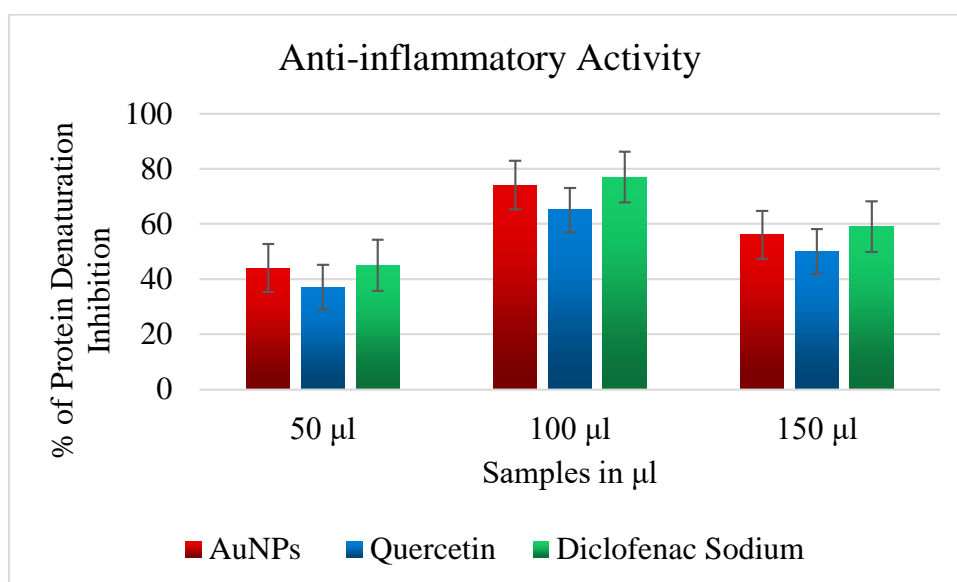


Figure No.9: The anti-inflammatory activity of biosynthesized gold nanoparticles, quercetin and Diclofenac sodium was tested by protein denaturation inhibition by Bovine Serum Albumin. All the values are calculated by taking the mean \pm standard deviation of 3 independent experimental designs.

Conclusion

Our study describes simple, inexpensive, and environmentally friendly agro-industrial by-products mediated by AuNPs using CSOC crude extract. The biosynthesized AuNPs were visualized by nanotechnology characterization techniques. Furthermore, the AuNPs had tremendous anti-inflammatory activity against the bovine serum albumin protein denaturation inhibition assay. At any rate, more experiments are expected prior to applying the AuNPs in bio-nanotechnology and biomedical sciences.

Competing Interests of Authors

The authors proclaim that they have no contending interests.

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