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Gold Nanoparticles Biosynthesized from Synephrine Extracted from *Citrus aurantium* Peels and its Cytotoxicity Assay

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Abstract: Nanotechnology is one of the most productive fields of study in current science.Nanoparticles (NPs) and nanomaterials are finding new uses at a rapid rate. The aim of the study that synthesis of nanoparticles from peels, which are considered *Citrus auriantum*. In this study synephrine was extracted from C. *auriantum* peels and biosynthesized gold nanoparticles from it, then characterization of synthesized gold nanoparticles (AuNps) by UV-visible spectroscopy Fourier Transform Infrared (FTIR) and FESEM which confirm the presence of AuNps in diameter size range to (20.8-34.3) nm and determined their toxicity assay on lymphoid human cell in 24 ,48 and 72 hours least cytotoxicity value was in 3.013 in 10 mg/ml for *Citrus auriantum* extract in 24 hours. It was concluded that synephrine extracted has no toxic effect inhibition rate of this maximum 5.698 in AuNp biosynthesized in 20 mg/ml concentration in 72 hours.

Keywords: Gold nanoparticles, synephrine, C. *aurantium*

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Introduction

Nanotechnology is one of the m ost productive fields of study in current science.Nanoparticles (NPs) and nanom aterials are finding new uses at a rapid r ate.nanomaterials have been facilitated by recent advances in the production of nanoparticles (1) Due to their potential for use in the treatment of cancer, research on AuNPs has increased recently. Their distinctive physicochemical characteristics related to size (1-100 nm) and higher surface area to volume ratio increase their reactivity with other molecules and allow them to pass through biological barriers (2). Additionally, by more precisely targeting the drug delivery system, AuNPs can be employed as drug carriers to enhance drug delivery and minimize side effects. Recent developments in the creation of AuNPs have demonstrated that they may also be excellent promising viral targeting and diagnostics possibilities (3,4) and that they may be utilized as standalone substances or as carriers for the administration of other medications or vaccines. In addition ,to cytotoxicity, the exposure to AuNP has been found to induce cell cycle arrest in several cell phases (5, 6) According to (7) AuNPs blocked the S phase, causing oxidative stress, G0/G1 cell cycle arrest, and apoptotic cell death. Using modified AuNPs, similar outcomes have also

been documented (8). According to some theories, the size, shape, surface charge, composition, concentrations, and cell type of AuNPs all affect how poisonous they are. *Citrus aurantium* L., sometimes referred to as Bitter orange, sour orange, or bitter orange, is an evergreen tree that can reach a height of 5 meters. It is renowned for its fragrant white blossoms and is thought to have originated in Syria and eastern Africa before being grown in the United States, Spain, and Italy (9).

Numerous investigations on the bioactivity of *Citrus aurantium* compounds have been conducted(10) .Synephrine is alkaloid secondary metabolites chemically formula ($C_9H_{13}NO_2$), synephrine the main component of various citrus species, including *citrus sinesins* and *Citrus aurantium* (often known as bitter orange), and it shares structural similarities with ephedrine and adrenaline.Synephrine is being used mo re frequently to replace the use of ephed rine in food supplements for weight loss and sports (energizing, muscle-enhancing chemicals). frequently in combination with caffeine among other chemicals Ortho o-, para p-, and meta m are the three positional isomers of synephrine) (11).

Material and methods

Collection of plant samples

This study included the use peels of C. *aurantium* was collected from Diyala, Iraq. The plant was identified in the herbarium at the University of Baghdad Department of Biology, College of Science.

Plant peels were dried and proce ssed through a mechanical grinder to cre ate powder, it kept at 4 C° for further investigation. extraction of synephrine according to (12) *C. aurantium* peels weighing 100g were hexane defatted for 24 hours, after which they were allowed to dry at room temperature. The defatted plant material, which was kept in a thimble, then put into the Soxhlet extractor. In a 1-liter round bottom flask equipped with a Soxhlet extractor, 500 ml of 80% methanol was utilized . For another 12 hours, the extraction was continued. The extract was first shaken and dissolved in 2N hydrochloric acid on a water bath, filtered, and concentrated under reduced pressure to dryness using a rotary evaporator at a temperature 40°C. The resulting filtrate was then shaken with chloroform to remove unwanted components. Alkaloidal bases were released from the acidic aqueous layer by raising its pH with ammonia, which was then concentrated under vacuum and subjected to identification, isolation, and purification methods(13).

Using TLC to identify plant components

The extract was investigated using TLC on premade silica gel GF254 (20 x 20 cm) plates with a 0.25 mm thickness from MERCK 2% ninhydrin in n-butanol spraying reagent was used for detection. The standard for synephrine was bought from Chengdu Biopurify Phytochemicals (11).

Synephrine identification using HPLC

Synephrine was estimated both qualitatively and quantitatively using HPLC. (Knauer/Germany), HPLC analysis was performed. by comparing the retention time obtained from examined, materials with standards compound under identical chromatographic conditions, Acetonitrile, water, and trifluoroacetic acid (5:95: 0.01) served as the mobile phase in a C18 column with a 150mm 4.

6mm/5um internal diameter. and the flow rate was 0.6 ml/min with UV detection. 220 nm detector (11).

Biosynthesis of gold nanoparticles: a green and eco-friendly approach

In order to synthesis gold nanoparticles, *C. aurantium* peel extract in the bio reduction of HAucl4 The peels are cleaned in distilled water, cut into small pieces, and then cooked in the universal solvent (distilled water) to produce extract. By employing various techniques including filtering and centrifugation, the extract can be further refined. Simply combining the extract with the auric salts solution at room temperature causes them to transform into nanoparticles in a quick, easy, and environmentally friendly process. (14).

Characterization of biosynthesised gold nanoparticles

An essential stage in Characterization of the particles is done

Ultraviolet-visible spectroscopy

An ultraviolet-visible spectrophotometer (UV-Vis) refers to absorption spectroscopy. The samples were measured by UV-VIS double beam spectrophotometers from 400-800 Wave length (15).

Fourier transform infrared (FTIR)

The functional groups present on A uNPs were confirmed through the utilizatio n of Fourier transform infrared (FTIR) spectroscopy within the spectral region of 400–4000 cm−1(16).

Characterization of AuNPs using field – emission scanning electron microscopy (FESEM)

Use electrons rather than light sources to investigate the topography of things. Electrons produced by the field emission source are accelerated in the direction of a strong electrical field gradient. The primary electrons are concentrated in a high vacuum column, where an electronic lens deflects the blasted electrons onto each object. Each item emits secondary electrons in this way. The velocity and angle of secondary electrons are strongly influenced by the surface characteristics of any item. A detector captures these electrons and produces an electrical signal as a result. A video-scan is made of this signal. The signal undergoes a transformation that results in the generation of a video-scan image. This image can then be observed on a monitor and subsequently stored for subsequent processing (17).

Testing of toxicity of synephrine and gold nanoparticles

Are tested on lymphoid blood cell lines After we created lymphocytes, looked at them under a microscope to make sure they were growing normally.

After being distributed in a 96-hole multi-hole plate, the cells were exposed and treated with three concentrations (10,15,20) mg/ml of produced AuNPs using a micropipette (18,19).

Result and discussion

TLC detection

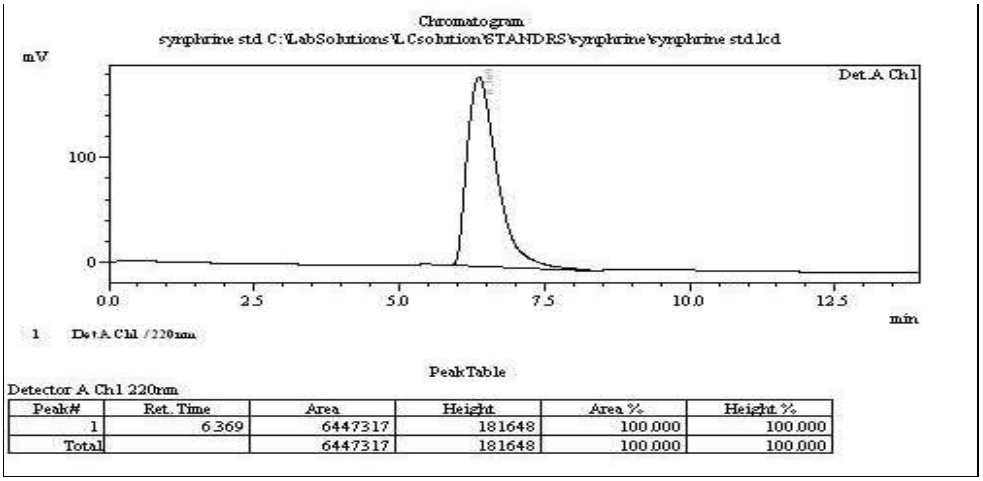
The existence of synephrine in the extract of Iraqi *Citrus aurantium* fruit peels was confirmed by the appearance of a single round compact spot on TLC plates as shown in (figure 1) .in five various developing solvent systems, sharing the same color and Rf for stander 0.95 and for extract 0.87 values.(TLC) depends on the separation principle. The separation relies on the relative affinity of compounds towards both the phases. The compounds in the mobile phase move over the surface of the stationary phase. The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly

while the other compounds travel fast. Therefore, the separation of the mixture is attained. One of the major advantages of TLC is the minimal sample preparation normally required. However, the result that's agree with(18) the existence of a possible matrix effect should be constantly taken into account. Since *C. aurantium* alkaloids can interact strongly with the free silanols present on the surface of the stationary phase, peak tailing of the analytes is often observed. Spraying it by ninhydrin to the samples and standards to suppress the ionization of the alkaloids and enhance peak resolution.

Figure (1) TLC result

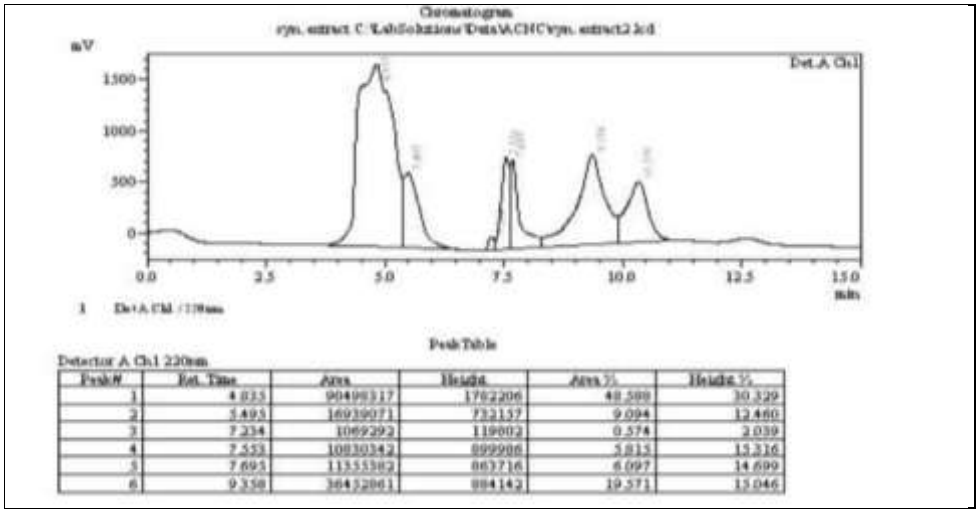
The HPLC detection

The sample and its container indicate its "on- column" time. As a result, different components of a sample elute at various perio ds. In this way, the sample ingredients are separated. Synephrine was further confirmed to be present in Iraqi *C. aurantium* fruit peels using HPLC. retention period (7.2 minute) for the synephrine extracted from Iraqi *C. auriantum* fruit peels, and (6.5 min) reference to the standard was in the chromatogram indicating that the peak was most likely synephrine, figure (2, 3) as in figure (3) the chromatogram shown a different separated peak related to synephrine structurally According to (19) octopamine, phenylephrine (m-synephrine), tyramine, N-methyltyramine, and hordenine, which may be present in bitter orange (*C. aurantium*). p-Synephrine was found to be the primary biogenic amine present in all materials tested, accounting for >80% of the total biogenic amine content in all samples.



Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.369	6447317	181648	100.000	100.000
Total		6447317	181648	100.000	100.000

Figure (2): HPLC of Synephrine stander



Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.035	90498317	1702206	48.589	30.329
2	5.495	16939071	732157	9.094	12.460
3	7.234	1069292	119002	0.574	2.039
4	7.553	10830342	899906	5.815	13.316
5	7.695	11355382	863716	6.097	14.699
6	9.350	36432061	884142	19.571	13.046

Figure (3): HPLC of Synephrine in the extracted *Citrus auriantum* peels.

Study using UV spectrophotometry

As shown in (Figure 4). The findings of The UV- visible absorption spectra demonstrate a unique method for the synthesis of Au NPs. The scale of wavelength was consistent between 400 and 800 nm, and there was an increase in intensity up to 10 minutes as a function of time without any change in the peak wavelength. The produced Au NPs' surface plasmon resonance (SPR) has a wavelength of 465 nm.

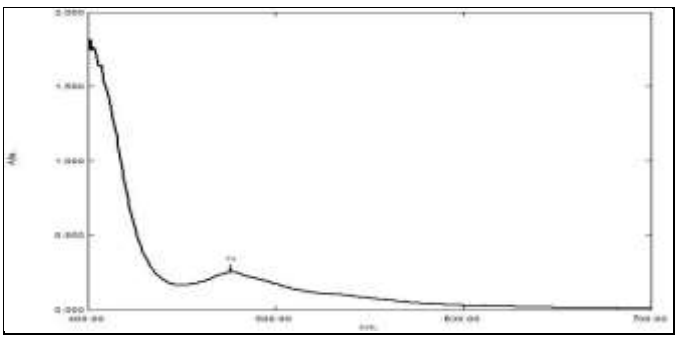
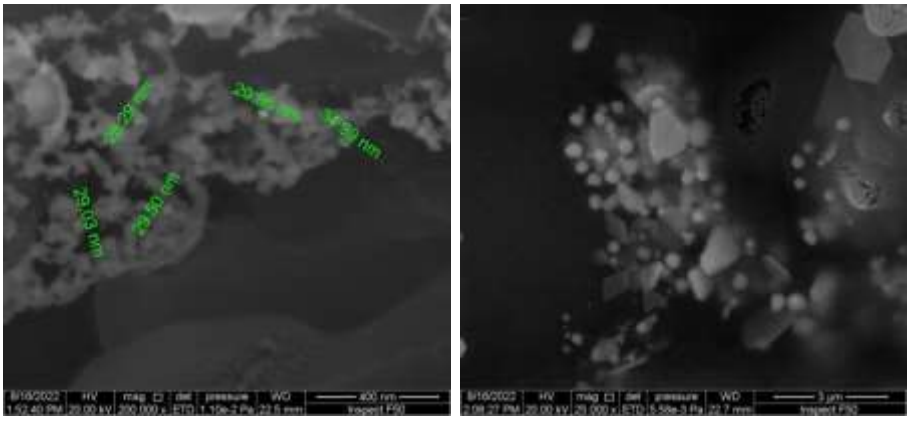


Figure (4): Synthesized Au NPs had a peak in their UV-visible spectrum at 470 nm.

FESEM analysis

Using FESEM, the surface morp hology of NPs was studied.Even inside t he aggregates, the produced Au NPs we re not in direct contact with one another. , as shown by the microscope analysis, demonstrating that the NPs were stabilized. The morphological shapes and dimensions of the aggregations were described, and it was found that the bulk of the biosynthesized Au NPs had spherical morphologies. The study demonstrates that the surface still maintains the decreasing process.After 10 min of reaction time at (pH 7.0), the histogram of the partic le size distribution of the Au NPs was pr oduced. The particle size range was determined by a representative FESEM micrograph to be (20.8-34.3) nm, as show in figure (5). It was shown that nucleation to generate new NPs and aggregation to make larger particles occurred concurrently by the coexistence of Au NPs in smaller and larger sizes induced by those created in the early and later stages of the process. The Au NPs spot-profile EDX.



Figure(5): FESM images show the diameter and spherical shape of the produced Au NPs.

The Fourier transform infrared (FTIR)

Is a type of spectroscopy that may recognize changes in the overall composition of biomolecules by detecting changes in functional groups. The vibration and rotation of molecules impacted by an infrared wavelength are measured using FTIR. Information about the existence of their interactions can be determined by identifying structural changes in molecule binding. Transmittance FTIR, attenuated total reflectance (ATR-FTIR), and micro-spectroscopy FTIR are three of the most popular FTIR-based methods for characterizing materials (20). Figure (6,7) below demonstrates.

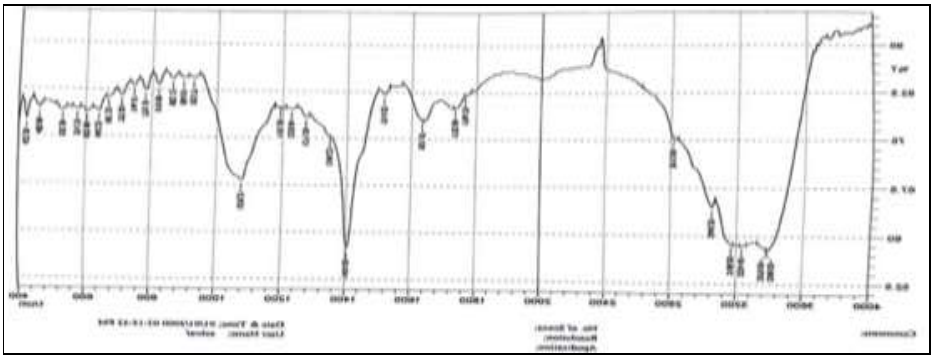


Figure (6): C. *auriantum* FTIR analysis

C. *auriantum* FTIR analysis (Figure 6) demonstrated the presence of an aliphatic peak at (2813), a broad peak for the hydroxyl groups at (3170–3394), and a single peak for the secondary amine group at (3029). The presence of meta compensation on the ring, which creates a peak that is regarded as the compound's fingerprint between (1667-2000), is the most significant group in this molecule.

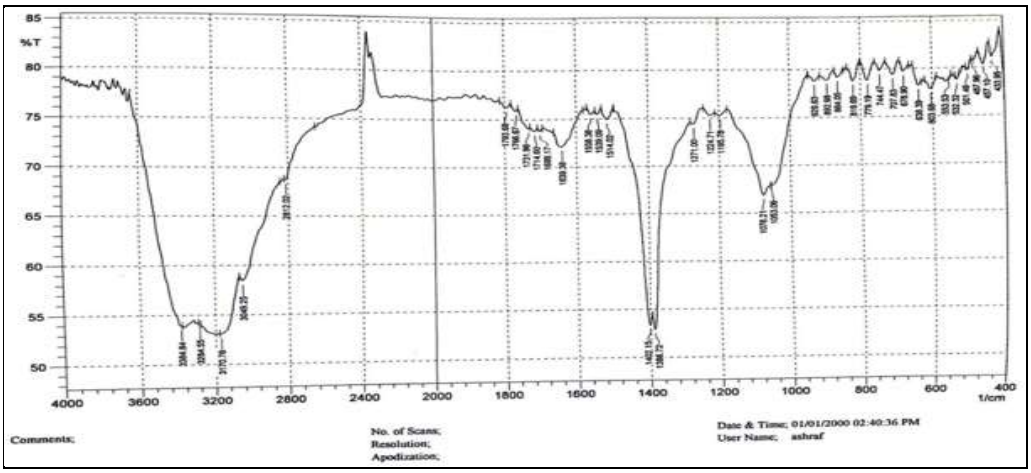


Figure (7): FTIR spectrum of synthesized Au NPs

FTIR study of the production of AuNp in *C. auriantum.* In (figure 7) showed the presence of aliphatic aggregates at 2814, while the hydroxyl groups appeared in the form of a broad peak at (3160-3390), while the secondary amine group appeared in the form of a single peak at (3049). The most important group in this compound is the presence of meta compensation on the ring, and this constitutes a peak that is considered a fingerprint of the compound between (1631-1764) FTIR analysis clearly shows that cap- ping and reducing of NPs by biomolecules present in peels extract of. *C.auriantum* could be responsible for prolonged stability.

Table (1): Cell death percentage (cytotoxicity assay) measured on human blood lymphoid exposed to 10,15,20 mg/ml of plant extract and biosynthesis AuNps for 24 ,48,72 hours

In 24 hr				
Inhibition rate % Conc. Treatment	**10 mg/ml**	**15 mg/ml**	**20 mg/ml**	**Mean**
***C.auriantum* extract**	3.013	3.311	4.038	3.454
***C. auriantum* biosynthesis from AuNps**	3.246	4.286	4.144	3.892
In 48 hr				
***C. auriantum* extract**	4.562	3.888	5.009	4.486
***C.auriantum* biosynthesis from AuNps**	4.238	4.700	4.837	4.592
In 72 hr				
***C. auriantum* extract**	5.016	4.568	5.436	5.007
***C. auriantum* with AuNps**	4.717	5.103	5.698	5.173
lsd5%	0.146**	0.169**	0.293**	
P-value	0.001	0.004	0.001	

The cytotoxicity assay for gold nanoparticles (AuNPs) and plant extracts on blood lymphoid cells

The toxicity assay for gold nanoparticles (AuNPs) and plant extracts; provided some information on their effects on human blood cells, and the mechanisms involved remain sparse.

The experiments measured the death of human blood lymphoid cells percentage after exposing to different solutions of gold nanoparticles at different concentrations for 24 ,48 and 72 hours. The study that's the least cytotoxicity value in 3.351% in 10 mg/ml for *C. auriantum* extract in 24 hr. which give indicate that synephrine extracted have not toxic effect inhibition rate of this maximum 5.698 in AuNp biosynthesized in 20 mg/ml concentration in 72 hr . increased with an increase in its concentration and exposure time (21). According to research by (22), nanoparticles disturb cellular components and the mechanism of cell death by increasing the permeability of the cell wall and reactivating the oxygen atom in an interactive manner (23). Since p-synephrine is present in regularly consu med citrus fruits, a maximum limit for p-synephrine in dietary supplements may be established on the quantity of p-synephrine consumed via the regular diet in the absence of a health- based advice value.Strong cytotoxic effects on cells reproduced by the entire plant and alkaloidal crude extract, and the effects were dose dependent(24).

Conclusion

The conclusion was the importance of the biological method for synthesis of nanoparticles from peels, which are considered waste of *Citrus auriantum* plant, Using plant waste is a good recycling way .in addition the biologically generated of gold nanoparticles very effective , low toxicity ,less cost and less time , and considered a safe methods for the environment. Furthermore, since the biogenic components of plants and microorganisms themselves function as stabilizing and capping agents, there is no need to add any additional stabilizing agents. the time needed for the biosynthesis of gold nanoparticles is less.

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