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Synthesis, spectral characterization and free radical scavenging activity of zinc-genistein nano complex

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Metal-flavanoid complexes could be of interest in the medical field. The most studied dietary phytoestrogen (plant compounds that may resemble estrogen or modify its effects in the human body) is genistein, a naturally occurring isoflavone. Zinc, an essential trace element, is involved in various physiological processes in the human body and can protect against oxidative stress in certain tissues and conditions. The formation of metal flavonoid complexes can improve the stability and bioavailability of flavonoids while influencing their biological activity. The purpose of this study is to quantify the antioxidant capabilities of the zinc-genistein complex (ZGC) compared to flavonoids alone. Zinc chloride and genistein were combined in a modified chelation process to create ZGC. FTIR (Fourier transform infrared spectroscopy), UV (ultraviolet-visible spectroscopy), ^{1}HNMR (proton nuclear magnetic resonance), atomic absorption spectra (AAS), mass spectrometry (MS) and elemental analysis were used to characterize the metal-flavanoid complex produced. When the characterized ZGC was tested for its antioxidant activity, it was found that ZGC had an IC_{50} value of 119.32 mcg, while genistein had an IC_{50} value of 908.34 mcg. This indicates that ZGC has greater antioxidant activity than genistein alone.

Keywords: Antioxidant, Atomic Absorption Spectra, Bioavailability, Genistein, Metal flavonoid complex, Zinc

Oxidative stress can contribute to not only tissue damage but also leads to various metabolic disorders[1]. Flavonoids are a class of phenolic compounds found in various plants and are known for their diverse biological activities and health benefits. They act as anti-oxidants and have been connected to a variety of possible health-promoting actions, including anti-inflammatory, anti-cancer, and cardiovascular protective characteristics.

Genistein is a naturally occurring isoflavone, a type of flavonoid, found in several plants, especially soy and soy products. It is one of the best-known and most studied dietary phytoestrogens, which are plant compounds that can mimic or modulate the effects of estrogen in the human body. Genistein's chemical structure is similar to that of estrogen, making it capable of attaching to estrogen receptors and acting their estrogenic or anti-estrogenic effects, depending on context. Genistein has many potential health benefits, such as antioxidant, anti-cancer, and estrogenic properties[2]. Due to its estrogenic properties, Genistein is effective in hormone-related conditions, such as menopausal symptoms, hormone-dependent cancers, and hormonal imbalances[3,4]. Zinc, an essential trace element, is involved in many physiological functions in the human body. Research has linked zinc supplementation to antioxidant effects and examined zinc's potential defense against oxidative stress in certain tissues and situations[5-7].

A metal flavonoid complex refers to a coordination complex formed between a metal ion and a flavonoid molecule. Due to their potential therapeutic uses, these complexes have generated a great deal of attention in neutraceutical research. By fusing the advantageous traits of flavonoids with unique properties of metal ions, novel metal complexes were developed with enhanced bioactivity and pharmacological properties[8,9]. The stability and bioavailability of flavonoids can be improved through the development of metal flavonoid complexes, which can also influence their biological activities. In addition, compared to their free ligands,

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metal flavonoid complexes may exhibit certain properties such as altered antioxidant capacity, altered cellular uptake, and certain interactions with biological targets[10-12].

The possible applications and characteristics of metal flavonoid complexes may differ depending on the specific flavonoid, metal ions, and coordination environment involved in this field of study, which is still under investigation[13,14]. Extensive research is needed to fully understand the potential benefits and hazards of novel compounds.The current work attempts to evaluate and synthesize the antioxidant studies of the zinc-genistein nano complex (ZGC). However, nanotechnology revolutionized the medical field where defensive and therapeutic applications of nanotechnology are being used in present days[15-18]. Nanotechnology is not limited to medical areas, it also paves the way for mitigations of environmental degradation and finds uses in sustainable agriculture[19-21].

Materials and Methods

Chemicals and Reagents

Sigma-Aldrich is where genistein, zinc chloride, and ethanol are obtained. Only analytical-grade chemicals were purchased. In addition, analytical-grade reagents utilized in the research were acquired from Sigma-Aldrich.

The glassware was thoroughly cleaned with 10% nitric acid. Rinsed twice with running tap water and once with distilled water. The cleaned glassware was sterilized in a convection oven for 5 h at 80°C before use.

Synthesis of Zinc-Genistein Complex

An accurate weight of 0.272 grams (0.1 M) of genistein was added to a round bottom flask (RBF) containing 20 mL of ethanol. To dissolve the genistein in the ethanol, the RBF is shaken very hard. 68.14 mg (0.05 M) $ZnCl_2$ was added to the ethanolic solution and dissolved in ethanol. Tris HCl buffer solution is used to adjust the pH of the solution to 8.0[22-24]. The solution was kept under reflux at 70°C for the reaction while being stirred at 600 rpm using a magnetic stirrer. The solution's color changed from light yellow to brown after 8 h of reaction, indicating the formation of the complex. Using the mobile phase of toluene, ethyl acetate, and formic acid in the ratio of 5:4:0.2, respectively, thin layer chromatography (TLC) was used to monitor the reaction process at this stage. Once the reaction was complete, the solvent was evaporated, leaving yellow-brown crystals. After two cycles of recrystallization in ethanol, the crystals were dried and stored at 5°C.

Characterization of Zinc-Genistein Complex

Fourier Transform Infra-Red (FT-IR) spectroscopy

Infrared spectra (IR) of ZGC and pure genistein were recorded using a Fourier transform infrared spectrophotometer (Shimadzu, LC 2010NAHT, Japan), which digitized the data at 4 cm^{-1} resolution between 4000 and 400 cm^{-1}.

UV-visible spectroscopy

To determine the absorption maxima (max), UV-visible spectral studies of ZGC and genistein were performed using a UV-visible spectrophotometer (Shimadzu, UV-1700 series, Japan). The absorption maxima of ZGC and genistein were measured in the range of 200 nm and 400 nm wavelengths using a proper dilution of 10 mcg/mL solutions in methanol, and the spectra were recorded.

Atomic absorption Spectroscopy (AAS)

The zinc content of ZGC was calculated using the dry ash method. A porcelain dish containing one gram of sample was heated to 600°C for 5 h, or until the sample burned completely to ash. After collection, the ash was diluted with five milliliters of nitric acid. After taking 1 mL of the above nitric acid solution, 10 mL of deionized water was added. This solution was tested using conventional zinc dilutions as the sample. One milliliter of the zinc standard solution, *i.e.* 100 mcg/mL, was placed in a 10 mL volumetric flask and used as a stock solution. 0.5 mL, 1.0 mL, 1.5 mL, 2.0 mL, and 2.5 mL of the stock solution were obtained and diluted with deionized water in different 10 mL volumetric flasks, yielding dilutions of 5 mcg /mL, 10 mcg/mL, 15 mcg/mL, 20 mcg/mL and 25 mcg/mL, respectively. These dilutions are used as a reference for the AAS sample. The absorbance of the sample and standard dilutions was determined using an atomic absorption spectrophotometer (Shimadzu, AA-6300, USA).

Elemental analysis

The CHNS/O microanalyzer (Thermo Scientific Flash 2000 Organic Elemental Analyzer Waltham, MA, USA) was used to determine the elemental composition. An elemental analyzer was used to examine the compositions of various elements, including carbon, hydrogen, sulfur, nitrogen, and oxygen. A thermal conductivity detector was used to determine the corresponding element after an accurately weighed 0.5 mg sample was heated to 1000°C.

^{1}H-Nuclear mass resonance (NMR) spectroscopy

To study the binding mechanism and validate ZGC structure, the sample's ^{1}H NMR spectra were examined on a 400 MHz NMR spectrometer (Bruker, AC-300 Spectrometer, Germany) in methanol. 100 mg of the sample was dissolved in methanol and the NMR spectrum was acquired at a fundamental frequency of 400 MHz, using methanol as solvent.

High-Resolution mass spectroscopy (HR-MS)

Using a high-resolution liquid chromatography-mass spectrometer (Agilent Technologies, 1260 infinity USA), the HR-MS spectrum was obtained to determine the precise molecular weight of ZGC. The average molecular weight of the material was determined using electron spray ionization (ESI) and direct mass infusion.

Particle size analysis

Zetasizer (Malvern, ZS 90, UK) was used to measure the polydispersity index and particle size. Before the measurement, 1 mg of ZGC was suspended in deionized water and sonicated. The polydispersity index and mean particle size were measured at room temperature with a scattering angle of 90°C.

Radical Scavenging activity

To determine the scavenging of radicals by ZGC, the DPPH (2,2-diphenyl-1-picrylhydrazyl) method was employed. A stock solution of 1000 mcg/mL DPPH was prepared from which various concentrations (1000 mcg/mL, 500 mcg/mL, 250 mcg/mL, 125 mcg/mL, 61.25 mcg/mL) were prepared. The same concentrations of sample (ZGC) and standard (genistein) were prepared using dimethyl sulfoxide (DMSO) as solvent. All the dilutions were filled in 96-well plate under standard conditions. After approximately 20 min of incubation at 37°C, the absorbance of the well plate was measured at a wavelength of 490 nm. The formula below was used to calculate the inhibition percentage.

$$\%\,\text{Inhibition} = \frac{(\text{Absorbance of Control - Absorbance of Sample})}{\text{Absorbance of Control}} \times 100$$

Results and Discussion

Synthesis of Zinc-Genistein Complex

Genistein and zinc chloride were combined in a modified chelation process to create a genistein-zinc complex. To create metal complexes with greater antioxidant activity than genistein alone, genistein can chelate metal ions. Completion of the reaction was first observed by the change in the color of the solution from light yellow to brown color and then examined by TLC method using mobile phase made up of 5 parts of toluene, 4 parts of ethyl acetate, and 0.2 parts formic acid, with the difference in the spots appeared.

Fourier Transform Infra-Red (FT-IR) spectroscopy

Infrared spectra (IR) of ZGC and pure genistein were recorded with the use of an infrared spectrophotometer that used the Fourier transform to digitize the data between 4000 and 400 cm^{-1}at a resolution of 4 cm^{-1}. The major functional groups found in genistein are visible in the IR spectrum as peaks at different wavenumbers. The O-H groups are represented by a strong peak at 3408.59, the C-OH groups by a peak at 1272.76, the C-H bonds by a peak at 3058.51, the C=C bonds by a peak at 1501.90 and the C=O groups through a peak at 1650.87. Zinc conjugation resulted in small variations in the wavenumbers of the hydroxyl (OH) and ketone (C=O) groups in the ZGC IR spectra. The zinc functional group at 788.49, indicating that conjugation of zinc and genistein has occurred, is visible in the ZGC at 1650.87 and 788.49 due to the absence of ketone groups. Figures 1 and 2 shows the IR spectra of Genistein and ZGC.

Uv-visible spectroscopy

UV-visible spectral studies of ZGC and genistein were performed (Fig. 3A & B). The absorption maxima of ZGC and genistein were measured in the range of 200 nm and 400 nm wavelengths. UV spectra of genistein show absorption peaks that correspond to the electronic shifts of the molecule. UV absorption maxima for genistein are often observed between 260 and 270 nm. UV spectra of zinc ions (Zn^{2+}) generally lack well-defined peaks in the ultraviolet spectrum as they are generally colorless, and do not absorb UV light very strongly. The genistein ligand interacting with zinc ions would influence the UV properties in the UV spectra of genistein-zinc complexes. A significant peak was observed at 262 nm in the absorption maximum of pure genistein. However, ZGC showed a broad peak ranging from 257 to 265 nm, indicating that zinc and genistein are conjugated.

Atomic absorption spectra

The absorbance of the sample solution (ZGC) and the dilutions of the zinc standard solution (5 mcg/mL, 10 mcg/mL, 15 mcg/mL, 20 mcg/mL and 25 mcg/mL) were determined in an atomic absorption spectrophotometer. measured at 213.9 nm (Fig. 4).

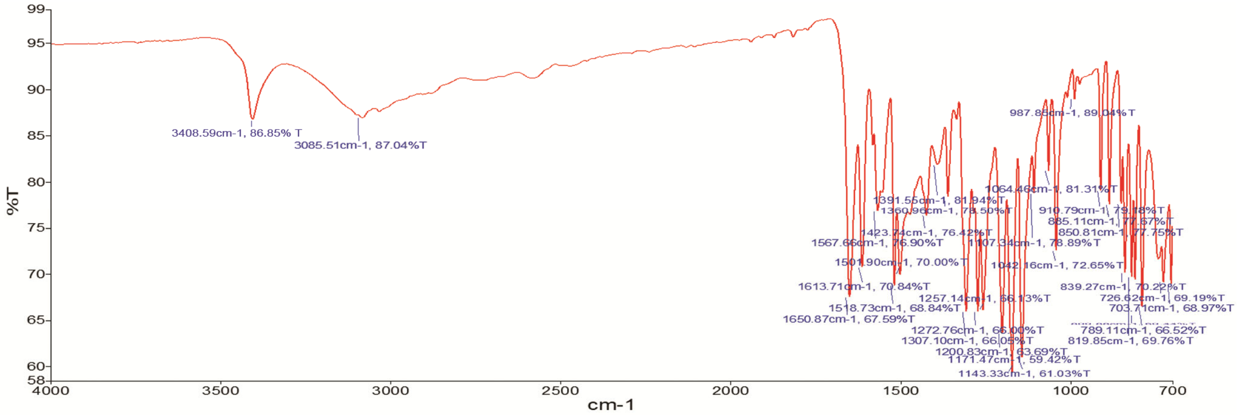


Fig. 1 — Genistein IR- 3408.59 (O-H str), 1272.76 (C-OH str), 1143.33 (C-O-C str), 3058.51 (C-H str AlK), 1501.90 (C=C str), 1650.87 (C=0 str)

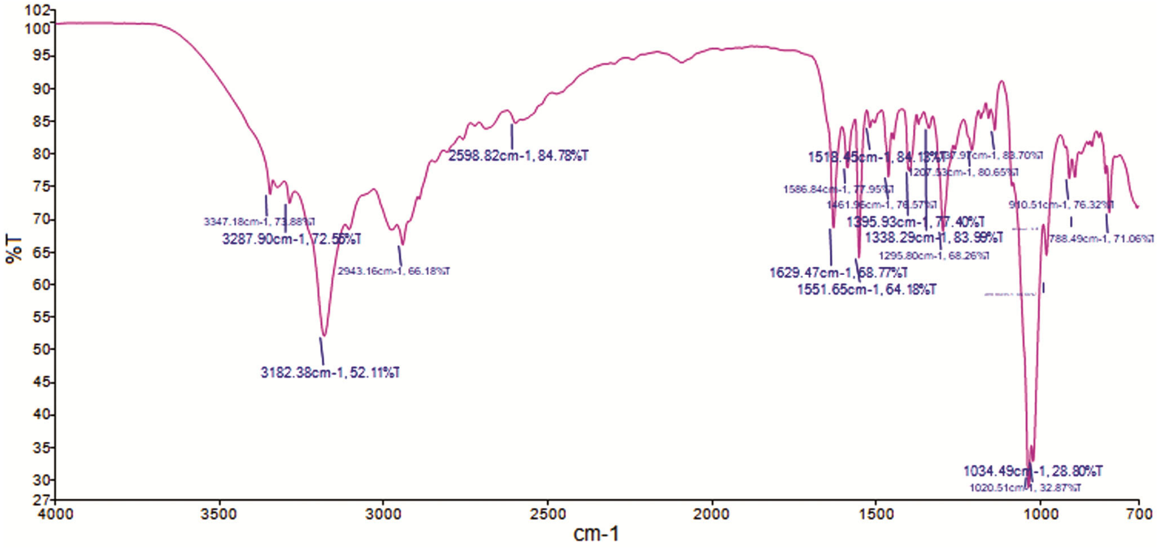


Fig. 2 — ZGC IR - 3182.38 (O-H str), 1295.80.53 (C-OH str), 1034.49 (C-O-C str), 2943.16 (C-H str Alk), 1551.65 (C=C str), 788.49 (Zn-O)

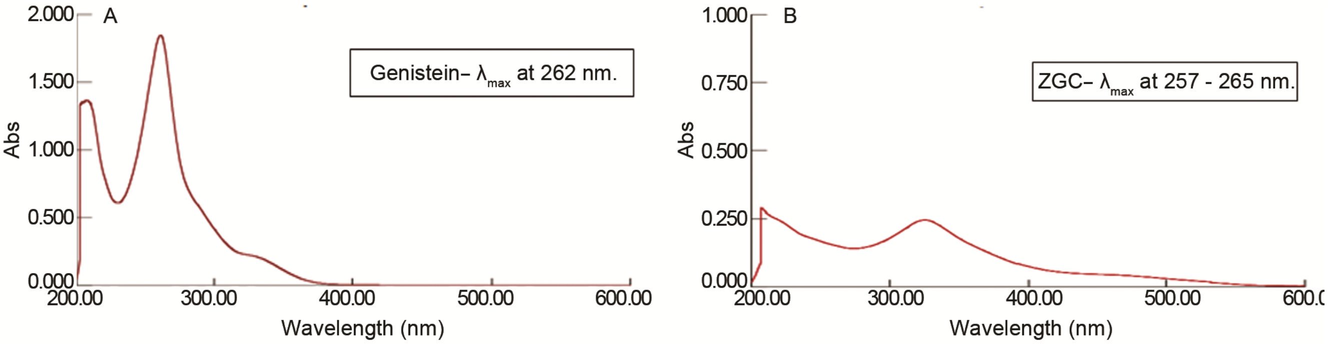


Fig. 3 — UV-Visible spectra for (A) Genistein; and (B) ZGC

Table 1 shows the absorbance values for the sample and subsequent dilutions. To determine the amount of zinc in one gram of ZGC, a graph was created based on the concentrations and absorbance values. The absorbance value of the sample was determined to be 1.016, which is comparable to 20 mcg/mL. This leads to an estimate of 1.9 ppm in the supplied sample.

Elemental analysis

The elemental composition was determined using the CHNS/O micro analyzer. ZGNC ($C_{30}H_{20}O_{10}Zn$) was found to have the elemental compositions of carbon 33.90%, hydrogen 11.21%, and oxygen 19.19%, respectively.

^{1}H-Nuclear mass resonance (NMR) spectroscopy

To study the binding mechanism and validate ZGC structure, the sample's^{1}H NMR spectra were examined on a 400 MHz NMR spectrometer in methanol. The OH absorption bands of the zinc-genistein complex shifted to a lower frequency compared to pure genistein, according to the spectrum. This could be because the metal ions removed the phenolic hydrogen atoms from the flavonoid, leading to a significant increase in conjugation in the zinc-genistein complex. Figure 5 shows the ^{1}H-NMR spectra of ZGC. The predicted structure for ZGC is given in (Fig. 6).

Table 1 — Atomic Absorbance of zinc standard and sample

Sl. No.	Concentration	Absorbance value	Parts per million (ppm)
1	5 mcg /mL	0.824	0.5
2	10 mcg /mL	0.951	1.0
3	15 mcg /mL	0.990	1.5
4	20 mcg /mL	1.017	2.0
5	25 mcg /mL	1.061	2.5
6	Sample (ZGC)	1.016	1.9

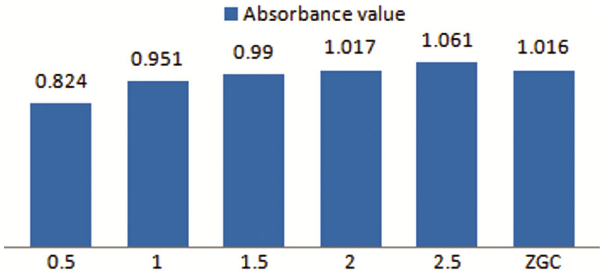


Fig. 4 — AAS for Standard Zinc and ZGC

1H NMR (300 MHZ, DMSO) δ 8.0 (s, IH, OH1), 8.4 (s,1H, OH2), 3.9(m, 5H, Ar-H1), 3.5 (m,5H, Ar-H2),7.5 (d,2H, CH), 6.9(d,2H, CH), 6.1 (d,2H, CH), 5.0 (d,2H, CH).

High-Resolution mass spectroscopy (HR-MS)

Using a high-resolution liquid chromatography-mass spectrometer, the HR-MS spectrum was obtained to determine the precise molecular weight of ZGC. The molecular ion peaks of ZGC by HR-MS (ES+) shown in (Fig. 7) were found to be by the

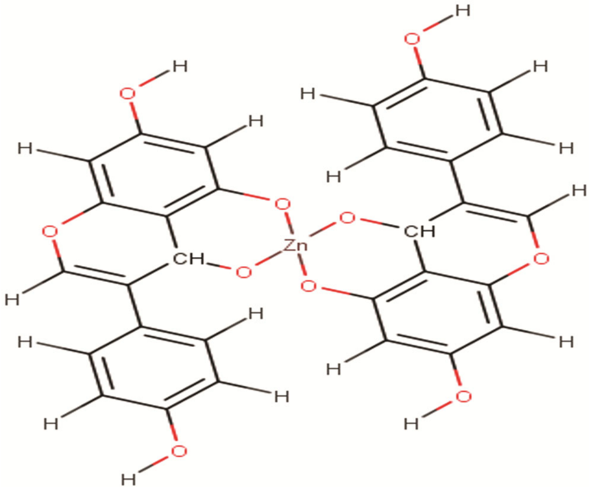
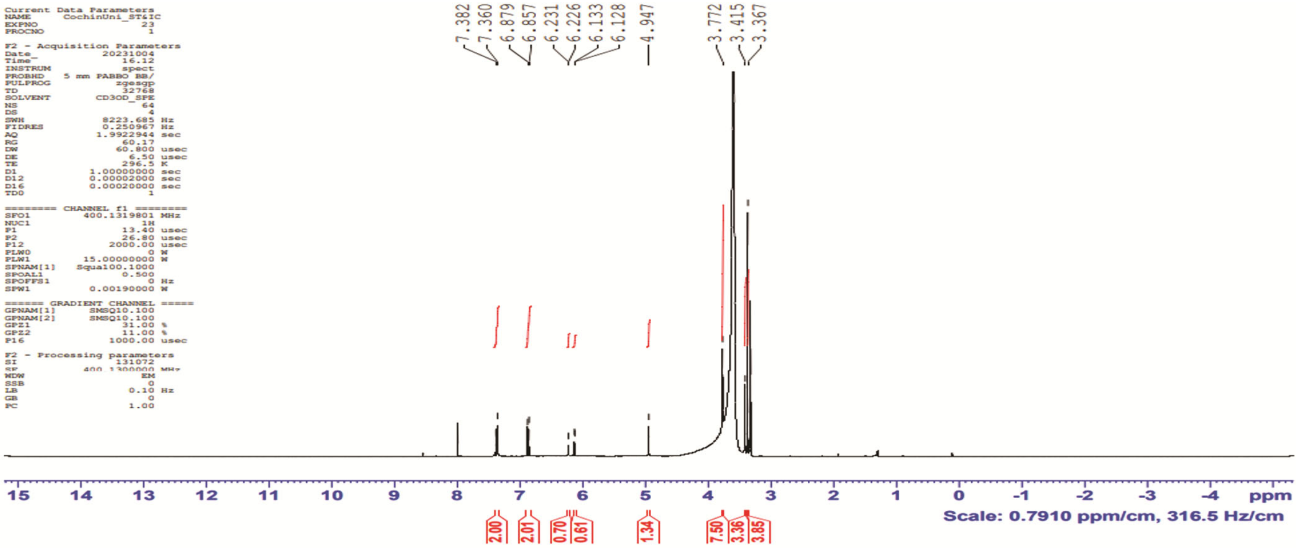


Fig. 6 — Predicted structure of ZGC



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PROCNO                  1

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PULPROG            zgesgp
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SOLVENT        CD3OD_SPE
NS                     64
DS                      4
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FIDRES           0.250967 Hz
AQ              1.9922944 sec
RG                  60.17
DW                 60.800 usec
DE                   6.50 usec
TE                  296.5 K
D1             1.00000000 sec
D12            0.00002000 sec
D16            0.00020000 sec
TD0                     1

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SFO1          400.1319801 MHz
NUC1                   1H
P1                  13.40 usec
P2                  26.80 usec
P12               2000.00 usec
PLW0                    0 W
PLW1          15.00000000 W
SPNAM[1]    Squa100.1000
SPOAL1              0.500
SPOFFS1                 0 Hz
SPW1           0.00190000 W

====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPZ1                31.00 %
GPZ2                11.00 %
P16               1000.00 usec

F2 - Processing parameters
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SF            400.1300000 MHz
WDW                    EM
SSB                     0
LB                   0.10 Hz
GB                      0
PC                   1.00
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Fig. 5 — ^{1}H-NMR spectra of ZGC

Table 2 — Antioxidant activity of Genistein and ZGC

Sl. No.	Sample	Concentration (mcg/mL)	% Inhibition
1	Genistein	1000	52.80
		500	38.55
		250	31.66
		125	30.99
		62.5	29.76
		31.25	25.59
2	ZGC	1000	92.64
		500	83.41
		250	75.16
		125	74.63
		62.5	41.74
		31.25	40.32

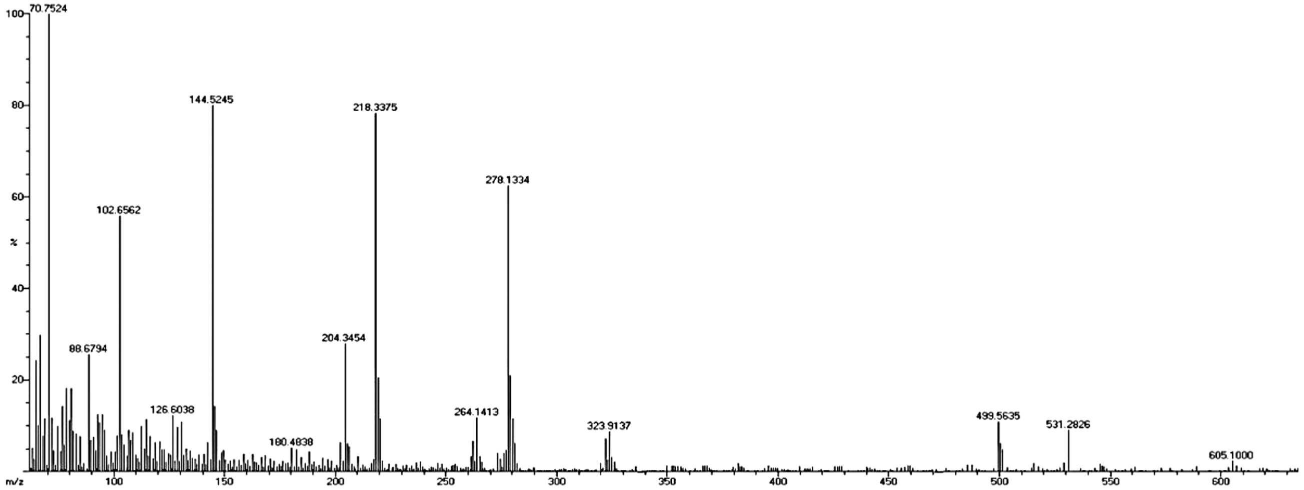

Fig. 7 — HRMS Spectra of ZGC

molecular formula $C_{30}H_{20}O_{10}$ and the molecular weight was calculated as 605.86 which resembles at 605.1 peak.

Particle size analysis

Zetasizer was used to measure the polydispersity index and particle size. Before the measurement, 1 mg of ZGC was suspended in deionized water and sonicated (Fig. 8). The polydispersity index and mean particle size were measured at a scattering angle of 90°C at room temperature. The mean hydrodynamic diameter of the particle was found to be 257.8 nm with a polydispersity index of 17.5%.

Radical Scavenging activity

The ability of a substance to 'capture' or neutralize free radicals in biological systems is called 'radical scavenging activity'. Extremely reactive chemicals called free radicals can damage tissues and cells. Antioxidants are known to eliminate free radicals. These consist of natural substances called polyphenols, flavonoids, and vitamins. ZGC used the 2,2-diphenyl-1-picrylhydrazyl (2,2-diphenyl-1-picrylhydrazyl) technique to measure its radical scavenging activity. At a wavelength of 490 nm, the absorbance of the sample and standard concentrations was measured and recorded as shown in (Table 2). The IC_{50} value of ZGC was determined to be 119 mcg, while the IC_{50} value of ZGC was determined to be 119 mcg, and the normal IC_{50} of genistein was 908.34 mcg. This indicates that the metal

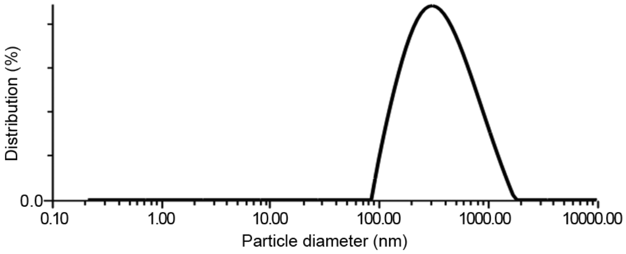

Fig. 8 — Particle size analysis of ZGC

flavonoid complex exhibits more activity than the flavonoid alone.

Conclusion

This work lays the foundation for further investigation and possible medical applications, adding to the expanding corpus of information on the Zinc-Genistein nanocomplex. The chelation approach was used to synthesize and characterize ZGC. Antioxidant effectiveness was assessed. According to the findings ZGC exhibited more antioxidant activity than genistein by itself. ZGC demonstrated more solubility than amorphous genistein because of its nanoparticle range and crystalline structure which can result in increased bioavailability even at lower doses. The results shown here open the door to more focused and efficient applications of Zinc-genistein complex.

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Conflict of interest

All authors declare no conflict of interest.

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