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Eco-friendly Synthesis of Zinc Oxide Nanoparticles by *Garcinia cambogia* and Evaluation of Their Obesity and Antimicrobial Activities

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Abstract

In the present synthesis, study of nanoparticles of zinc oxide using ethanolic extract of *Garcinia cambogia* leaves extract. Using of zinc oxide nanoparticles as a precursor in leaf extracts of this plant for NPs synthesis. The optical properties and structural of NPs were investigated by ultraviolet-visible(UV-Vis) spectrophotometer ,scanning electron microscope (SEM), transmission electron microscopy (TEM) and dynamic light scattering (DLS).

Liquid Chromatography-Electrospray Ionization-Mass (LC-ESI-Mass) in negative ion mode of ethanolic extract demonstrates the presence of six bioactive compounds identified as guanosine, prephenic acid, tetrahydroxy-cholanic acid, limocitrin, 6,7-dihydroxycoumarin-6-glucoside and hydroxy citric acid which they responsible for providing electrons for reducing zinc nitrate hexahydrate to ZnO-NPs.

The antibacterial activity of both ethanolic extract and eco-friendly synthesis Of ZnO-NPs. by *G.cambogia* were tested against two pathogenic bacterial strains ,*Staphylococcus aureus* ATCC6538 and *Escherichia coli* ATCC10536 by using disk diffusion method.

The ZnO-NPs showed antibacterial activity against *S. aureus* (40 mm) more than ethanolic extract (35 mm). It revealed significant antibacterial activity against *E. coli* (50 mm) compared to ethanolic extract (45 mm). Also, ZnO-NPs displayed more antibacterial activity than Ampicillin against *S. aureus* (266.6%) and *E. coli* (500%) and displayed more antifungal activity than Fluconazole against *C. albicans* (238.6%). However, ZnO-NPs showed higher antifungal activity against *C.albicans* (50.11 mm) than the ethanolic extract (45.37 mm).

Based on these findings, it is concluded that the synthesized ZnO-NPs is a more promising candidate than the alcoholic extract of *G. Cambogia* leaves against pathogenic bacterial, fungal strains.

The administration of *G. cambogia* and synthesized ZnO-Nps as a weight-reduction treatment is intended to give the important knowledge for both scholarly and broad public use regarding the effects of the plant.

Keywords: *Garcinia cambogia*, eco-friendly synthesis , ZnO-NPs, LC-ESI-Mass analysis, antimicrobial activity, Obesity .

1. Introduction

Garcinia cambogia (*G.cambogia*) plant, belongs to the family Clusiaceae was in the continent of Asia cultivated in Africa. Previous studies reported that this plant contains various organic acids, benzophenones, xanthones and hydroxy citric acid as major constituents which are used for weight loss. Hemorrhoids, dysentery, ulcers, diarrhea, and various cancers, including leukemia, are all treated with *G.cambogia* extract, an Indian medication. Initial research on seeds shown its antihistaminic, antifungal, anticancer, antibacterial, antiviral, antiulcerogenic, and vasodilatory properties [1]. Size of nano crystalline (less than 100 nm) was obtained and exhibit atom-like behaviors as a result of

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increased surface energy because of their huge surface area and wider band gap between the conduction band and the valence band when they are separated into nearly atomic size [2]. In many domains, including physics, biotechnology, chemistry, information technology , material science and environmental technology, transition metal oxides with semiconductors and nanostructures with dimensions in the nanometer empire are advantageous [3]. In recent years, ZnO is important semiconductor with technological interest tremendous, scientific and other biological processes and increasing awareness towards green chemistry. Nanomaterials techniques of natural products were used to reduce pollution sources [3]. Therefore, ZnO-NPs green synthesis was agreed out using *G. cambogia* as extract for the eco-friendly development of many technologies.

ZnO-NPs have many applications in all aspects of life in general. Its ethanolic extract are rich in bioactive compounds as reducing agent to reduce Zinc nitrate hexahydrate to ZnO-NPs, which were analyzed and characterized using,Dynamic light scattering (DLS), UV–visible spectroscopy, Scanning electron microscopy (SEM), Zeta potential and Transmission electron microscopy (TEM).

Furthermore, ethanolic extract of *G. cambogia* and synthesized ZnO-NPs have antimicrobial activities and cause weight loss

2. Materials and Methods

All essential chemicals used are analytical grade and purchased from Sigma-Aldrich and Merck (Germany).

2.1. Collection of the Plant

Fresh leaves of 250 gm of *G. cambogia* were collected from Zagazig - Sharqia, Egypt in March 2022. Alaaeldin Sayed Ewase, a professor of plant taxonomy at the Ministry of the Environment, generously authenticates it.. Voucher specimens of the authenticated plant were deposited at the herbarium of the National Research Center (NRC), Giza, Egypt.

2.2. Extraction.

The extraction of *G. cambogia* leaves were performed using organic solvent extraction by Soxhlet extraction method. This extraction was done by taking 100 gm of dried powder plant and was were analyzed and tentatively identified using the Maslynx 4.1 program.

2.5. Biosynthesis Of Zinc Oxide Nanoparticles

placed into a glass thimble then extracted with 350 ml of different solvents separately (defatted by petroleum ether (60-80^{o}) then 95% ethanol). The extraction procedures continue until the solvent in the Soxhlet device' siphon tube turns colorless.. The extracts (3.5, 9.64g) respectively, were kept in refrigerator at -4 ^{o}C for their future use

2.3. Paper Chromatography:

In order to conduct comparative experiments on the *G.cambogia* leaf extract under consideration, BAW was used for the first dimension and 15% AcOH was used for the second dimension.Whatman (IMM) was subjected to two-dimensional paper chromatography (TDPC).

2.4. Liquid Chromatography Electrospray Ionization-Mass Spectrometry (LC-ESI-Mass).

The ethanol extract was detected by using an inverse stage C_{18} column (ACQUITY UPLCBEH C_{18} 1.7 µm particle 5 mm Column) at the Drug Discovery and Development Research Centre (Ain Shams University, Giza, Egypt). The sample solution (100 µg/mL) was prepared using MeOH grade solvent, filtered through a membrane disc filter (0.2 µm) and then analyzed using LC-ESI-MS. The sample volume (10 µl) was injected into the LC-ESI-MS apparatus. The portable sample stage was degassed and filtered with a 0.2 µm filter membrane disc prior to injecting the sonication. Gradient mobile phase elution was carried out with acetonitrile and water that had been acidified with 0.05% formic acid as the eluents at a flow rate of 0.2 mL/min. The negative ion mode used the following parameters: 50 L/h cone gas flow, 30 eV cone voltage, 3 kV capillary voltage, 440°C desolation temperature, and 900 L/h desolation gas flow are the conditions.. The ESI negative ion mode was used to identify mass spectra in the m/z range of 100 to 1000. By comparing retention time (Rt min) with mass range and external data, peaks and spectra

The synthesis was performed by adding 5mg of *G. cambogia* leaves ethanol extract continued to 100 ml distilled water on 900 ml of freshly prepared aqueous (0.01 M) Zn $(NO_3)_2.6H_2O$ (zinc nitrate hexahydrate)at 60°C for 12 hours on a hot plate with a magnetic stirrer (1000 rpm).

The ZnO-NPs were isolated and purified by centrifugation at 10000 rpm for 30 minutes, followed by dispersion of the nanoparticle pellet in ethanol and dried in an oven at 50°C. Change in color occur from pale yellow to white powder obtained confirms the of ZnO-NPs formation .

2.6. Characterization Of Zinc Oxide Nanoparticles

The ZnO-NPs have been examined using a digital pH meter (Eutech Cybersacn pH 300). The Rigol ultra-3660 UV-vis spectroscopy was performed in the 200-1200 nm range. Following that, Zeta potential and the dynamic light scattering were used to measure the size distribution of ZnO-NPs. SEM was used to assess the surface area, size, and shape. One drop of the sample was applied to a glass slide after the ZnO-NPs solutions had been ultrasonically processed at room temperature for 15 minutes. After the gold coating had dried, the glass slide was inspected with scanning electron microscope (Germany's Zeiss Evo-MA 10). ZnO-NPs powder was sonicated, placed into a copper grid while suspended in ethanol, allowed to dry, and then examined by TEM.

2.7. Antimicrobial Activity

The antimicrobial activities of ethanolic extract of *G.cambogia* and ZnO-NPs against *Escherichia coli (E. coli)* ATCC10536 and *Staphylococcus aureus (S. aureus)* ATCC6538 were evaluated using the disc diffusion method [4]. Briefly, the ethanolic extract and ZnO-NPs were dissolved in DMSO, and a solution of 1 mg/mL concentration was produced. The Müller-Hinton agar medium (Mha, Chaitanya Agro Biotech Pvt.Ltd.) plates were streaked with the tested bacterial strains using sterile cotton swabs. Then, sterilized Whatman filter papers no.1 (Merck) with a diameter of 6 mm were impregnated with appropriate extract and placed on the agar surface plates using sterile forceps .To establish total contact with the agar surface, the object was gently pressed down. The diameter of each inhibitory zone was measured with a ruler and recorded in millimeters after the plates were incubated aerobically at 37 °C for 24 hours.. Moreover, the antimicrobial activity of ethanolic extract of *G. cambogia* and ZnO-NP against *Candida albicans* (*C. albicans*) (ATCC 66027) was estimated. The Czapex-Dox agar medium (Merck) was seeded with *C. albicans* and poured onto sterile petri dishes and allowed to solidify. After that, 5 mm diameter holes were punched by a sterile cork-borer, and 0.1 ml of each extract was inoculated. The diameter of the inhibition zone (in mm) was used to calculate the antibacterial activity after the inoculation plates were incubated at 30oC for 7 days.The complex's percent activity index was calculated using the following equation:

$$\% \text{ Activity index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100$$

2.8. Wight Loss :

The laboratory animal unit provided 28 male Sprague-Dawley rats (8 weeks old; 300-380g). All rats were housed and kept in stainless steel cages at a temperature of 25 °C, a relative humidity of 55%, and a light-dark cycle of 12 hours. Rats had a two-week acclimatization period before the trial began. The Care and Use of Laboratory Animals was followed throughout all study methods, which were authorized by the Ethics of Animal Use in study Committee (IACUC), Zagazig University, Egypt. The rats were fed commercial pellets food naturally but to increase the weight of these rats they will be fed commercial pellets food and sunflower seeds by (90:10) percent, rats weighing between 308-366 gm were used; the weight was already increased between 320-383 gm and then the rats were fed with commercial pellets food and *G.cambogia* by (90:10) percent and other rats were fed with commercial pellets food and ZnO-NPs in the same ratio.the experimental rats were randomly divided into 4 groups (7 rats per group). The experiment was conducted over 6 days

As the following: Group I (control): Normal control fed with commercial pellets food only (300-380gm); Groups II: Rats given commercial pellets food and sunflower seeds (325gm). Group III: Rats given commercial pellets food and *G. cambogia* extract (90:10) % (16 gm, orally) for 6 days. Group IV: Rats given commercial pellets food with ZnO-NPs (90:10)%(16 gm,orally). At the end of the experiment; plasma from blood samples were drawn and analyzed in specialized analysis laboratories (Almostafa Laboratory,Alsharqia,Egypt) to determine

cholesterol, phospholipids, triglycerides, free fatty acids, total lipids and lipoprotein enzymes [5].

Furthermore, Lipoprotein lipase enzyme gene encodes (LPL) act as a dual functions, homodimer of triglyceride hydrolase and ligand / bridging factor for lipoprotein receptor uptake. Very low-density lipoprotein (VLDL) is converted to intermediate-density lipoprotein (IDL) and then to Lipoprotein lipase (LDL) by catalysis. This cause LPL deficiency result in hyperlipoproteinemia (type I), while less extreme mutations in many disorders of metabolism of lipoprotein where LPL are linked. This enzyme was assayed by the method of Baginsky et.al., [6].

3. Results and Discussion

3.1. Investigation of Bioactive Compounds of G. cambogia using LC-ESI-Mass.

The analysis of ethanolic extract of *G. cambogia* and their compounds indicates by using Liquid Chromatography Electrospray Ionization-Mass (negative ion mode) were detected and mass fragmentation patterns compared with literature data (Fig.1). Six detected compounds were identified as; guanosine, prephenic acid, tetrahydroxy-cholanic acid, limocitrin, 6,7-dihydroxycoumarin-6-glucoside and hydroxy citric acid as shown in (Table 1). The high concentration of bioactive chemicals that are capable of supplying electrons for the reduction of zinc nitrate hexahydrate to ZnO-NPs, as shown in (Fig. 2), is what causes the green synthesis of ZnO-NPs by alcoholic extract of *G. cambogia* . The carboxyl and hydroxyl groups create a protective layer on the ZnO nanoparticles' surface during the procedure. The particles can be stabilized by the steric barrier this shielding layer can offer around them.

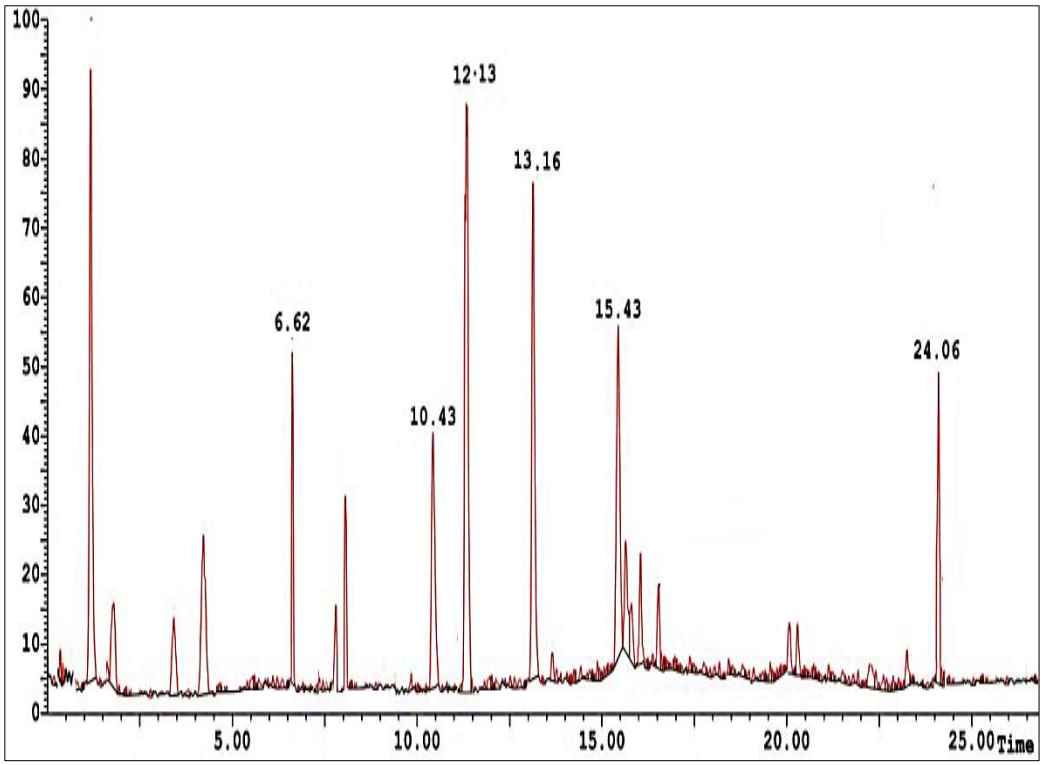

Fig. (1): LC-ESI-Mass Profile, of *G.cambogia* Ethanolic Extract.

Table (1) : Major Bioactive Compounds Identified By LC-ESI-Mass Of *G. Cambogia* Ethanolic Extract.

Peak	Compounds	$R_{t.min}$	Base Peak m/z	Exact Mass	Formula	Peak
1.	Guanosine	6.62	282.25	283.09 167	$C_{10}H_{13}N_5O_5$	157.63055
2.	Prephenic acid	10.43	225.20	226.04774	$C_{10}H_{10}O_6$	146.99469
3.	Tetrahydroxy-cholanic acid	12.13	423.25	424.28250	$C_{24}H_{40}O_6$	507.77061
4.	Limocitrin	13.16	345.15	346.06888	$C_{17}H_{14}O_8$	468.25989
5.	6,7-Dihydroxycoumarin-6-glucoside	15.43	339.35	340.28400	$C_{15}H_{16}O_9$	63.16640
6.	Hydroxy citric acid	24.06	208	209.07824	$C_6H_8O_8$	57.37039
Total						1401.19254

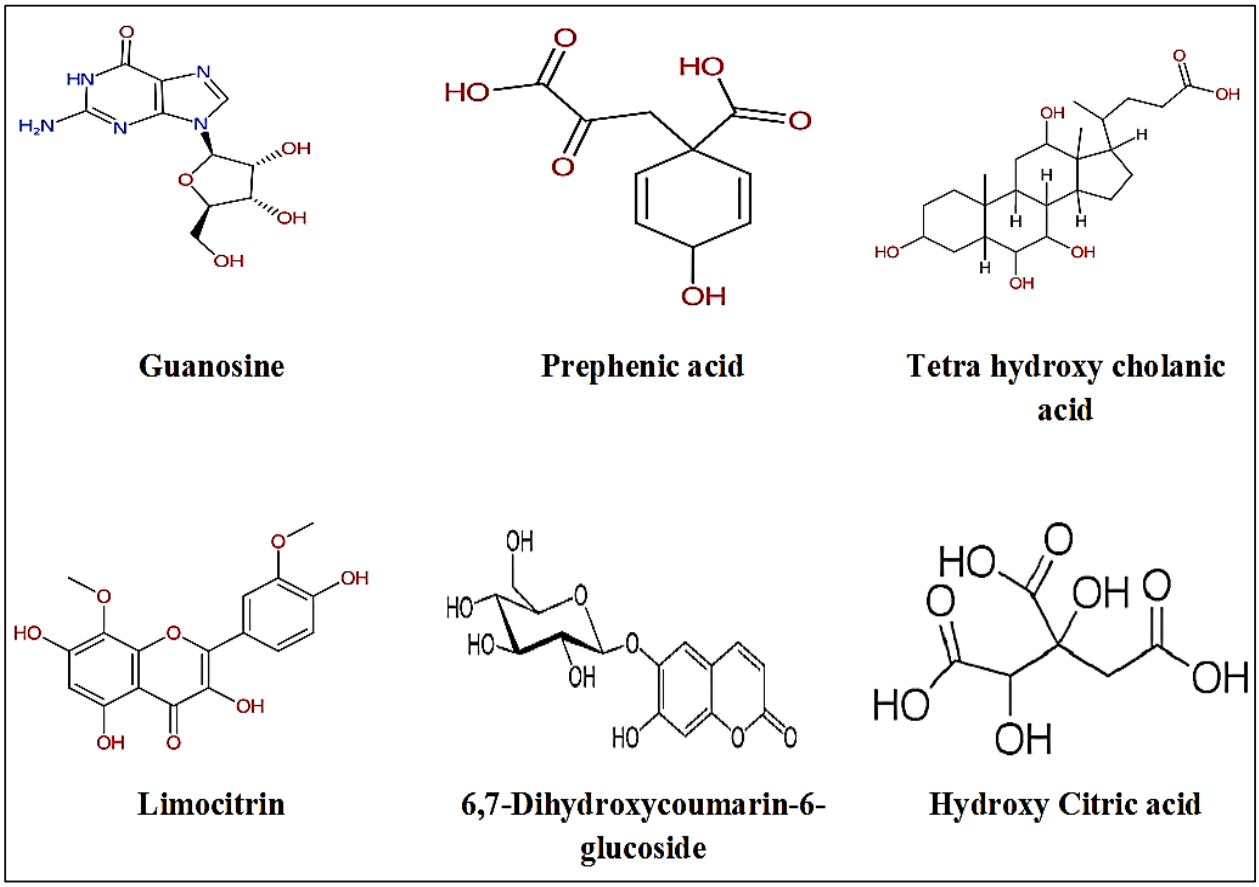

Fig. (2): Chemical Structures Of Bioactive Compounds In *G. Cambogia* Ethanolic Extract.

3.2. Green Synthesized ZnO-Nps Characterization

3.2.1. Change in Visual Color

The first indication of ZnO-NPs formation is a shift in the color of the reaction mixture. The color is changed from light yellow to white, suggesting that ZnO-NPs were formed, a similar visual observation was made in synthesized ZnO-NPs using *G. cambogia* leaves [7].

3.2.2. pH Reduction

Reaction mixture pH decreased from 11.07 to 6.58 in the presence of *G. cambogia* extract, suggesting a reduction of 0.01M (Zn (NO_3) $2.6H_2O$) during the formation of ZnO nanoparticles.

3.2.3. UV–Visible Spectrophotometric Analysis

By UV-vis spectroscopy ZnO-NPs formation was confirmed within the range 200–1200 nm. The green synthesized ZnO-NPs absorption spectrum showed a characteristic response at 200 to 350 nm which showed that ethanolic extract of *G. cambogia* leaves was able to synthesis ZnO-NPs (Fig.3).By UV-visible spectrum sharp peak confirms the zinc oxide nanoparticle at the absorption range between 260 and 280 nm. [8].

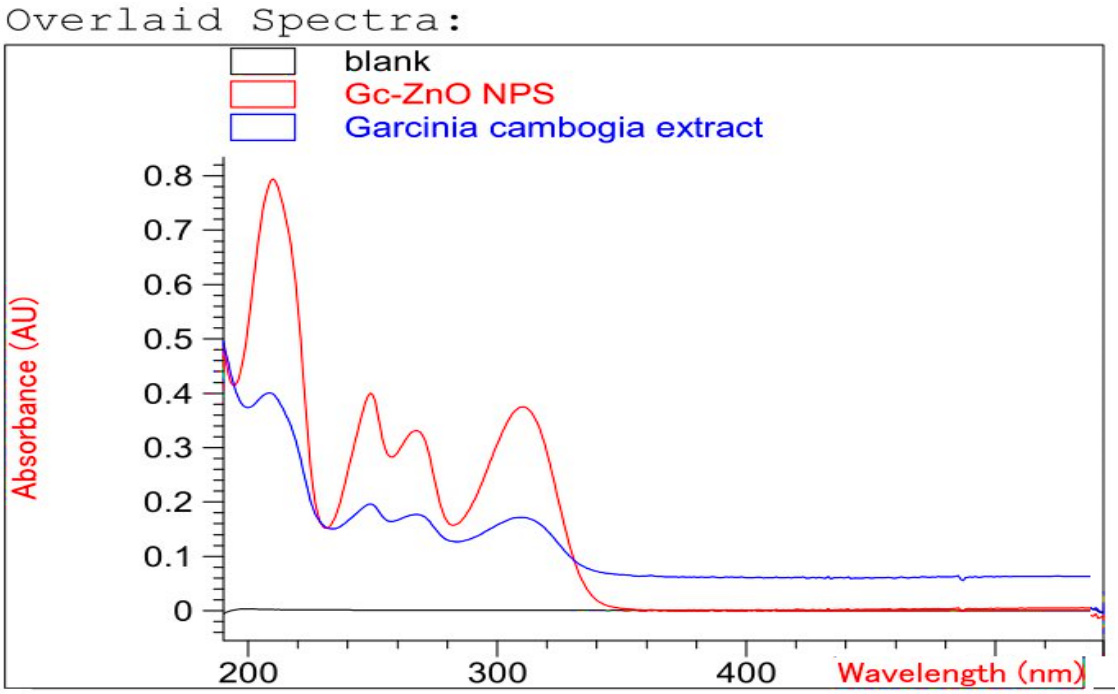

Fig. (3): The UV–visible Spectrum Of *G. Cambogia* Extract and ZnO-NPs

3.2.4. Scanning Electron Microscopy (SEM)

ZnO-NPs formed by *G. cambogia* leaves extract have a somewhat rod shape, as seen in SEM images (Fig.4). This discovery is similar to the fact that rod-shaped ZnO-NPs were created using plant extract [8]. In addition, the spherical ZnO-NPs were observed by *Garcinia cambogia* [9]

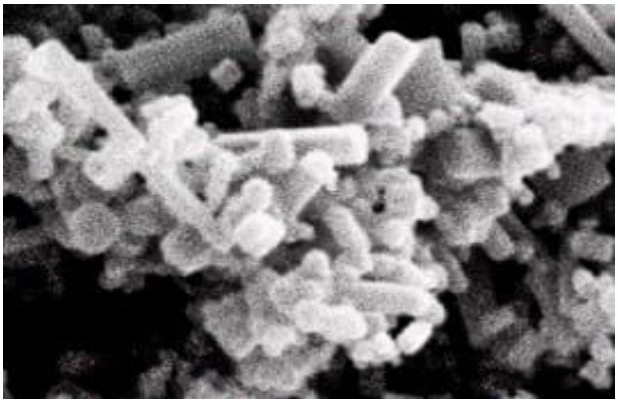

Fig. (4): SEM Analysis Of Synthesized ZnO-NPs

3.2.5. Transmission Electron Microscope (TEM)

TEM analysis may be used to evaluate the morphological properties, size and form of the produced ZnO-NPs (Fig.5). TEM images illustrating hexagonal forms shape and size of synthesized nanoparticles ranges 33 nm . Additionally, the majority of the produced nanoparticles have hexagonal shapes and range in size from 11 to 32 nm [8].

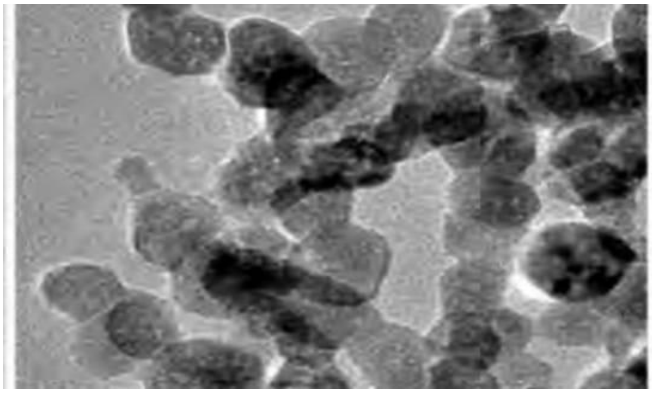

Fig. (5): TEM Images of ZnO-NPs

3.2.6. Dynamic Light Scattering and Zeta Potential

The size distribution and hydrodynamic diameter of ZnO-NPs were determined using dynamic light scattering analysis. The average particle size is 131.5 nm as demonstrated in (Fig .6). This finding is similar to the average particle size of produced ZnO-NPs from *G. cambogia* was determined to be around 133.8 nm using the dynamic light scattering approach [8]. The size of ZnO-NPs varied from 90 to 110 nm which is mostly present in aggregates [9]. The bioactive components in *G. cambogia* ethanolic extract suppress the formation of zinc oxide micro particles caused by agglomeration. The Zeta potential of ZnO-NPs was around 53 mV as indicated in (Fig.7). Also, synthesized ZnO-NPs derived from *G.cambogia* extract which demonstrated a Zeta potential of 57.9 mV and was responsible for stability [8].

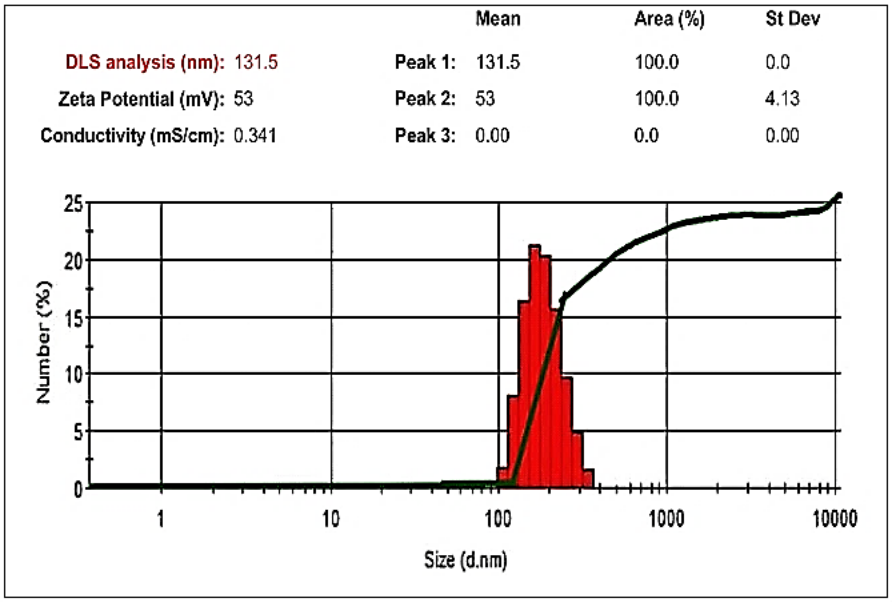

Fig. (6): Dynamic Light Scattering Analysis Of ZnO-NPs

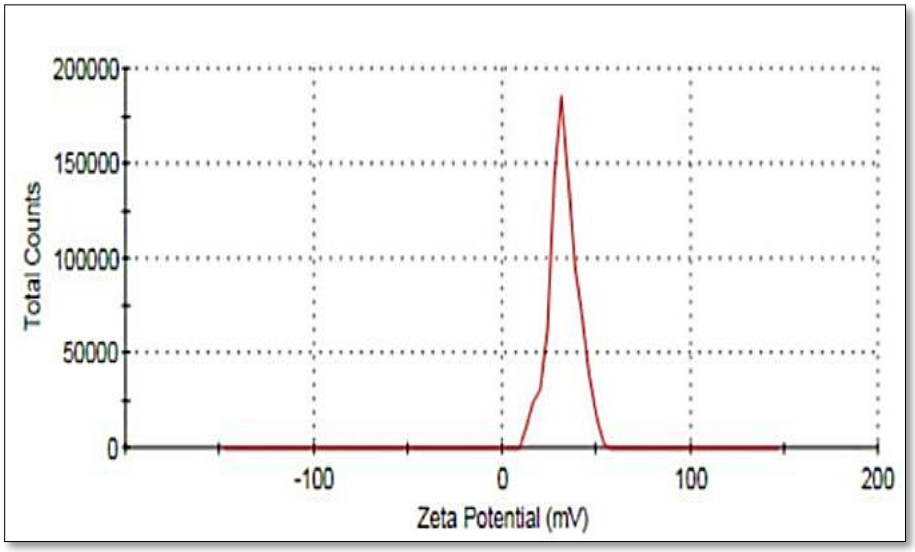

Fig. (7): Zeta Potential Of ZnO-NPs

3.3. Antimicrobial activity

The prevalence of microbial infections has risen over the last few decades, particularly bacterial infections, which cause a high death rate among immune-compromised patients [10]. Due to the limited number of antimicrobial medicines available, there is an urgent need to discover new antimicrobial drugs or chemicals [11].

In this study, diffusion agar method was used to evaluate the antibacterial and antifungal properties of ethanolic extract of *G. cambogia* and ZnO-NPs. The diameter of inhibition zone produced on a variety of harmful bacteria was measured and recorded in mm (Table 2). The antibacterial results were compared to the activity of commercially available standard antibiotic (Ampicillin), while for antifungal results were compared to the commercially available standard antifungal (Fluconazole).

Table (2) : Antimicrobial Activities of *G. cambogia* Ethanolic Extract and ZnO-NPs

Bacterial Microorganisms	*G. cambogia* Ethanolic extract		ZnO-NPs		Standard Antibiotics (Ampicillin)	
	Inhibition zone's diameter (mm)	Activity index (%)	Inhibition zone's diameter (mm)	Activity index (%)	Inhibition zone's diameter (mm)	Activity index (%)
***Staphylococcus aureus* ATCC6538**	**35**	**233.3**	**40**	**266.6**	**15**	**100 %**
***Escherichia coli* ATCC10536**	**45**	**450**	**50**	**500**	**10**	**100 %**
Fungal Microorganism					**Standard Antifungal (Fluconazole)**	
***Candida albicans* ATCC 66027**	**45.37**	**216**	**50.11**	**238.6**	**21**	**100 %**

It was found that ZnO-NPs exhibited high antibacterial activity against *S.aureus* (40 mm) than *cambogia* ethanolic extract (35 mm). Also, ZnO-NPs showed significant antibacterial activity against *E. coli* (50 mm) compared to *G. cambogia* ethanolic extract (45 mm).

However, ZnO-NPs showed high antifungal activity against *C.albicans* (50.11 mm), while *G. cambogia* ethanolic extract showed lower activity (45.37 mm). Furthermore, the ZnO-NPs displayed higher antibacterial activity than regular use and long-term effects of particular substances [13].

ampicillin when tested against *S.aureus* (266.6%) and *E. coli* (500%) and displayed more antifungal activity than Fluconazole against *C.albicans* (238.6%). The better explanation for that may be due to the bioactive ingredients detected by LC-ESI-MS analysis in the ZnO-NPs, such as phenolics and flavonoids, may be amplified in the presence of ethanolic extract. Also, the higher extraction capacity of ZnO-NPs may be to blame, resulting in more active chemicals in the polar extracts. It is possible that certain compounds present in this extract are accountable for their antimicrobial properties, which supports the historical use of medicinal herbs in treating bacterial infections [12].

3.4. Weight Loss Activity:

3.4.1. Effect Of *G. Cambogia* Extract and its ZnO-NPs On Weight Loss And Biochemical Parameters In Laboratory Rats:

Obesity has become one of the most significant issues affecting public health in a number of nations today as a result of the sharp rise in cases over the past few years .

As a result, numerous weight-loss aids have emerged, including miracle diets and dietary supplements, although frequently little is known regarding the

According to previous research, *G. cambogia* helps to maintain weight by promoting fullness, inhibiting appetite by lowering the desire to eat, and stimulating the body's natural fat-burning processes [13,14].

In this study, the administration of ethanolic extract of *G. cambogia* as a weight-reduction treatment resulted in marked increase in the levels of total lipids, cholesterol, triglycerides, Phospholipids and free fatty acid while, the synthetized ZnO-Nps decreased in the levels of total lipids, cholesterol and free fatty acid as compared to those in normal rats.

The levels of Phospholipids remained unchanged, while triglycerides increased significantly in the synthetized ZnO-Nps administered rats.

On other hand, the level of Lipoprotein lipase enzyme decreased in the plasma after ethanolic extract of *G. cambogia* administration but increased significantly in the plasma after the synthetized ZnO-Nps administration as compared to those in normal rats as shown in (Table 3)

However the separation of plasma from heparinized blood samples was assayed by the method of *Hitz et al.,* [15]. Separation of plasma lipoproteins by a dual precipitation method [16]. Total cholesterol, phospholipids, triglycerides and free fatty acids were determined in the plasma [17].

Table (3): Effect Of *G. Cambogia* And ZnO-Nps on Weight Loss of Rats and Biochemical Parameters of Laboratory Rats

Parameters	Group I Normal control fed	Group II with food	Group III with extract	Group IV with ZnO-NPs
Weight of rats	337.9 ± 29.4 gm	351.0 ± 31.7gm	342.6 ± 32.6 gm	333.9 ± 29.7 gm
Total lipids	61.1 ± 1.2	75.1 ± 1.3	64.6 ± 2.0	59.7 ± 2.0
Cholesterol	4.4 ± 0.2	7.2 ± 0.3	5.5 ± 0.7	4.0 ± 0.2
Triglyceride	4.0 ± 0.2	9.3 ± 0.1	8.4 ± 0.1	5.4 ± 0.2
Phospholipids	34.7 ± 0.7	38.2 ± 0.8	37.3 ± 0.7	34.7 ± 0.6
Free fatty acid	9.7 ± 0.4	13.9 ± 0.8	12.0 ± 0.1	9.1 ± 0.2
Lipoprotein lipase enzyme	5.3 ± 0.1	3.3 ± 0.1	4.0 ± 0.1	5.5 ± 0.2

4. Conclusions

In the current work, we generated ZnO-NPs from the ethanolic leaves extract of *G. cambogia* rich in bioactive compounds was proven utilizing LC-ESI-MS analysis. As a result of the bioactive components of the extract acted as a reducing and stabilizing factor in the synthesis of ZnO-NPs. Several methods, including "pH analysis, UV-vis , SEM, and TEM," were used to validate the ZnO-NPs, demonstrating the presence of ZnO nanoparticles with particle size less than 20 nm.

Based on these findings, it is concluded that the synthesized ZnO-NPs is a more promising candidate than alcoholic extract of *G. cambogia* leaves against pathogenic bacterial , fungal strains,

The administration of *G. cambogia* and synthetized ZnO-Nps as a weight-reduction treatment is intended to give important knowledge for both scholarly and broad public use regarding the effects of this plant and its treatment of obesity and providing satisfying safer with less expensive alternatives to traditional therapeutic regimens with all of the promising characteristics of green-produced ZnO-NPs.

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