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Der Pharmacia Lettre, 2016, 8 (19):156-164
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Bioactivities of gold and iron oxide nanoparticles biosynthesized from the edible plant *Corchorus olitorius*

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ABSTRACT

The exploitation of different plant leaves extracts for the biosynthesis of nanoparticles is considered as a new and recent ecofriendly and cost effective technique because it doesn't involve any harmful chemicals beside its beneficial properties and broad spectrum applications in diverse fields. In this context gold nanoparticles (GNPs) and iron oxide nanoparticles (IONPs) were biosynthesized, for the first time, in one-step green method via reduction of gold aureate and ferric chloride solutions with 70% aqueous ethanolic (AE) extract of *Corchorus olitorius* (C. olitorius) leaves. Preliminary screening of the extract revealed the presence of a wide array of phytochemicals including phenols, flavonoids, tannins, proteins, carbohydrate, soluble sugars and polysaccharides. Quantitative estimation of these phytochemicals were 385.4 ± 3.6 , 564.61 ± 4.6 , 92 ± 2.0 , 766.9 ± 6.1 , 255.7 ± 5.7 and 131.1 ± 2.9 mg/ml, respectively. HPLC analysis of the phenolic and flavonoid contents of the leaves extract asserted the presence of gallic acid, chlorogenic acid, caffeic acid, rutin, p-coumaric acid, rosmarenic acid, 5-caffeoylquinic acid, quercetin 3-glucoside and myricetin in percentages of 59.47, 9.68, 2.32, 1.81, 1.53, 1.68, 1.40, 1.45 and 1.81, respectively. These phytoconstituents act as reductants and stabilizers for the biosynthesized gold and iron oxide NPs. The formed NPs were characterized by using UV-Vis absorption spectroscopy; Fourier transformed infrared spectroscopy (FT-IR); X-ray diffraction (XRD) and transmission electron microscopy (TEM). The biosynthesized NPs were found to exhibit high percentage scavenging activity of DPPH radical with percentage inhibition of 91.90 and 80.51% for GNPs and IONPs, respectively, at a concentration of 100mg compared to both effects of the leaves extract 76.62% and ascorbic acid 94.27% at the same concentration. In addition, the results obtained from the in vitro cytotoxic studies showed that these NPs exhibited the growth inhibitory property at IC_{50} % of 6.97 and 5.82 for GNPs and IONPs, respectively, against MCF 7 breast cancer cell lines in comparison with the leaf extract effect 9.77% and standard drug effect 3.95%. These data provide strong evidence that GNPs and IONPs should be studied further as potential novel free radical as well as cytotoxic agents.

Key words: biosynthesis, nanoparticles, *Corchorus olitorius*, HPLC, antioxidant, cytotoxicity.

INTRODUCTION

Many routes have been used for the biosynthesis of nanoparticles (NPs) using various biological materials as reducing agents such as marine organisms, microorganisms, and herbal extracts [1-5]. Among the most important bioreductants are plant extracts, which are low cost, readily available and comparably easier to handle [6]. This renders the plant-based nanoparticles synthesis is cost effective and can be easily scaled up to be used for large scale production. Moreover, biologically active molecules involved in the green synthesis of NPs act as functionalizing ligands, making these NPs more suitable for biomedical applications [7]. Besides, NPs are tiny in diameter and gaint in surface area, therefore they have unusual and fascinating properties, which are strongly affected by their morphology, size and structure [8].

Biomedical applications using gold nanoparticles (GNPs) and iron oxide nanoparticles (IONPs) have become a very active research area in recent years [9-16]. A large variety of possible biomedical applications have been examined, According to their unique chemical, physical, mechanical and thermal properties, and also by having suitable surface characteristics, superparamagnetic nanoparticles offer a great potential in many biomedical applications, such as cellular therapy, tissue repair, drug delivery, magnetic resonance [16-22].

Corchorus olitorius (Jew's mallow), belongs to the family *Tiliaceae* and genus of about 40-100 species, is a green edible plant which is grandly consumed either fresh or dry specifically in Egypt. Research discloses that this plant originated in Egypt and was the source of health and beauty of the Egyptian royalties [23]. Most of the bioactive phytochemical components in *C. olitorius* offer a diversity of health benefits, particularly for households in poor economic settings such as antioxidant, antibacterial, wound healing, anti-inflammatory or anticancer activity [24-30].

Because of insufficient information from literatures on the effect of *C. olitorius* on cytotoxic activity as well as the very little utilization of this plant in the synthesis of nanoparticles, The main intention of this study is, therefore, to (a) analyse qualitative and quantitative phytochemicals in the *C. olitorius* leaves, (b) utilize the ethanolic extract of the plant leaves for individually synthesis of gold and iron oxide NPs, (c) characterize, spectrally and microscopically, the biosynthesized NPs, and (d) evaluate the *in vitro* cytotoxic activities of the plant leaves extract alone as well as the plant extract containing, singly, GNPs and IONPs.

MATERIALS AND METHODS

Chemicals:

In our experiments, all chemicals used were of analytical grade and were used as received without any further purification. Whenever we refer to water it was used doubly distilled water.

Plant Materials:

Collection of the plant leaves. Fresh leaves of *Corchorus olitorius* were collected from the Center Aldnecat fields, Lake County, Egypt. The plant materials were washed repeatedly for 2-3 times with running tap water and once sterile with distilled water. The plant leaves were air-dried in the shade for seven days and homogenized to fine powder and stored in well closed bottles for further analysis in the laboratory.

Authentication of plant materials:

The plant was identified and authenticated by Mrs. Therese Labib, Consultant of plant taxonomy at the Ministry of Agriculture and Ex-director of the Orman botanical garden, Giza, Egypt. The specimen was labelled, numbered and annotated with the date of collection and locality.

Extraction of the plant leaves:

The dried, powdered leaves of *C. olitorius* (20 g) were then subjected to extraction in 100 ml ethanol (70%) using a Soxhlet apparatus. After complete extraction, the plant extract was then filtered out. The filtrate was concentrated to remove the solvent under reduced pressure at about 45°C. The dried extract gave a percentage yield of 22.3% was stored and then used for further laboratory analysis.

Qualitative phytochemical screening:

70% ethanolic *C. olitorius* leaves extract was evaluated by phytochemical qualitative reactions for usual plant secondary metabolites. The screening was performed for carbohydrates (reducing sugars), saponins, terpenoids/steroids, flavonoids, phenolic compounds, tannins, cardiac glycosids, proteins/ amino acids, alkaloids, and anthraquinones [31-33]. The precipitate formation or the colour intensity was used as analytical responses to these tests. The results obtained are tabulated in section 3.

Quantitative phytochemical analysis:

After the confirmation of the presence of the aforesaid constituents by preliminary qualitative phytochemical tests, the powdered plant leaves were taken for quantitative determination of primary metabolites (total soluble carbohydrates & proteins) and quantitative determination of secondary metabolites (total phenols, flavonoids & tannins) according to standard methods (Meda *et al.*, 2005; Quettier-Deleu *et al.*, 2000; Lowry *et al.*, 1951 and Umbriet *et al.*, 1959) [34-37].

HPLC analysis:

The residue of the hydroalcoholic extract of the investigated plant leaves was subjected to separation by HPLC with the following conditions: the rate of flow 1/min; Agilent 1100 series (Waldborn, Germany), quaternary pump

(G1311A), Autosamples Thermostated (G1329A), Degasser (G1322A), detector of variable wave length (G1314A); and column: Zorbax 300SB C18 column (Agilent Technologies, USA). Wavelengths Injection was performed at 280 nm for separation. Applying solvent system of methanol with 0.1% formic acid (solvent A); acetonitrile with 0.1% of formic acid (solvent B). The elution system was programmed as follows: starting at 30% solvent B, increasing to 60% over 10 min, increasing to 100% over 5 min, and then returning to 30% over 5 min. The injection achieved under ambient temperature.

Chemicals for nanoparticles biosynthesis:

For the green synthesis of gold and iron oxide nanoparticles, water soluble salts namely, HAuCl_4 and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were respectively employed as a precursor of nanoparticles, all purchased from Sigma-Aldrich Chemicals. The above chemicals were of analytical purity and were used without further purification.

Synthesis of gold nanoparticles (GNPs):

10 ml of the ethanolic plant extract was added to a definite amount of distilled water. Afterwards, 1ml of 0.1M chloroauric acid (HAuCl_4) solution was added to this extract solution and the total volume of this mixture was completed to 100 ml with water. The reaction mixture was kept under continued stirring for different durations (15, 30, 45, and 60 min) and at a constant temperature of 60°C using a magnetic stirrer. The visual change of colour from yellow to violet or red indicated the formation of gold nanoparticles (GNPs) (**Fig. 1A**).

Synthesis of iron oxide nanoparticles (IONPs):

0.93 g of ferric chloride hexahydrate was dissolved in 1000 ml water and stirred for 15 minutes, 10 ml of this solution was mixed with 10 ml of *C. olitorius* 70% ethanolic leaves extract, then the volume completed to 60ml using double distilled water and the procedure was completed as in synthesis of GNPs. The colour of the solution changes, within 10 minutes, from yellow to deep green evidencing the formation of IONPs (**Fig. 1B**).

Characterization of NPs:

Au and Fe_3O_4 NPs synthesized by the aforesaid green synthesis method were characterized by **1: UV-visible** spectrophotometer (Jasco V-670 UV-V) in the range of 450-600 nm for GNPs and 200nm-500nm for IONPs as depicted in Figures 2A and 2B. **2: FT-IR** The biosynthesized nanoparticles (Au & Fe_3O_4 NPs) thus obtained were purified by repetitive centrifugation at 8000 rpm for 15 minutes, followed by redispersion of the pellet of nanoparticles into 10 ml of deionised water. After drying, the dried nanoparticles were subjected to FTIR analysis by KBr pellet (FTIR grade) method in 1:100 ratios and spectrum was recorded in Nicolet Impact 400 FT-IR Spectrophotometer using diffuse reflectance mode. FTIR spectra were performed and recorded with a Fourier-Transform infrared spectrophotometer of type Nicolet 870 between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1} . **3: TEM** Transmission Electron Microscope (TEM- 2100 JEOL Japan) is used to determine the particle size, distribution and morphology of the formed nanoparticles. **4:XRD analysis** The oven dried nanoparticles were coated onto XRD grid and analysed by X-Ray diffractometer (6000-shimadzu-Japan). The powdered nanoparticles were stacked in the cubes of XRD and the result was taken with the XRD equipment at this conditions: X-ray tube target, copper potassium alpha radiation; voltage, 40.0 (kV); current, 30.0 (mA). Divergence slit, 1.00000 (deg); scatter slit, 0.00000 (deg); receiving slit, 0.30000(mm). Scanning drive axis, Theta-2Theta, scan range, 4.0000 - 90.0000 (deg); scan mode, continuous scan; scan speed, 8.0000 (deg/min); sampling pitch, 0.0200 (deg).

Antioxidant activity:

In order to investigate the *in vitro* free radical scavenging activity of the ethanolic leaves extract as well as the corresponding GNPs and IONPs colloidal solutions, DPPH assay was used as described in detail elsewhere [38].

In vitro cytotoxic activity:

In vitro cytotoxic activity will be determined using cell survival Sulpho Rhodamine-B (SRB) method as previously described by Skehan *et al* (1990) [39]. Its principle is based on the ability of the protein dye sulforhodamine B to bind electrostatically and pH dependent on protein basic amino acid residues of trichloroacetic acid-fixed cells. Under mild acidic conditions it binds to and under mild basic conditions, it can be extracted from cells and solubilized for measurement. The results of the SRB assay were linear with a cell number and cellular protein measured at cellular densities ranging from 1 to 100% of confluence. Its sensitivity is comparable with that of several fluorescence assays and superior to that of Lowry or Bradford. The signal-to-noise ratio is favourable and the resolution is 1000-2000 cells/well.

RESULTS AND DISCUSSION

Phytochemical analysis:

The healthful properties of edible plants are perhaps due to the presence of a variety of phytoconstituents such as polysaccharides, flavonoids, glycosides, phenols, saponins, tannins *etc*. The preliminary screening tests are useful in

the detection of these bioactive constituents and subsequently facilitate their quantitative estimation. The results of phytochemical screening depicted in **Table 1** revealed the presence of a wide array of chemicals including carbohydrates, saponins, steroids, flavonoids, phenols, tannins, proteins/amino acids, alkaloids and anthraquinones in AE extract of *C. olitorius* leaves. These results were in agreement with the previous results obtained by Ujah *et al* 2014 [40]. The vast majority of these phytoconstituents was quantitatively determined and illustrated in **Table 2**. reveals that the leaves extract contained the highest concentration of proteins (766.9 mg/ml) followed by flavonoids (564.61 mg/ml), phenolics (385.4 mg/ml), total carbohydrates (255.7mg/ml; including 124.6 mg/ml soluble sugars and 131.1 mg/ml polysaccharides) and tannins (92 mg/ml). The HPLC analysis of this extract determined nine compounds (**Table 3**). As highlighted in **Table 3** the main compounds detected were gallic acid (59.47%), chlorogenic acid (9.68%) which is previously identified in *C. olitorius* by Salawu *et al*, 2009 [41] and caffeic acid (2.32%).

Table 1: Preliminary phytochemical constituents detected in 70% aqueous ethanolic leaves extracts of *C. olitorius*

Phytochemical constituents	Results
Carbohydrates (Reducing sugars)	+++
Saponins	+
Terpenoids	-
Steroids	+
Flavonoids	++
Phenols	++
Tannins	+
Cardiac glycosids	++
Proteins/Amino acids	+
Alkaloids	++
Anthraquinones	+

(+) present, (-) absent

Table 2: Quantitative analysis of phytochemicals (mg/ml) in 70% aqueous ethanolic leaves extract of *C. olitorius*

Parameter	Result	λ_{\max}
Total phenolics (mg GAE/g of extract)	385.4±3.6 mg/ml	765nm
Total flavonoids(mg Qur/g of extract)	564.61±4.6 mg/ml	510nm
Total tannins	92±2.0	725nm
Total protein	766.9±6.1	750nm
Total carbohydrate	255.7±5.7	620nm
Total soluble sugars	124.6 ±1.8	620nm
Total polysaccharides	131.1± 2.9

Table 3: HPLC of phenolics and flavonoids composition of 70% aqueous ethanolic extract of *C. olitorius*

Compounds	Concentration%	Rt
Gallic acid	59.47	8.53
Chlorogenic acid	9.68	8.95
Caffeic acid	2.32	9.65
Rutin	1.81	10.13
P-Coumaric acid	1.53	10.72
Rosmarinic acid	1.68	11.32
5-caffeoylquinic acid	1.40	12.08
Quercetin 3-glucoside	1.45	12.80
Myricetin	1.81	14.19

Results are expressed as relative area percentage

Characterization of the biosynthesized NPs:

UV-visible analysis. UV-visible spectroscopy is widely used method to investigate the optical properties of the synthesized nanoparticles. The change of the colour from yellow to violet in case of GNPs and yellow to deep green in case of IONPs as depicted in **Fig. 1A&B** indicates the formation of metal and metal oxide nanoparticles, respectively. Increasing the colour intensity with time indicates the successive growth of nanoparticles as Matheswaran Balamurugan, 2014, explained [42]. Analysis was done in the range of 450-600 nm and the maximum absorbance was observed at 530 nm for GNPs formation. On the other hand, IONPs analysis was done in the range of 200-500nm and the maximum absorbance was observed at 280-330 nm regions as illustrated in **Fig. 2A&B**. These results were quite similar to those reported for GNPs by Elia *et al*, 2014 [21] and for IONPs by Latha *et al*, 2014 [44].

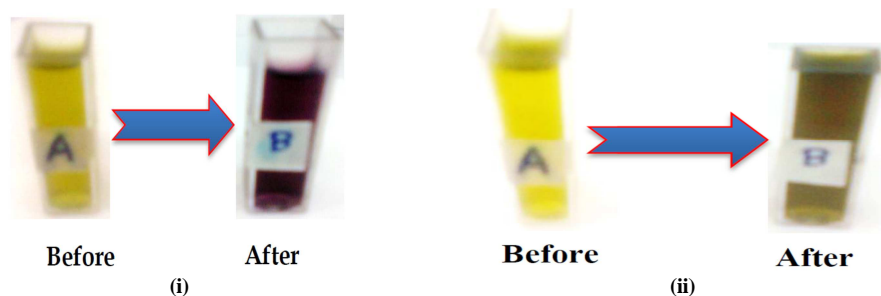


Fig. 1: Colour change of the plant extract before and after mixing with (i) Au solution and (ii) FeCl_3 solution

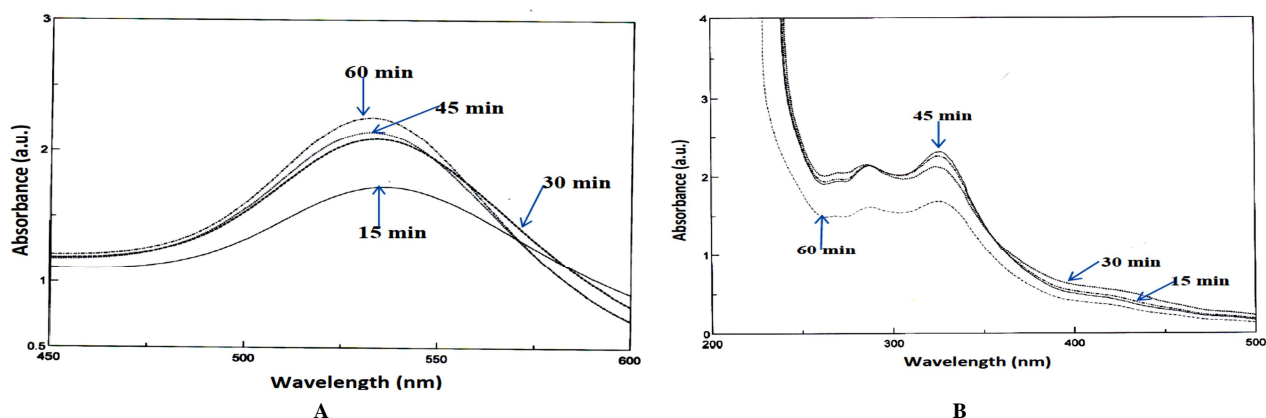
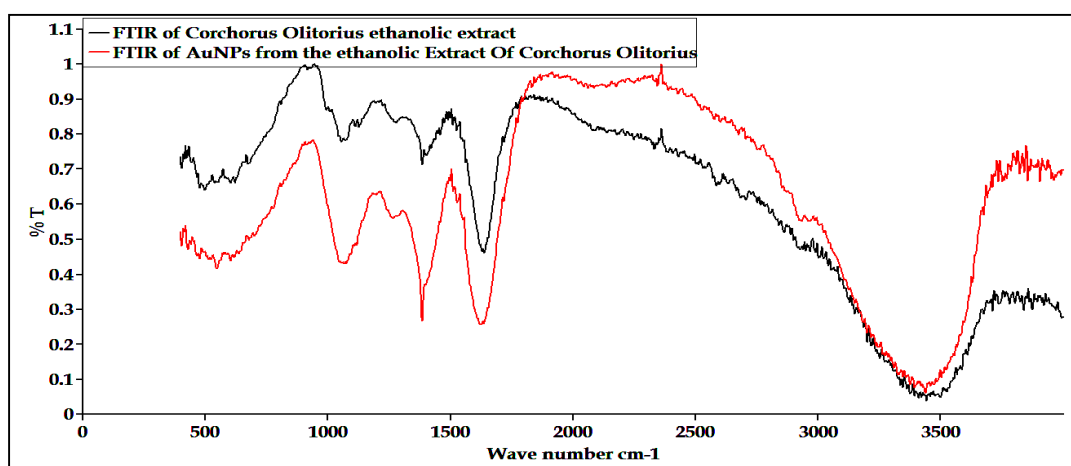


Fig. 2: UV-visible absorption peak of nanoparticles biosynthesized from *C. olitorius* aqueous ethanolic extract at constant temperature 60°C : (A) GNPs; (B) IONPs

FT-IR spectroscopy. Functional group's determination and their role in the synthesis of GNPs and IONPs in 70% AE extract of *C. olitorius* leaves were performed using FT-IR analysis. These examinations were accomplished as well to characterize any chemical changes that take place during the synthesis of NPs. The FTIR data in **Fig. 3A&B** disclose the presence of the following groups for ethanolic extract of *C. olitorius* leaves before biosynthesis; 3129.13 cm^{-1} -OH, 1645.43 cm^{-1} -C=C-, 1410.09 cm^{-1} -N-H, 923.99 cm^{-1} -C-C-, 1512.33 cm^{-1} C_6H_6 , 1211.41 cm^{-1} -C-O, 2106.43 cm^{-1} -alkynes. From above data one can confirm the presence of different chemical groups in this extract and FTIR in **Fig. 3A&B** after the formation of GNPs and IONPs showed that these absorption bands shifted towards higher wave number with the loss in intensity as previously mentioned [45,46].



(A)

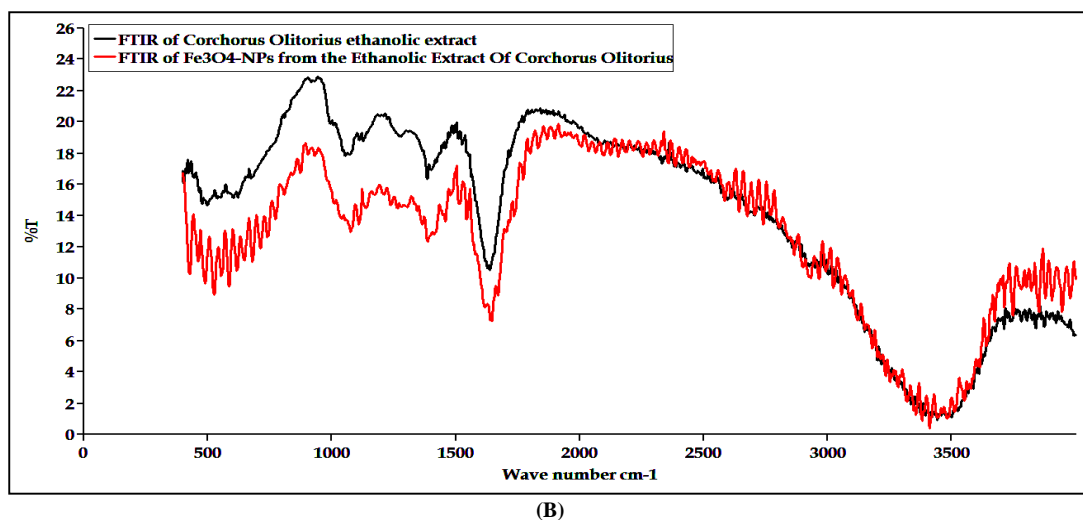


Fig. 3: FTIR spectra of nanoparticles biosynthesized from *C. olitorius* aqueous ethanolic extract at constant temperature 60°C: (A) GNPs ; (B) IONPs

TEM. The formed nanoparticles were characterized by TEM measurements. It is evident from **Fig. 4A&B** that the average size of the GNPs was 22 nm while that of IONPs was 8.24 nm, GNPs were monodispersed having a triangular, spherical and hexagonal shape. For IONPs this image discloses, the particles formed were needle in shape, and almost uniform, dispersed with a mean size in the range 7-8 nm. These results were comparable with GNPs and IONPs of similar sizes biosynthesized by different methods in literature [47-49].

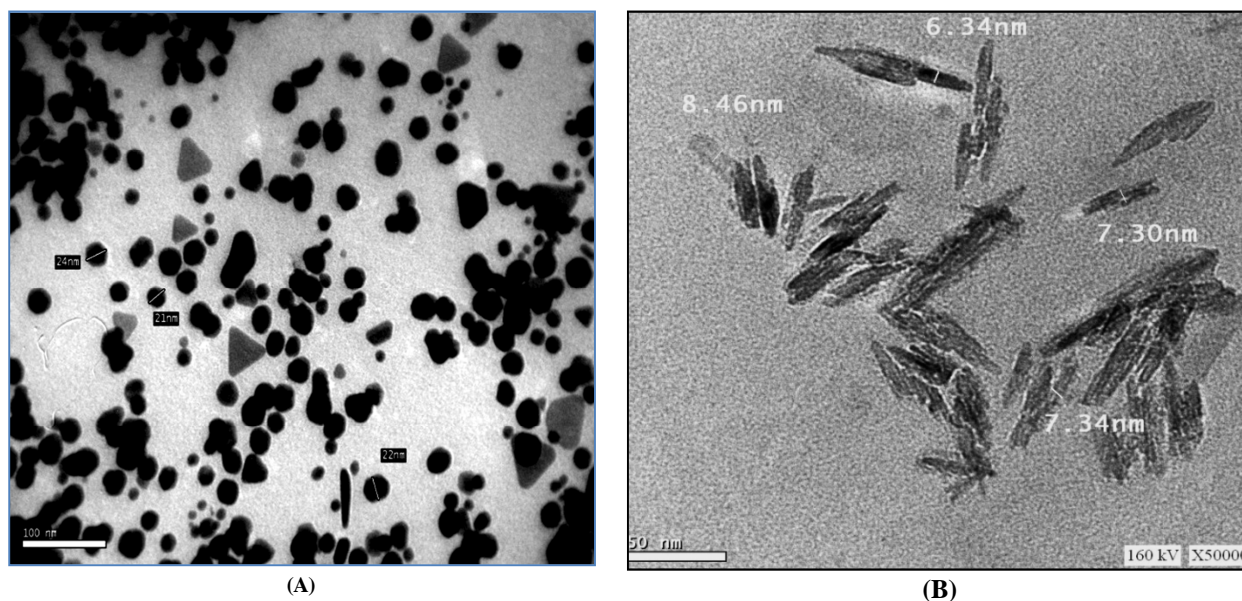
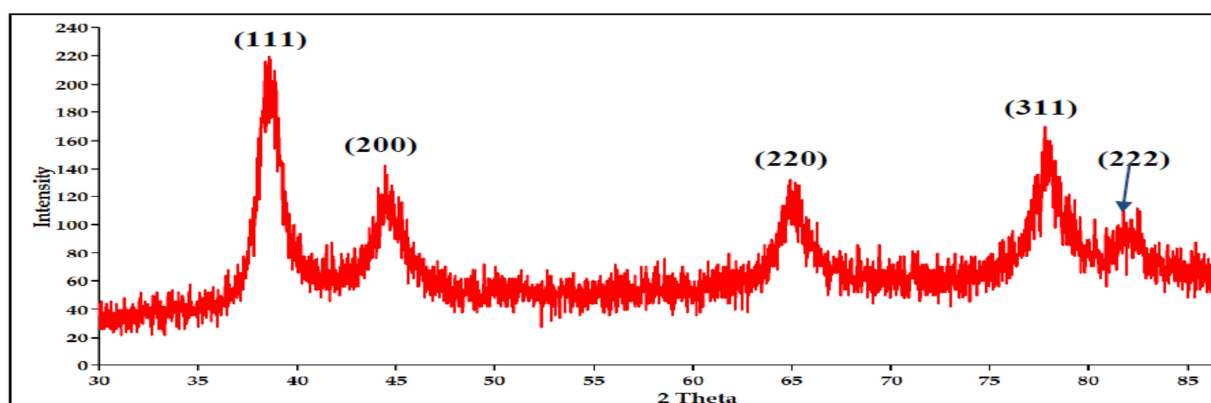
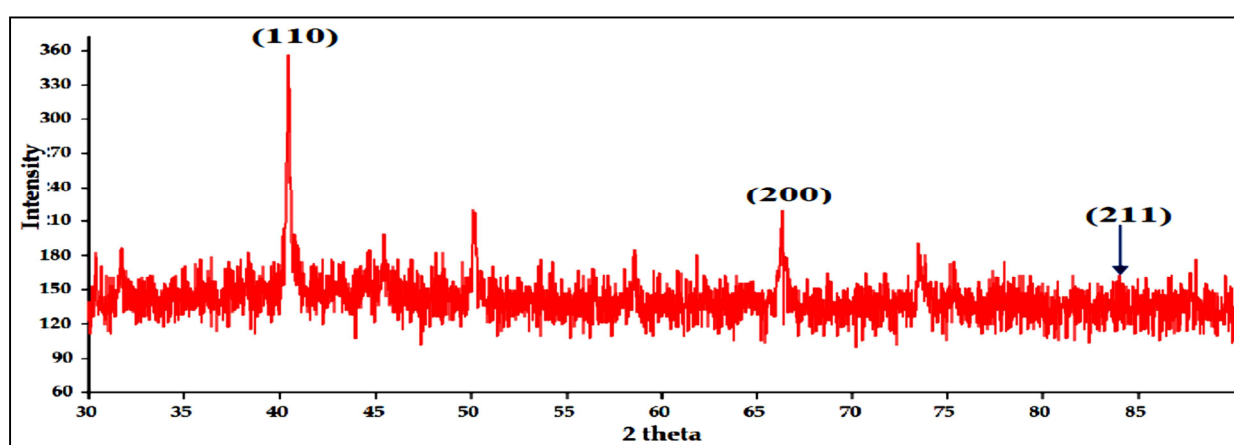


Fig. 4 : TEM images of nanoparticles biosynthesized from *C. olitorius* aqueous ethanolic extract at constant temperature 60°C : (A) GNPs ; (B) IONPs

XRD analysis. X-ray diffraction analysis was done to affirm the appearance of GNPs was illustrated in **Fig. 5A**. The peaks at 2θ values of 38.5°, 44.7°, 64.9° and 77.8° corresponding to the crystal planes of (111), (200), (220) and (311) of gold nanoparticles respectively. Previous works were found to have similar reports of XRD for gold nanoparticles synthesized using plant extract [44,50,51] and these confirm our results. Meanwhile **Fig. 5B** affirms the appearance of IONPs that indicate that there exist strong diffraction peaks with 2θ values of 30°, 35.5°, 43°, 57° and 63° that corresponds IONPs [52-54].



(A)



(B)

Fig. 5: X-ray diffraction of nanoparticles biosynthesized from *C. olitorius* aqueous ethanolic extract: (A) GNPs ; (B) IONPs**Antioxidant activity:**

Ethanolic leaves extract as well as the corresponding GNPs and IONPs were examined for the antioxidant activity at different concentrations (10,50 and 100 mg) by the DPPH method as depicted in **Table 4**. The outcome of this experiment showed that the free radicals were scavenged in a dose- dependent manner. The maximum percentage inhibition was obtained at 91.90, 80.51 and 76.62% for GNPs, IONPs and ethanolic leaves extract, respectively, at the highest concentration (100 mg) incomparable with the standard ascorbic acid whose maximum was 94.27% at the same concentration. This was in agreement with the previous results which reported that the hydrophilic extracts of *C. olitorius* considerably scavenged DPPH free radicals and this activity was ascribed to their high phenolic and flavonoidal contents [55-57]. On the other hand, the key factors which may be associated with the GNPs and IONPs antioxidant activity include (i) plant-based reductant biological molecules on the reactive surface of these nanoparticles; (ii) active redox cycling on the surfaces of GNPs and IONPs due to transition metal-based NP; and (iii) particle- DPPH free radical interactions.

Table 4: Antioxidant activity of GNPs and IONPs biosynthesized from 70% aqueous ethanolic extract of *C. olitorius*

Concentration mg	% of Antioxidant activity			
	GNPs	IONPs	Plant aqueous ethanolic extract	Ascorbic acid
10	57.14	50	23.37	43.47
50	82.72	61.03	62.33	80.85
100	91.90	80.51	76.62	94.27

In Vitro cytotoxic activity:

There is a dearth of information on the use of *C. olitorius* leaves extract as an anticancer agent. In addition, there was no work has been carried out on the GNPs and IONPs usage as anticancer activity against human breast cancer cell lines (MCF 7). Therefore, the present study attempts to fill this research gap by investigating and comparing the

effects of ethanol extract of *C. olitorius*, GNPs and IONPs prepared from it on the growth of human breast cancer cell lines (MCF 7). The results obtained in **Table 5** showed that both the ethanolic plant extract and the prepared GNPs and IONPs significantly inhibited the proliferation of MCF 7 cells in a dose-dependent manner. This could be principally due to phytochemical constituents of the *C. olitorius* leaves [58,59] as well as the inhibiting effects of these nanoparticles [60-63]. MCF 7 cells, treated with IONPs at a concentration higher than 6.25 µg/mL, displayed a strong reduction in cell viability (11.28%) and with 5.82 IC₅₀% than those treated with GNPs (40.59% cell viability, 6.97% IC₅₀%), and plant ethanolic extract (46.84% cell viability, 9.77 IC₅₀%) when compared with the standard drug 5-flu (8.19% cell viability, 3.95 IC₅₀%).

Table 5: Cytotoxic effect of GNPs and IONPs biosynthesized from 70% aqueous ethanolic extract of *C. olitorius* against MCF7 breast cancer cell lines

Concentration µl/well	Viability %				IC50%			
	GNPs	IONPs	Plant ethanolic extract	Standard drug	GNPs	IONPs	Plant ethanolic extract	Standard drug
0	100	100	100	100				
6.25	51.82	46.76	76.34	22.87				
12.5	40.59	11.28	46.84	8.19				
25	39.41	6.90	45.76	7.63	6.97	5.82	9.77	3.95
50	35.99	4.66	43.49	6.30				
100	21.55	3.69	23.14	5.93				

CONCLUSION

In conclusion, biosynthesis of GNPs and IONPs from *C. olitorius* showed promising results for biomedical applications. The results shown by high throughput techniques like UV-visible spectroscopy, FTIR, TEM and XRD indicate the successful formation of both GNPs and IONPs. The present investigation suggests that the biosynthesized GNPs and IONPs are exerting an *in vitro* cytotoxic effect on human breast cancer cell line MCF-7. This effect proved the potential application of both GNPs and IONPs synthesized from *C. olitorius* leaves aqueous ethanol extract.

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