

Novel multitasking gold nanoparticles biosynthesized by *Cassia fistula*: antifungal, anti-obesity, anti-diabetic, and anti-ulcer activities

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Abstract

In this study, an aqueous extract of *Cassia fistula* leaves (CFLE) was employed for the biosynthesis of gold nanoparticles (CFL-AuNPs). The CFL-AuNPs were characterized and evaluated for antifungal, anti-obesity, anti-diabetic, and anti-ulcer activities *in vitro*. The characteristic change in color from colorless to wine red and the UV-visible absorption at 560 nm confirmed the phytosynthesis of CFL-AuNPs. The particles were anisotropic, with spherical and irregular shapes. Fourier transform infrared spectroscopy (FTIR) analysis revealed peaks that correspond to the -OH compound of phenols or alcohol, -NH₂, -N-H amines of protein, and -C=O/-C-O of carbonyl groups. CFL-AuNPs were active against *Aspergillus flavus* (50.70%), *A. fumigatus* (47.73%), *A. niger* (44.29%), and *Fusarium solani* (47.65%). Similarly, CFL-AuNPs exhibited lipase inhibitory activity of 88.93±0.81% with an IC₅₀ of 121.38 µg/ml comparable with standard Orlistat (89.46±0.50%) having an IC₅₀ of 120.51 µg/ml showing anti-obesity potential. CFL-AuNPs also inhibited alpha-glucosidase activity by 42.93±4.12%. Proton potassium (H⁺/K⁺-) ATPase inhibitory assay of CFL-AuNPs showed activity of 84.60±9.54% at IC₅₀ <75 µg/ml which was more efficient than acetaminophen (IC₅₀ of 187.6 µg/ml) with promising anti-ulcer activity. The phytosynthesized CFL-AuNPs exhibited a multitasking nature as demonstrated by the antifungal, anti-obesity, anti-diabetic, and anti-ulcer activities, making it a promising candidate for further study as a potential therapeutic agent for the treatment of multiple diseases. As far as we are aware, this is the foremost report on the *in vitro* evaluation of the anti-ulcer activities of AuNPs.

Introduction

Nanotechnology is a recent advancement in technology that is concerned with the creation, manipulation, and characterization of materials with dimensions ranging from 1-100 nm. It has continued to gain relevance in many fields ranging from agricultural science, food science, computing, chemical, fuel, and energy science, electronics, radiography, microbiology, and pharmaceuticals to biomedical sciences [1, 2]. These vast applications have triggered the possibility of the various ways in which nano-sized materials (nanoparticles) can be synthesized, which include physical, chemical, or biological methods. Both physical and chemical methods can produce pure and well-defined nanoparticles successfully; however, they are expensive and potentially dangerous to the ecosystem [3].

Among these methods, the sustainable green (biological) method has continued to gain much attention owing to its numerous distinguishing properties. This method is swift, economical, and environmentally friendly; it permits the usage of very small amounts of chemicals accompanied by limited toxic by-products [4]. The method involves the use of biological molecules ranging from microorganisms (bacteria, fungi, and microalgae), enzymes, animal metabolites, plant extract/biomass, to agrowastes as reducing and capping agents in the synthesis of nanoparticles [5–7]. However, the plant-mediated synthesis of nanoparticles (NPs) has attracted much research attention due to the ease of preparation of NPs, abundance of plants, the possession of wide arrays of bioactive compounds, and their many applications in biomedical fields. Different nanoparticles including gold (Au), silver (Ag), titanium (Ti),

copper (Cu), and zinc (Zn), among others, have been synthesized using plant materials of different species [8–10].

Gold nanoparticles (AuNPs), amidst the metallic nanoparticles, have been extensively studied for their significant biocompatibility, low toxicity, and unique optical and electronic properties, which make them useful in a variety of biomedical applications [4]. In contrast to silver, gold is not readily oxidized; as such AuNPs can be used for long-term biomedical applications. They have been studied for different applications such as antimicrobial, antioxidant, anti-diabetic, anticoagulant, anticancer, anti-obesity, bio-imaging, biosensing, catalytic, larvicidal, thrombolytic, and photothermal [11–16]. The use of plants in the synthesis of AuNPs is gaining much attention as it offers a cost-effective, eco-friendly and biocompatible alternative to chemical and physical methods [17].

In an attempt to expand the biomedical applications of AuNPs from natural resources, *Cassia fistula*, also known as the Golden shower, of the family Leguminosae was selected in this study. *Cassia fistula* is a medium-sized deciduous tree with characteristic bright yellow flowers and gray bark that blossoms during the rainy season. It has so many beneficial constituents that account for its medicinal values [18, 19]. All plant parts, including the leaf, stem, root, pod, fruit, and flower, are rich in phytochemicals that influence many therapeutic applications, particularly in traditional medicine. *Cassia fistula* leaves have been reported for their flavonoids, tannins, triterpenoids, saponins, steroids, anthraquinones, glycosides, carbohydrates, reducing sugars, amino acids, and proteins content [20]. Several research studies have reported *Cassia fistula* as a medicinal plant possessing properties such as anti-microbial [20], anti-ulcer [21, 22], anti-cancer [23], anti-tussive [24], anti-itching [25], anti-diabetic [26], anti-inflammatory [27], wound healing [28], and insecticidal activities [29].

Furthermore, in recent times, this magic plant, *Cassia fistula*, has gained applications in the green synthesis of nanoparticles such as silver [30], zinc oxide [31], copper oxide [32], and gold nanoparticles [16] with antimicrobial and anti-cancer activities. However, there is no report in the literature related to the *in vitro* anti-obesity and anti-ulcer activities of *C. fistula*-mediated nanoparticles. Though, in recent times, some biosynthesized AuNPs with anti-obesity activities have been reported [13, 33–35], limited reports exist on the evaluation of the anti-ulcer activities of AuNPs [36]. Therefore, this study investigates the biosynthesis of AuNPs using the aqueous extract of *C. fistula* leaves and evaluates their *in vitro* antifungal, anti-obesity, anti-diabetic, and anti-ulcer activities. The findings of this study may open up new possibilities for the development of a single AuNPs-based therapeutic for the treatment of multiple diseases.

Materials and methods

Collection and identification of plants Sample

All collections and experimental research on *Cassia fistula* were conducted in full compliance with the National Policy on the Environment, 2016 (<https://faolex.fao.org/doc/pdf/nig176320.pdf>). Necessary

permissions were obtained from the Botanical Garden of the Department of Pure and Applied Biology, Faculty of Pure and Applied Sciences, Ladoke Akintola University of Technology, and all research activities were carried out with the utmost consideration for the preservation of the plant species, biodiversity, and the natural environment. Fresh and healthy leaves of *Cassia fistula* (CFL) were collected from the Botanical Gardens, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The leaves were taxonomically identified and authenticated. The leaves were cleaned, separated, shredded into pieces, and air-dried for three weeks under ambient conditions ($30 \pm 2^\circ\text{C}$). The dried leaves were milled into powder using an electric blender, sieved using a mesh of 0.5 mm to obtain fine powder, and then stored in an airtight container at room temperature.

Preparation of *Cassia fistula* leaf extract and biosynthesis of AuNPs

The extraction of *Cassia fistula* leaves was done using procedures similar to the method reported by Mohanta *et al.* [37], whereby 0.5 g of pulverized *Cassia fistula* leaf was soaked in 100 ml of sterile distilled water and heated for 1 h at 60°C . The mixture was purified by centrifugation at 4000 rpm for 15 min. The supernatant was then filtered through Whatman filter paper No. 1 and stored at 4°C until further use. The biosynthesis of AuNPs was carried out according to the modified methods of Daisy and Saipriya [38]. Exactly 20 ml of 1 mM gold chloride solution was reacted with 2 ml of *Cassia fistula* leaf extract (CFLE) at room temperature. The timely development and stabilization of color as a function of the formation of AuNPs was monitored.

Characterization of the CFL-AuNPs

The complete formation of AuNPs was monitored visually and quantitatively through the measurement of the absorbance spectra with the use of a UV-visible spectrophotometer. The visual monitoring was done to observe the colour change of the solution, while the absorbance response of the bio-reduction of the Au^+ ions in aqueous solution to Au^0 was monitored using a UV-visible spectrophotometer (Shimadzu UV 1800) in the wavelength range of 250–800 nm. The functional group of the biomolecules responsible for the synthesis, capping, and stabilization of the CFL-AuNPs was ascertained by Fourier-transform infrared (FTIR) spectroscopy using an IRAffinity-1S spectrometer (Shimadzu, UK). The morphological characterization of the biosynthesized CFL-AuNPs was also investigated using a transmission electron microscope (TEM) to ascertain the shape and size of the CFL-AuNPs, while elemental composition was obtained via energy-dispersive X-ray (EDX) spectroscopy [13].

Antifungal activity of CFL-AuNPs

The antifungal activities of the CFL-AuNPs against some food grain fungal isolates were determined using the mycelial growth inhibition method [39]. CFL-AuNPs prepared in different concentrations were incorporated into potato dextrose agar (PDA) and inoculated with agar plug of 6 mm of 48 h-old cultures of *Aspergillus niger*, *A. flavus*, *A. fumigatus*, and *Fusarium solani*. Control experiments were also set up with PDA plates exposed only to the extract without the CFL-AuNPs. All the plates were incubated at $28 \pm 2^\circ\text{C}$ for 72 h. The radial fungal growths in all the plates were measured, and the percentage growth inhibitions were calculated thus:

$$\% \text{ Fungal inhibition} = \frac{D_{\text{control}} - D_{\text{test}}}{D_{\text{control}}} \times 100$$

Where D_{control} is the diameter of fungal growth on the PDA plates without the test agent, and D_{test} is the diameter of the test sample.

Anti-obesity activity of CFL-AuNPs

The *in vitro* inhibition of the pancreatic lipase was chosen as an anti-obesity parameter, and as such, the pancreatic lipase inhibitory property of the CFL-AuNPs was investigated using the porcine pancreatic lipase inhibitory assay [40, 41]. A stock solution (1 mg/ml) of the CFL-AuNPs in 10% DMSO was prepared separately, from which different concentrations were also prepared. Stock solutions of porcine pancreatic lipase enzyme (1 mg/ml) and 20.9 mg p-nitrophenyl butyrate (PNPB) in 2 ml of acetonitrile were also prepared. Then, 0.1 ml of freshly prepared porcine pancreatic lipase was added to 0.2 ml of CFL-AuNPs at different concentrations, made up to 1 ml with the addition of Tri-HCl solution (pH 7.4), and pre-incubated at 25°C for 15 min. Thereafter, 0.1 ml PNPB was added and incubated for another 30 min at 37°C. Pancreatic lipase activity was determined by measuring the hydrolysis of PNPB to p-nitrophenol (pNP) at 405 nm using a UV-visible spectrophotometer. Control experiments were also set up for both plant extract and the standard anti-obesity drug Orlistat using the same concentrations and procedures.

Anti-diabetic activity of CFL-AuNPs

The *in vitro* anti-diabetic activity evaluation of CFL-AuNPs was performed using the α -glucosidase inhibition assay [42]. The reaction mixture contained 70 μ l of 50 mM phosphate buffer (pH 6.8), 10 μ l of test samples (CFLE/CFL-AuNPs) in different concentrations, and 10 μ l of α -glucosidase (0.057 unit). The mixture was incubated at 37 °C for 10 min and an initial absorbance at 400 nm was determined. The reaction was then initiated by the addition of 10 μ l of substrate (0.5 mM P-nitrophenyl- α -glucopyranoside, pNPG) and incubated at 37 °C for 30 min. The absorbance of the resulting p-nitrophenol (pNP) was determined at 400 nm using a Synergy HT microplate reader. Control experiments contained only enzyme and substrate, and α -glucosidase inhibition was calculated thus:

$$\% \text{ Glucosidase Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Where control is the total enzyme activity without inhibitor, and the test is enzyme activity in the presence of the test agent.

Thereafter, IC_{50} values (concentration at which there is a 50% enzyme catalyzed reaction) were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

Anti-ulcer activity of CFL-AuNPs

The anti-ulcer activity of CFL-AuNPs was evaluated *in vitro* using proton potassium (H^+/K^+)-ATPase inhibitory activity as described by Yadav *et al.* [43]. H^+/K^+ -ATPase is a key enzyme in inducing acidity, it is located on the apical secretory membrane of parietal cells. H^+/K^+ -ATPase was prepared from the

mucosal scrapings of healthy rats [44]. The rats were sacrificed, and the stomachs were gently rinsed with tap water. The fundic mucosae were scraped off, homogenized in ice-cold phosphate buffer (pH 7.4), and centrifuged for 20 min at 18,000 rpm. The supernatant obtained was centrifuged again for 60 min at 100,000 rpm. Thereafter, the pellet was homogenized by suspending it again in the buffer. $H^+/-K^+$ -ATPase was then prepared using Ficoll-sucrose discontinuous density gradient centrifugation. The determination of protein was done using Lowry's method [45].

Assay of $H^+/-K^+$ -ATPase activity

A reaction mixture (1 ml) containing 2 mM $MgCl_2$, 10 μg membrane protein, 40 mM Tris-HCl buffer (pH 7.4), and different concentrations of CFL-AuNPs (75, 150, 225, 300, 375 $\mu g/ml$) was set up. The reaction was then instigated with the addition of 2 mM ATP Tris salt and incubated at 37 °C for 20 min. The reaction was stopped by adding 1 ml of ice-cold trichloroacetic acid (10% v/v). The amount of inorganic phosphate released from ATP was determined using a spectrophotometer at 400 nm [43]. The $H^+/-K^+$ -ATPase activity was evaluated in the presence and absence of different doses of the aqueous leaf extract (CFLE), its phytosynthesized gold nanoparticles (CFL-AuNPs), and acetaminophen. Inhibition was estimated as the percentage inhibition against maximal stimulation, while IC_{50} values were obtained from a typical dose-response curve.

Statistical analysis

All experiments were carried out in triplicates, and results were expressed as the mean $\pm$ standard error. Statistical analysis was performed using analysis of variance (ANOVA).

Results and Discussion

Biogenic synthesis of AuNPs

Cassia fistula leaf (CFLE) extract mediated the synthesis of AuNPs within 10 min with the emergence of a wine-red colour, the intensity of the colour gradually increased and finally stabilized within 30 min of synthesis (Fig. 1). Several colour changes have been reported in the biosynthesis of AuNPs ranging from purple, reddish brown, ruby red, dark green, pinkish, and yellowish [46, 11, 8, 14]. The formation of CFL-AuNPs as indicated by the development of colour may be due to the excitation of surface plasmon resonance of the reduced Au^+ particles, which was enabled by the bioreducing potential of the aqueous leaf extract of *Cassia fistula*. The CFL-AuNPs synthesized maintained high stability, void of any form of aggregation or deterioration over time. The peak absorbance exhibited by the biosynthesized CFL-AuNPs at 560 nm (Fig. 2) was within the range reported by several authors [11, 8, 13, 14].

FTIR spectra of CFL-AuNPs (Fig. 3) showed peaks at 3992, 2393, 1653, 1391, and 1081 cm^{-1} that correspond to O-H stretch, and H-bond, O = C = O, N-H stretch, C-H stretch, and C-N stretch, respectively [47]. This result revealed that alcohols, phenols, alkanes, aldehydes, carbonyl groups, and aliphatic amines that were present in the extract of *Cassia fistula* influenced the biofabrication of the AuNPs. The

transmission electron micrograph of CFL-AuNPs synthesized from leaf extract of *Cassia fistula* and the EDX spectrum is shown in Fig. 4. The CFL-AuNPs were irregularly spherical, with sizes of 10–50 nm. The EDX showed prominence of elemental gold with 75.2% wt.

Antifungal Activities of Phytosynthesized Gold Nanoparticles

The phytosynthesized CFL-AuNPs inhibited the growth of *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium solani*, and *Aspergillus niger* at tested concentrations of 75 and 100 µg/ml (Fig. 5). The percentage fungal growth inhibitions obtained were 50.70, 47.73, 47.65, and 44.29% for *A. flavus*, *A. fumigatus*, *F. solani*, and *A. niger*, respectively. Conversely, the aqueous extract of *Cassia fistula* leaves and gold chloride solution showed little or no growth inhibition against the test isolates. Results obtained in this study are similar to AuNPs phytosynthesized by aqueous extract of *Opuntia ficus-indica* which produced fungal growth inhibition of 42–64% at a concentration of 200 µg/ml against *Candida albicans*, *A. niger*, *A. flavus*, *A. fumigatus* and *F. solani* [8]. However, the AuNPs biofabricated using fungal xylanase of *A. niger* L3 (NEA) and *Trichoderma longibrachiatum* L2 (TEA) displayed higher antifungal activities of 74–84% against *A. niger*, *A. flavus*, and *A. fumigatus* [14]. Also, Jayaseelan *et al.* [48] also reported the antifungal potency of AuNPs synthesized from an aqueous extract of *Abelmoschus esculentus* seed against *A. niger*, *A. flavus*, *Puccinia graminis tritici*, and *Candida albicans*. The biosynthesized AuNPs exhibited maximum growth inhibition against *P. graminis* (17 mm) and *C. albicans* (18 mm). The antifungal potency of AuNPs synthesized using leaf extracts of *Annona muricata* was between 30 and 60% against *A. flavus*, *C. albicans*, *F. oxysporium*, and *Penicillium camemeri* [49].

The fungal growth inhibitory potentials of AuNPs can be attributed to the acidification of the intracellular environment by inhibition of H⁺-ATPase in the fungal cells [50]. Antifungal properties of nanoparticles have also been documented to be particle size dependent; the smaller the particle size, the higher the antifungal property [51]. The small size of the particles enhances easy penetration into the fungal cell wall, cumulating into a series of events that eventually lead to the death of fungal cells [14].

Anti-obesity activity of CFL-AuNPs

The pancreatic lipase inhibitory activities of CFL-AuNPs are reported in Table 1. CFL-AuNPs showed excellent dose-dependent pancreatic lipase inhibitory activity comparable with Orlistat standard. CFL-AuNPs demonstrated maximum lipase inhibitory activity of 88.93 ± 0.81% at an IC₅₀ value of 121.3 µg/ml which was similar to Orlistat's IC₅₀ value of 120.5 µg/ml with the highest inhibitory activity of 89.46 ± 0.50%. The lipase inhibitory activity exhibited by CFL-AuNPs was higher than that of CFLE, which had a maximum inhibitory activity of 33.44 ± 0.93%.

Table 1
Pancreatic lipase inhibitory activities of CFL-AuNPs

Concentration ($\mu\text{g/ml}$)		Percentage lipase inhibition (%)					IC ₅₀
		50	100	150	200	250	
Sample	CFLE	28.69 $\pm$ 1.16	33.20 $\pm$ 0.58	27.46 $\pm$ 0.58	46.72 $\pm$ 1.16	33.44 $\pm$ 0.93	-
	CFL-AuNPs	17.62 $\pm$ 0.58	59.43 $\pm$ 0.58	64.92 $\pm$ 1.16	74.59 $\pm$ 1.16	88.93 $\pm$ 0.81	121.3
	Orlistat	42.63 $\pm$ 0.21	55.91 $\pm$ 0.10	74.11 $\pm$ 0.25	88.86 $\pm$ 0.04	89.46 $\pm$ 0.50	120.5

Similarly, Anand *et al.* [52] reported the *in vitro* lipase inhibitory activities of *Ficus racemosa* extract. The aqueous extract showed prominent pancreatic lipase inhibitory potential in a dose-dependent manner, ranging from 12 to 46%, with maximum inhibitory activity at a concentration of 25 $\mu\text{g/ml}$. In another study, hydroethanolic leaf extract of *C. fistula* exhibited pancreatic lipase inhibitory activity with an IC₅₀ of 30.5 $\pm$ 2.8 $\mu\text{g/ml}$ and 17.31 $\pm$ 1.18 $\mu\text{g/ml}$ [53]. Also, the n-hexane leaf extract of *C. bakeriana* Craib showed high inhibition of pancreatic lipase activity with an IC₅₀ of 25.27 $\pm$ 8.78 $\mu\text{g/ml}$ [54]. From the present study, it was evidently clear that the biofabricated CFL-AuNPs improved the inhibition of pancreatic lipase, which can make them suitable for the control of obesity through enzyme inhibition. As far as we are aware, this is the foremost report on the pancreatic lipase inhibitory activity of *Cassia fistula*-mediated AuNPs. However, AuNPs synthesized with *Smilax glabra* rhizome were reported for their anti-obesity property in a high-fat diet and streptozotocin-induced obese diabetes rat model, where tremendous body mass and weight reduction in diabetic and obese rats treated with the synthesized AuNPs were recorded [13].

Pancreatic lipase is responsible for the digestion of fats and triglycerides, and its inhibition is one of the therapeutic strategies in the management of obesity. It is also a key approach used to determine the potency of natural products as anti-obesity agents. The mechanism involves the inhibition of dietary triglyceride absorption, as this is the main source of excess calories [55]. Obesity is a recognized risk factor in the development of metabolic disorders such as, hyperlipidemia, diabetes mellitus, hypertension, asthma, certain cancers, and cardiovascular disease [40], while also being recognized as a major health problem in humans [56].

Alpha-glucosidase inhibitory activity of CFL-AuNPs

The inhibitory effect of CFL-AuNPs on α -glucosidase activity is shown in Table 2. CFL-AuNPs demonstrated a moderate α -glucosidase inhibitory potential in a concentration-dependent manner with a maximum inhibitory percentage of 42.93 $\pm$ 4.12%. This result suggests that CFL-AuNPs are more potent inhibitors of α -glucosidase activity than CFLE alone, which had the highest activity of 4.17 $\pm$ 2.24%. This study is similar to previous studies that have investigated the inhibitory effects of plant-mediated AuNPs on α -glucosidase. AuNPs synthesized from peels of *Padina boerghesii* were reported to be potent

inhibitors of α -glucosidase with an IC_{50} of 24 $\mu\text{g/ml}$ [42]. El-manawaty and Gohar [57] reported the methanolic bark extract of *Cassia fistula* with excellent α -glucosidase inhibition at an IC_{50} of 7.80 ± 1.9 ppm, while the dichloromethane fraction of *C. bakeriana* was also reported to be active in the inhibition of α -glucosidase activity with an IC_{50} of 359.55 ± 2.90 $\mu\text{g/ml}$ [54].

Table 2

Alpha-glucosidase inhibitory activities of CFL-AuNPs

Sample	Concentration ($\mu\text{g/ml}$)	Percentage Inhibition
CFLE	75	2.99 ± 0.89
	150	2.66 ± 0.29
	225	4.02 ± 1.05
	300	4.17 ± 2.24
	375	$3.13 \pm 0.82XXX$
CFL-AuNPs	75	1.54 ± 1.04
	150	6.37 ± 4.38
	225	8.63 ± 7.10
	300	39.57 ± 8.37
	375	42.93 ± 4.12

Inhibitors of α -glucosidase have been used as treatment for type 2 diabetes, by delaying the digestion of carbohydrates, which leads to a reduction in postprandial hyperglycemia [58]. It is also worth mentioning that this inhibitory effect may also have implications for the management of obesity, as α -glucosidase inhibitors have been proposed to have anti-obesity effects by regulating carbohydrate metabolism and thus reducing calorie intake [54, 58].

Anti-ulcer activity of CFL-AuNPs

$H^+/-K^+$ -ATPase is a key enzyme in inducing acidity, it is located on the apical secretory membrane of parietal cells. The proton-potassium ATPase inhibitory activities of the phytosynthesized gold nanoparticles (CFL-AuNPs) are shown in Table 3. CFL-AuNPs demonstrated outstanding $H^+/-K^+$ -ATPase inhibitory activities in a concentration-dependent manner ranging from 55.02 ± 0.00 to $84.60 \pm 9.54\%$ with an IC_{50} value lower than 75 $\mu\text{g/ml}$. However, CFLE also showed $H^+/-K^+$ -ATPase inhibitory activity with a maximum percentage inhibition of $76.47 \pm 0.49\%$, which is lower than the inhibitory percentage of CFL-AuNPs for the same concentration. This indicates that the CFL-AuNPs have an increasing effect on the inhibitory activity of CFLE on $H^+/-K^+$ -ATPase. It can be observed that the IC_{50} for acetaminophen standard was 187.6 $\mu\text{g/ml}$, while for CFLE and CFL-AuNPs, the IC_{50} was lower than 75 $\mu\text{g/ml}$. This means that CFLE and CFL-AuNPs were more potent inhibitors of $H^+/-K^+$ -ATPase activity than acetaminophen. This

finding is consistent with previous studies that have investigated the effects of some plant materials on H^+/K^+ -ATPase activity. Yadav *et al.* [43] reported the inhibitory effect of methanolic extract of *Cissus quadrangularis* on H^+/K^+ -ATPase activity, with an IC_{50} value of 38 $\mu\text{g/ml}$. However, there is a paucity of information on the use of AuNPs as anti-ulcer agents. The only existing report by Abdulla *et al.* [36] evaluated AuNPs biosynthesized by the rhizome extract of *Zingiber officinale* as offering gastroprotection against ethanol-induced ulcers in rats. The study concluded that the gastroprotective activity of the AuNPs may be due to their antioxidant activity, increased levels of mucus secretion, and endogenous enzymes. Therefore, *in vitro* results from the present study provided additional data on the efficacy of AuNPs in acting as anti-ulcer agents by inhibiting H^+/K^+ -ATPase activity.

Table 3

H^+/K^+ -ATPase inhibitory activities of AuNPs

Concentration ($\mu\text{g/ml}$)		Percentage H^+/K^+ -ATPase inhibition (%)					IC_{50} ($\mu\text{g/ml}$)
		75	150	225	300	375	
Sample	CFLE	63.49 ± 0.24	68.69 ± 0.24	68.17 ± 0.49	70.76 ± 0.24	76.47 ± 0.49	< 75
	CFL-AuNPs	55.02 ± 0.00	62.11 ± 0.24	66.09 ± 0.49	75.61 ± 0.24	84.60 ± 9.54	< 75
	Acetaminophen	33.88 ± 0.01	44.03 ± 0.16	53.90 ± 0.09	65.20 ± 1.57	73.89 ± 0.07	187.6

It is important to note that H^+/K^+ -ATPase, which is found in parietal cells, is the enzyme primarily responsible for the acidification and subsequently ulcerations in the stomach. Part of the symptoms of gastroduodenal ulcers is a condition known as hyperchlorhydria, which is characterized by uncontrolled hypersecretion of hydrochloric acid from parietal cells of the gastric mucosa through a proton pump [59]. Therefore, the control of acid secretion by inhibitors of H^+/K^+ -ATPase is essential for the treatment of the disease.

Conclusion

The use of multiple medications for treating a single disease or for multiple diseases in a single patient can be burdensome. Since many of the medications are chemically based, they tend to interact with their specific receptors and other biochemical, which have the potential to influence the effects of other medications [60]. However, the development of a single drug with therapeutic potential for many diseases can be much more relieving. For instance, an obese person is prone to diabetes, while a diabetic patient may also be affected by diseases such as peptic ulcers and microbial infections. Therefore, the multitasking nature of CFL-AuNPs as demonstrated by their antifungal, anti-obesity, anti-diabetic, and anti-ulcer activities, makes them a promising candidate for further study as a potential therapeutic agent for the treatment of multiple diseases.

Declarations

Conflicts of Interest

Authors declare no conflict of interest.

Data availability Statement

All data generated or analyzed during this study are included in this published article.

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Figures

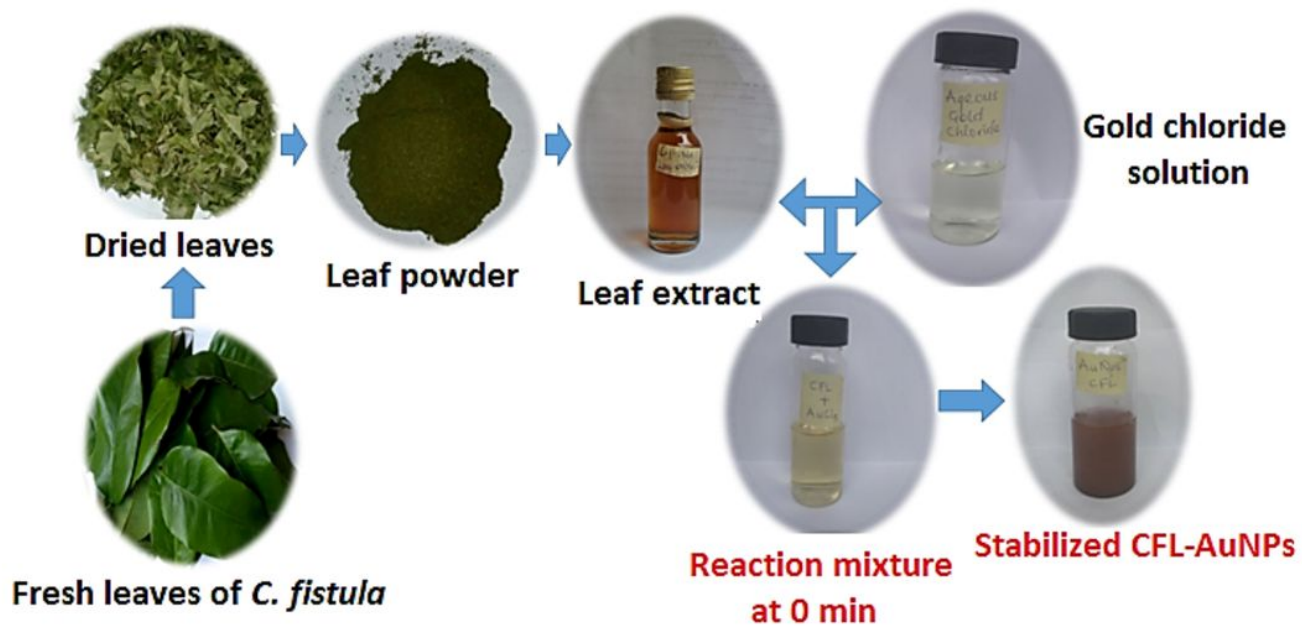


Figure 1

Biogenic synthesis of CFL-AuNPs

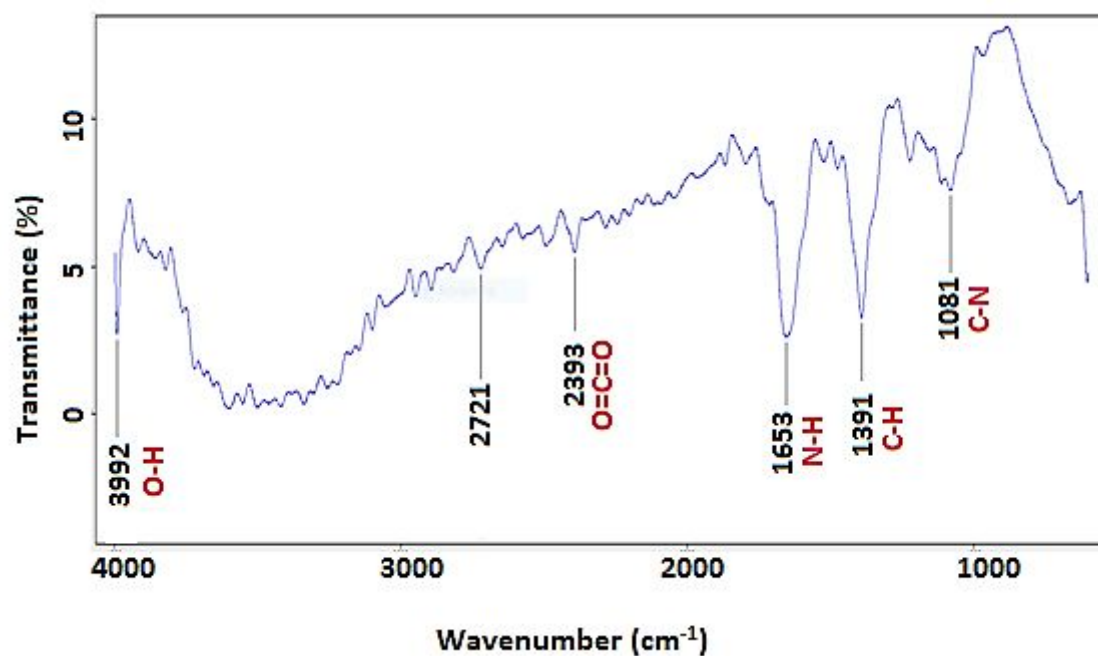
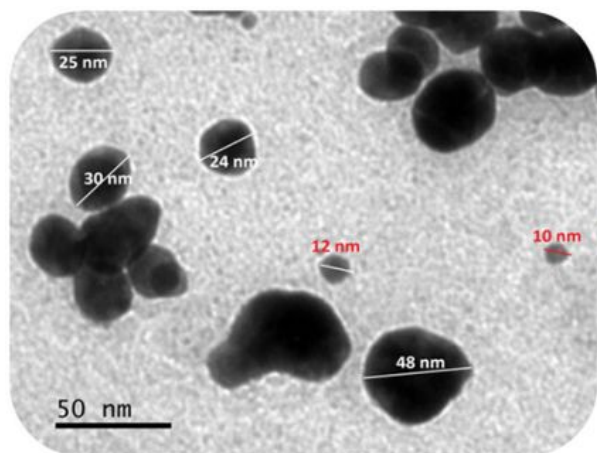
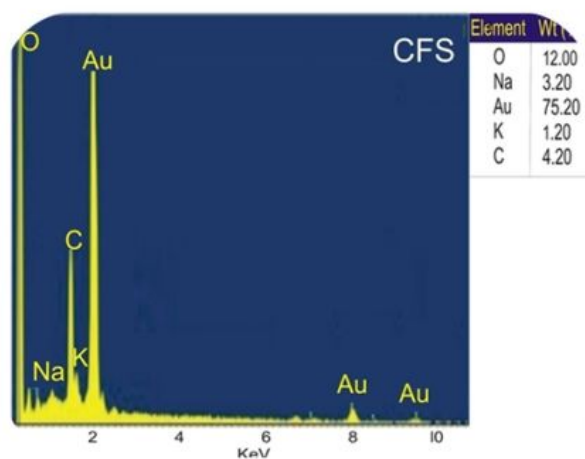


Figure 2

Biogenic synthesis of CFL-AuNPs



- Nearly spherical
- 10-50 nm



- 75% wt Au
- O, Na, K & C from extract

Figure 3

FTIR spectrum of CFL-AuNPs

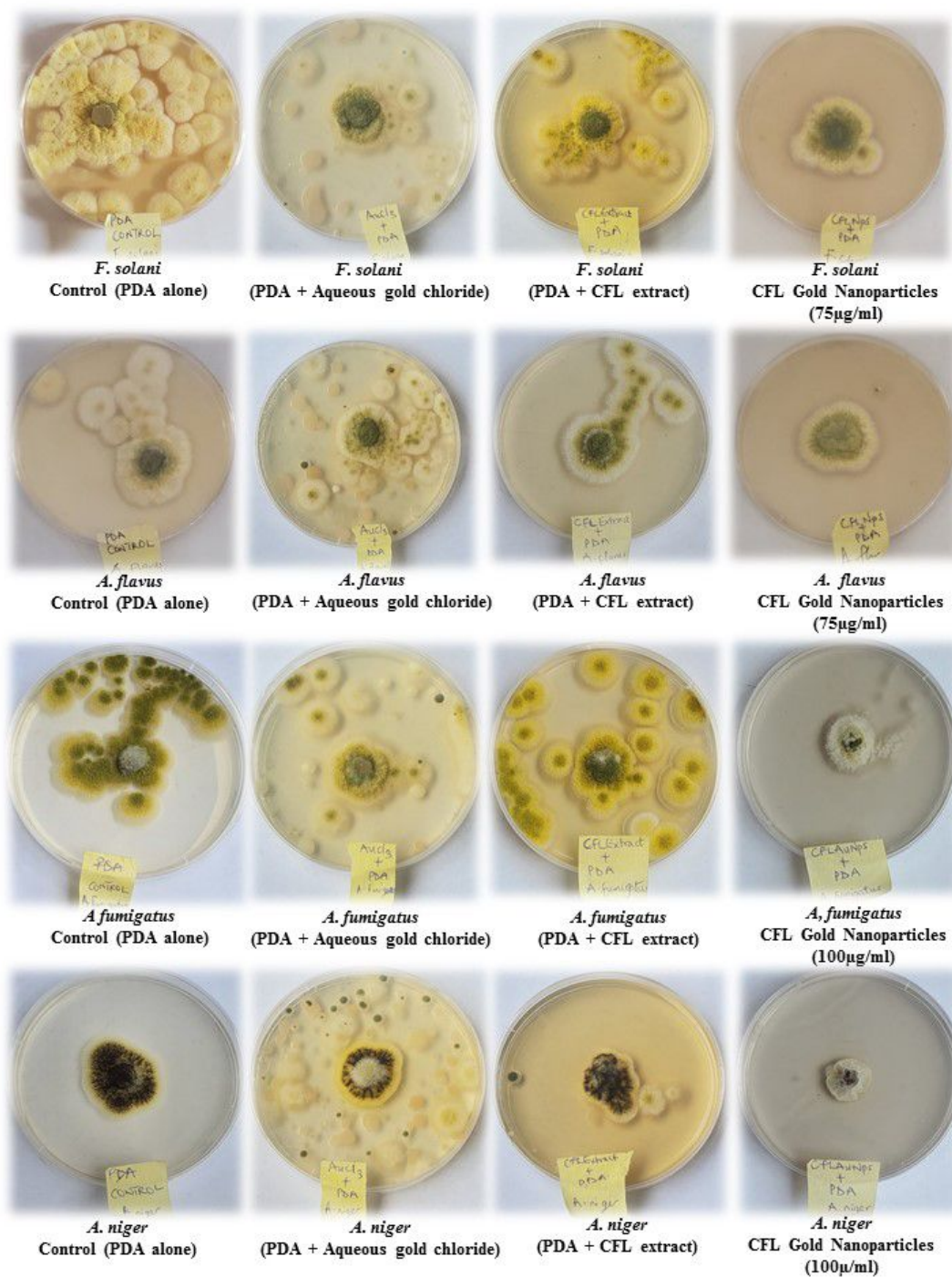


Figure 4

Transmission electron micrograph and EDX spectrum of the phytosynthesized

CFL-AuNPs