

# Synthesis of Gingerol-Metals Complex and in-vitro Cytotoxic Activity on Human Colon Cancer Cell Line

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**Introduction:** Herbs are excellent sources of medicinal substances, and their curative abilities have been recognized to treat many ailments and are used for example as antioxidants, analgesics, anti-inflammatories, antipyretics, and many other medicinal uses. The properties of natural compounds and their health effects have been studied extensively, especially those that originate from plant sources such as ginger. The ginger plant contains many chemical compounds, such as 6-gingerol, which is characterized by containing active groups such as carbonyl and hydroxide, which can be attached to metal molecules. This is what was done in this study, where the formation of complexes with a group of metals was studied and their effect on cancer cells was investigated. These complexes will open new horizons for further study of medicinal uses.

**Methods:** The synthesis of gingerol-metal complexes was carried out by conjugating gingerol molecules with Ag, Au, Cd, Co, Cu, Ni, and Zn metal ions. The extracted gingerol was transferred to culture tubes and deionized water-DMSO were added followed by sonication. The tubes were incubated at 90°C for two days as well as the control sample. The samples were then filtered and the complex solutions were transferred into new tubes for further studies. Different characterization techniques such as FT-IR, UV-vis spectroscopy, FESEM, and EDX are used to confirm the formation of the complexes. The in vitro of the complexes was tested by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay against the human colorectal cancer cell lines HCT116 and HT29 which exhibited strong cytotoxicity.

**Results:** The gingerol-metal complexes showed an enhancement as an anticancer agent compared to the control. The in vitro anticancer activity showed that the Ag-gingerol complex showed the most activity among the other complexes.

**Discussion:** Gingerol-metal complexes can inhibit cancer cells, noting that the potency of the complex depends on the type of metal used.

**Keywords:** ginger, gingerol, gingerol-complexes, colon cancer, tumor cell line

## Introduction

The discovery of new materials is a major challenge in the treatment of deadly diseases and is of particular interest to researchers. The number of fatalities which is attributed to cancer diseases increases on daily basis. Since the discovery of cisplatin, the first metal-based chemotherapy drug, metal complexes with metal-carbon bonds have been used as antitumor agents.<sup>1</sup> Thousands of platinum analogs have been developed, and cisplatin or derivatives like carboplatin or oxaliplatin are used to treat around half of all cancer patients who get chemotherapy.<sup>2</sup> Despite this, the high toxicity and resilience of tumor cells have motivated the evolution of innovative metal-based treatments currently being tested in clinical studies. In addition, several transition metals, such as copper or ruthenium, have sparked a lot of attention as possible substitutes for platinum-based anticancer drugs in recent years.<sup>3</sup>

The development of new lead compounds for cancer therapy depends on their capacity to target various pathways (eg, apoptosis induction, cell cycle arrest, or proteasome inhibition) which makes them very promising therapeutics. DNA damage repair pathways are one of the significant ways in cancer treatment. As the human cells are bound to continuous DNA damage which subsequently leads to change or damage in genome transcription or replication. Hence, DNA repairing materials trigger the affected spots to detect the damaged sites and activate pathways for treating the damaged cells. However, one of these agents' most distinguishing characteristics is their adaptability.<sup>4</sup> Toxicological prevention is a term used to describe the process of avoiding exposure to many of the compounds listed in the literature that have yet to be tested in animal models for toxicity. Herbs and natural products are excellent sources of medicinal substances, and their healing capabilities and value have long been recognized.<sup>5</sup> Natural bioactive compounds' characteristics and health effects, particularly those originating from plant sources such as oregano, have been extensively investigated. Phytochemicals are active compounds found in medicinal plants that are not required for normal human body function but have health or illness-relieving properties.<sup>6</sup> Gingerol is one of the most prevalent and widely used chemicals in ginger.<sup>7</sup> The fresh roots of ginger contain a significant amount of 6-gingerol [5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one].<sup>8</sup>

Ginger's strong and physiologically active compounds are 6-gingerol, 6-shogaol, zingerone, phenolics, and flavonoids. 6-gingerol is the most prevalent bioactive molecule in ginger with antioxidant, analgesic, anti-inflammatory, and antipyretic properties.<sup>9,10</sup> For example, In vitro tests were conducted by Gwang Hun Park and others to assess the anti-cancer effects of ginger leaf and then clarify any probable underlying mechanisms. They revealed that GL exposure reduced the viability of human colorectal cancer cells (HCT116, SW480, and LoVo cells) and caused apoptosis in a dose-dependent manner.<sup>11</sup> Sahdeo Prasad and Amit K Tyagi discussed the evidence for ginger extract's chemopreventive and chemotherapeutic potential.<sup>12</sup> The active ingredients of ginger have been evaluated using in vitro, animal models, and patients. The active ingredients in ginger, such as 6-gingerol and 6-shogaol, have anticancer properties that are effective against GI cancer. A number of signaling molecules, including STAT3, ERK1/2, Akt, NF-B, COX-2, PI3KTNF, MMP-9, and other cell growth regulating proteins, are modulated by ginger, which is thought to have anticancer properties.<sup>12</sup> Additional research employing ginger's crude ethanolic extract in vitro and in animals indicates promising anticancer efficacy against Cholangiocarcinoma (CCA) without any discernible toxicity.<sup>13</sup> Wang et al reviewed the biological aspects of 6-gingerol in vitro and in vivo. They point out that 6-gingerol is the primary pharmacologically active component of ginger.<sup>14</sup> Through its impact on several biological pathways related to apoptosis, cell cycle regulation, cytotoxicity, and angiogenesis suppression, 6-gingerol has been demonstrated to have anticancer properties. Thus, 6-gingerol has attracted a lot of interest as a possible therapeutic agent for the prevention and/or treatment of numerous diseases due to its effectiveness.<sup>14</sup> In this work, for the first time, the gingerol-metals complex was synthesized based on the production of metal complexes between two molecules of gingerol. The interaction of the metal ion solution with the molecule gingerol in the suitable medium was synthesized using Ag, Au, Cd, Co, Cu, Ni, and Zn ions to produce metal complex compounds and investigate their effect on the inhibition of cancer cells

## Experimental Work

### Instruments

FT-IR (Fourier Transform Infrared Spectroscopy) measurements were recorded under vacuum optics, using a VERTEX 70v BRUKER spectrophotometer (Bruker, Ettlingen, Germany) with a resolution of 4  $\text{cm}^{-1}$  and 64 scans. The absorbance for the samples was determined using UV-3600 (Shimadzu, Kyoto, Japan). High-resolution shape and morphology imaging and elemental analysis were studied using Field Emission SEM (JSM-7800F, Jeol) and the metal microanalysis was determined using Energy Dispersive X-ray Spectroscopy (OXFORD Instrument, X-Max<sup>N</sup>).

### Chemicals

Silver sulfate was obtained from Scharlau Chemie (Spain). Nickel nitrate, copper(II) sulfate, Cadmium sulfate-hydrate, and Cobalt chloride hexahydrate were procured from Merck (UK.). Gold(III) chloride hydrate, Zinc sulfate heptahydrate, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (UK). Double DI water was utilized throughout the experiment which was obtained using a Milli-Q water filtration system.

## Extraction and Synthesis

The extraction process was applied according to Ghasemzadeh et al<sup>15</sup> method with some modifications to improve the separation process. 200g of fresh ginger was transferred to a 500 mL round-bottomed flask connected with a condenser which was followed by further addition of 200 mL ethanol and the temperature was maintained at 90°C for 24 H. After that, the mixture was filtered using microfilter paper. The light brown ethanolic extract was taken into a 500 mL flask and left to concentrate (evaporate) at room temperature under a vacuum. The purification and separation were carried out according to the Tao et al technique<sup>16</sup> with some modifications as the silica gel column chromatography used was a 30 cm (Height), 10 cm i.d. (Rotaflo, England) column packed with activated silica gel at 130°C (40–75 µm) and the final eluent for extraction was an acidic mixture of acetic acid in methanol (5:95). The final product was kept at 4°C until it is used.

## Synthesis of Gingerol-Metals Complexes Using Gingerol

Gingerol-metals were synthesized as follows: Initially, 0.139822 g of the extracted product was transferred to culture tubes Soda-lime glass with DIN thread and P.P. screw cap 22 mL, deionized water, and DMSO were added (4:1) and sonicated for 45 min. The pH of the solution in each tube was adjusted to pH7. Then, 5 ml of a specific metal solution was gradually added (Table 1). The tubes were incubated at 90°C for two days as well as the control sample. Afterward, the tubes were taken from the incubator and cooled down to ambient temperature. The samples were then filtered using (FL091, Filter paper, 110 mm) and the gingerol-metals complexes solutions were transferred into new tubes and coded as (CT, S1, S2, S3, S4, S5, S6, and S7). The tubes were cooled at 4°C and stored.

## Cell Culture and Cell Lines

The human colon cancer cell lines HTC116 and HT29 were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 50 units/mL penicillin G, and 50 µg/mL streptomycin (GIBCO) at 37 °C in a humidified atmosphere with 5% CO<sub>2</sub>.

## Cytotoxicity Assay

HTC116 and HT29 colon cancer cell lines, which were purchased from ATCC (Manassas VA, USA), were plated at a density of 6000 cells/well in a 96-well plate was incubated at 37°C overnight and treated in triplicate with various concentrations of complex Materials (S1-S2-S3-S4-S5-S6-S7). After 48 h of treatment with the complex except for s1, it was incubated for 24 h to measure the cell viability based on the ability of viable cells to convert a soluble tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, MTS) to a formazan product by using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI, USA) [34]. Soluble MTS formazan product was estimated using an ELISA microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 490 nm wavelength. The 50% inhibitory concentration (IC<sub>50</sub>) values for the complexes exhibiting antiproliferative activity were calculated using publicly available software at AAT Bioquest website (<https://www.aatbio.com/tools/ic50-calculator>).

**Table 1** The Metals Solution Utilized in Complexes

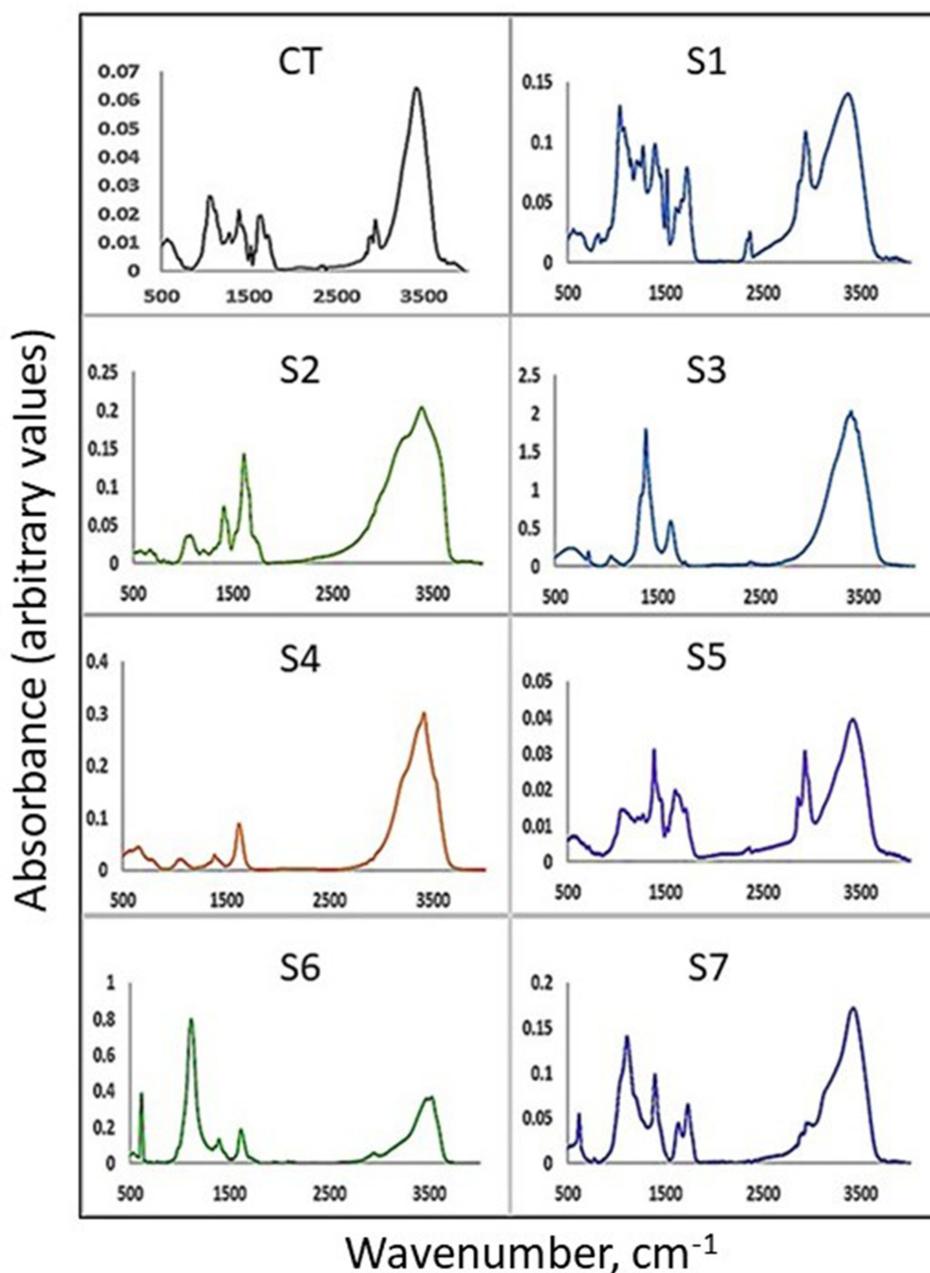
No.	Metal Solution	PH	Net Concentration	MW
1	Ag <sub>2</sub> SO <sub>4</sub>	6.3	0.01M	169.87 gmol <sup>-1</sup>
2	HAuCl <sub>4</sub>	5.5	0.01M	357.80 gmol <sup>-1</sup>
3	CdH <sub>2</sub> O <sub>5</sub> S	6.9	0.01M	226.49 gmol <sup>-1</sup>
4	CoCl <sub>2</sub> .6(H <sub>2</sub> O)	6.6	0.1M	237.93 gmol <sup>-1</sup>
5	CuSO <sub>4</sub>	5.2	0.1M	159.60 gmol <sup>-1</sup>
6	Ni(NO <sub>3</sub> ) <sub>2</sub>	6.1	0.01M	182.70 gmol <sup>-1</sup>
7	ZnSO <sub>4</sub> 7H <sub>2</sub> O	6.9	0.01M	287.56 gmol <sup>-1</sup>

## Results and Discussion

### Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of CTR (control sample, gingerol) and gingerol-metals complexes are illustrated in Figure 1, and the obtained bands are in Table 2. The gingerol shows bands at  $3419\text{ cm}^{-1}$  which is attributed to hydroxy group stretching for phenol.<sup>17,18</sup>

Stretching of the C-H atoms in symmetric and asymmetric is responsible for the absorption bands at about  $2940$  and  $2875\text{ cm}^{-1}$ , respectively.<sup>19</sup> The hydrogen-bonded carbonyl appeared at  $1719\text{ cm}^{-1}$  and the band at  $1628\text{ cm}^{-1}$  corresponds to C=O stretching<sup>20</sup> and the methyl group band appeared at  $1424\text{ cm}^{-1}$ . The C-O stretching vibration<sup>21</sup> is attributed to the distinctive peak at  $1387\text{ cm}^{-1}$ . The hydroxy group showed a band at  $1156\text{ cm}^{-1}$ , whereas the stretched hydroxy group appeared at  $1118\text{ cm}^{-1}$ . The characteristic peak at  $712\text{ cm}^{-1}$  is ascribed to the C=C in the benzene ring.<sup>22-24</sup> Metal complexes in the instance of gingerol-associated metals caused the appearance of new bands and the displacement of existing bands from their initial positions to lower



**Figure 1** The I.R. spectra of the control sample and gingerol-metals complex ; CT, S1, S2, S3, S4, S5, S6, S7.

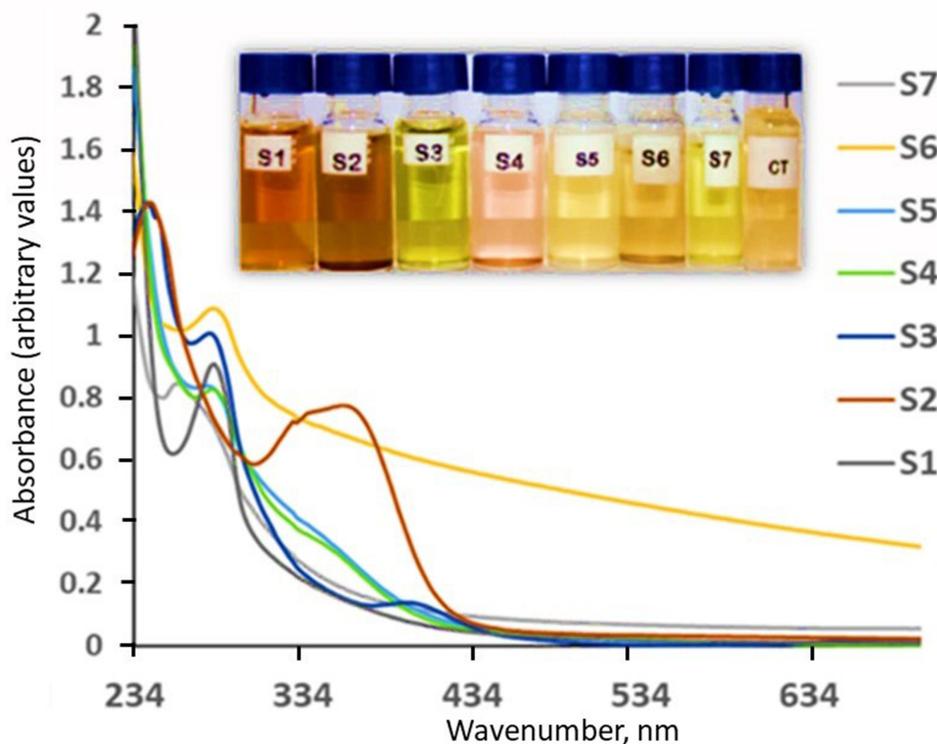
**Table 2** The I.R. Bands for the Control Sample and Gingerol-Metals Complexes

Sample Code	I.R. Peaks $\text{cm}^{-1}$
CT	3419, 2940, 2875–2901, 2834, 1714, 1628, 1424, 1387, 1277, 1156, 1118, 1059, 712, 579, 552
S1_Ag	3379, 2928, 2866, 2366, 2328, 1726, 1663, 1624, 1516, 1461, 1430, 1397, 1277, 1256, 1232, 1156, 1116, 1081, 1038, 818, 567
S2_Ni	3397, 3223, 1758, 1724, 1658, 1614, 1444, 1414, 1310, 1191, 1081, 1048, 814, 728, 691, 569
S3_Cu	3383, 2420–2440, 1636, 1389, 1338, 1059, 832, 662, 554, 549
S4_Cd	3415, 3271, 1630, 1393, 1075, 797, 667, 769, 552, 574
S5_Co	3399, 2922, 2850, 2411, 2393, 2336, 1716, 1642, 1601, 1536, 1461–1403, 1363, 1281, 1220, 1128, 1087, 1054, 716, 579
S6_Au	3528–3456, 2922, 2085, 1956, 1620, 1461, 1403, 1116, 787, 616, 549, 569
S7_Zn	3748, 3434, 2932–2877–2844, 2366, 2101, 1736, 1620, 1387, 1205, 1112, 779, 614, 569, 552

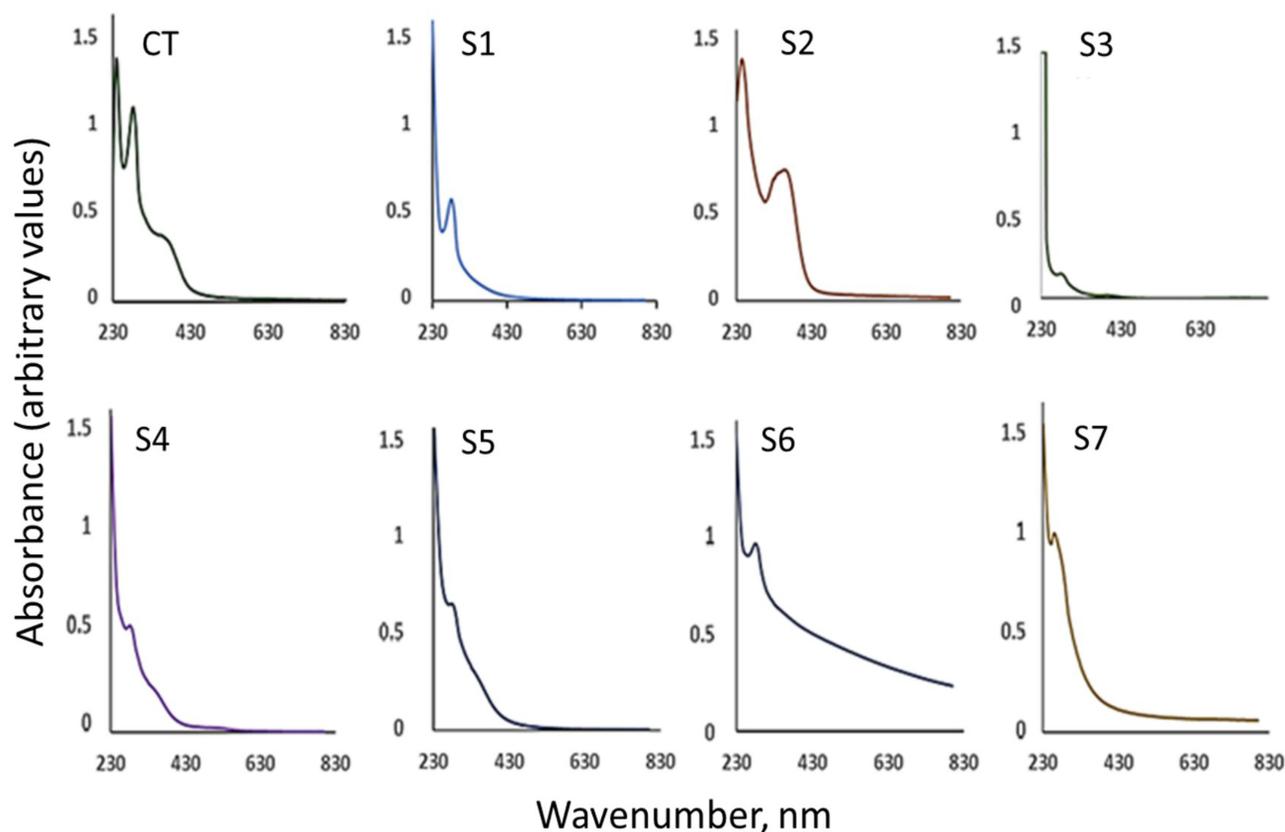
values. For example, the band at 2940 was shifted to 2928  $\text{cm}^{-1}$  and the characteristic peak at 2875 was shifted to 2866  $\text{cm}^{-1}$  and the 1714  $\text{cm}^{-1}$  band shifted to 1726  $\text{cm}^{-1}$ . A new band has emerged at 2366  $\text{cm}^{-1}$  and 2328  $\text{cm}^{-1}$ , which is attributed to the hydroxy-metal bond (O-M). C-H aromatic band occurred at 1663  $\text{cm}^{-1}$  and the 1628  $\text{cm}^{-1}$  band shifted to 1624  $\text{cm}^{-1}$ . The new band at 1516  $\text{cm}^{-1}$  could be attributed to C-O-M and the band at 1461  $\text{cm}^{-1}$  is ascribed to C-H bending.<sup>10</sup> The band at 1424  $\text{cm}^{-1}$  shifted to 1430  $\text{cm}^{-1}$  and the band at 1277  $\text{cm}^{-1}$  was in the same position which is attributed to C-H aromatic. The C-H bending is accountable for the band at 1461  $\text{cm}^{-1}$ . The 1424  $\text{cm}^{-1}$  band shifted to 1430  $\text{cm}^{-1}$  and the C-H aromatic corresponds to the band at 1277  $\text{cm}^{-1}$ . The band at 1256  $\text{cm}^{-1}$  is ascribed to C-O stretching and the band at 1118  $\text{cm}^{-1}$  was relocated to 1116  $\text{cm}^{-1}$  and is consigned to the vibration of C-O.<sup>25</sup> The new band at 1516 and bands at around 567, 574, and 549  $\text{cm}^{-1}$  are attributed to the C-O-M band and the band at 1232  $\text{cm}^{-1}$  is indicated to the formation of (M-O) bond<sup>26</sup> which confirms the formation of organometal complexes.

## UV-Visible Spectroscopy Study

The production of complexation between metal ions and gingerol to form an gingerol-metals complex has been identified using UV-visible spectra. The wavelengths measured ranged from 230 nm to 800 nm. Figure 2 depicts the spectra of



**Figure 2** The UV-Vis spectra of the metal complexes with gingerol (gingerol-metals complex). The Photo image on the top is the actual color of each complex compared to the control on the last right (C.T).



**Figure 3** The UV-Vis spectra of the gingerol-metals complex separately with the UV control, where it is noted that there is a shift on the peaks due to the formation of the complexation.

samples S1 to S7 including the control sample. All investigated samples had similar absorption for bands (I) and (II) in the interspecific study, which could be attributed to absorption overlap in this region. However, as compared to the control (gingerol) **Figure 3** shows considerably different absorption values at max 243 nm in the band (II) region. The spectra of the S1 sample indicated a shift in the maximum absorption peak wavelength. The spectral examination of S1-S7 revealed absorption peaks at 230 and 242 nm, with a bit of shift in the band depending on the metal ion which is typical of complex formation.<sup>27,28</sup>

### High-Resolution Field Emission SEM and Metal Microanalysis

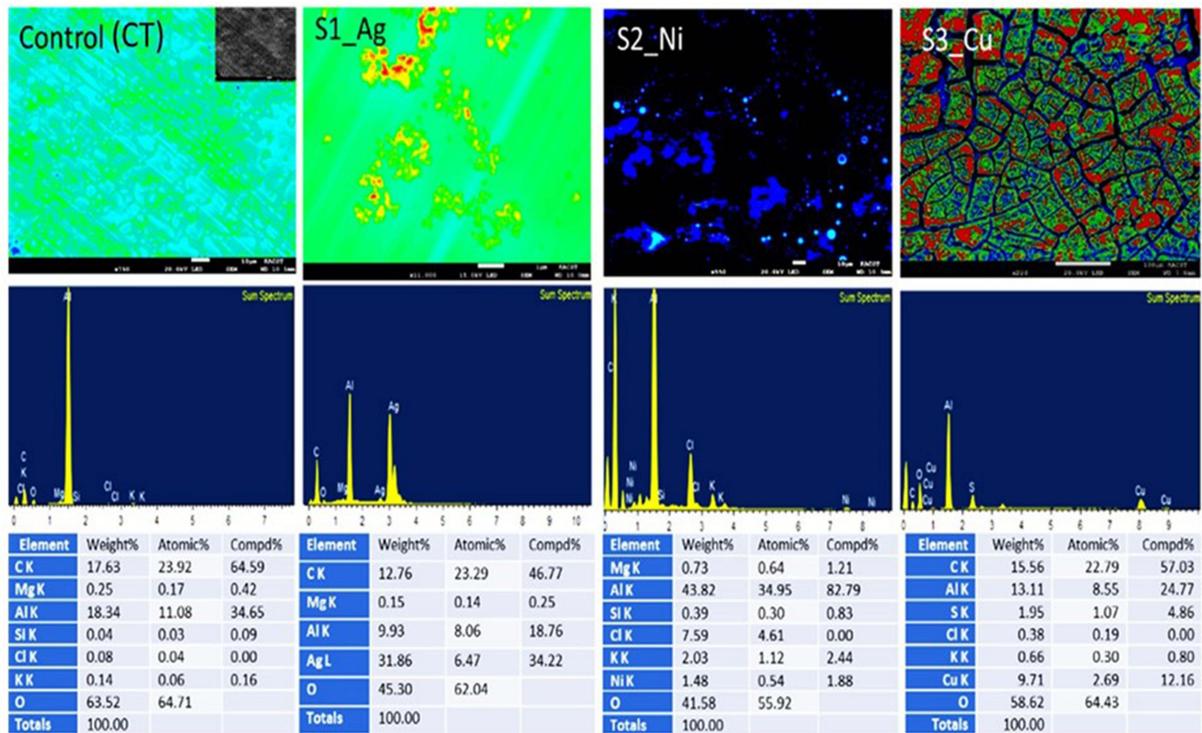
The morphology of the dried complexes and the metal microanalysis results are shown in **Figure 4**. Several peaks have been identified for the metals and organic compounds. The initial peak at  $K\alpha = 0.277$  keV corresponds to carbon and the secondary peak at  $K\alpha = 0.52$  keV represents oxygen. The peak at  $K\alpha = 2.984$  keV characterizes Ag. The peaks at  $K\alpha$  7.47 keV and  $L\alpha$  0.851 keV are assigned for Ni. Cu showed two peaks at 8.04 keV for  $K\alpha$  and 0.93 keV for  $L\alpha$ . The peak at 3.133 keV is attributed to Cd and the peak at 6.92 keV and 0.76 keV is ascribed to Co  $K\alpha$  and  $L\alpha$ , respectively. The Au showed only a clear peak at 2.210 keV and Zn showed a peak for  $K\alpha$  at 8.630 and  $L\alpha$  at 1.012 keV. These results confirm the presence of metal in the structure of the complex.

The previous results confirm the formation of the metal complexes and the suggested structure is shown in **Figure 5**, which is consistent with other studies of the metal complexes.<sup>29–31</sup>

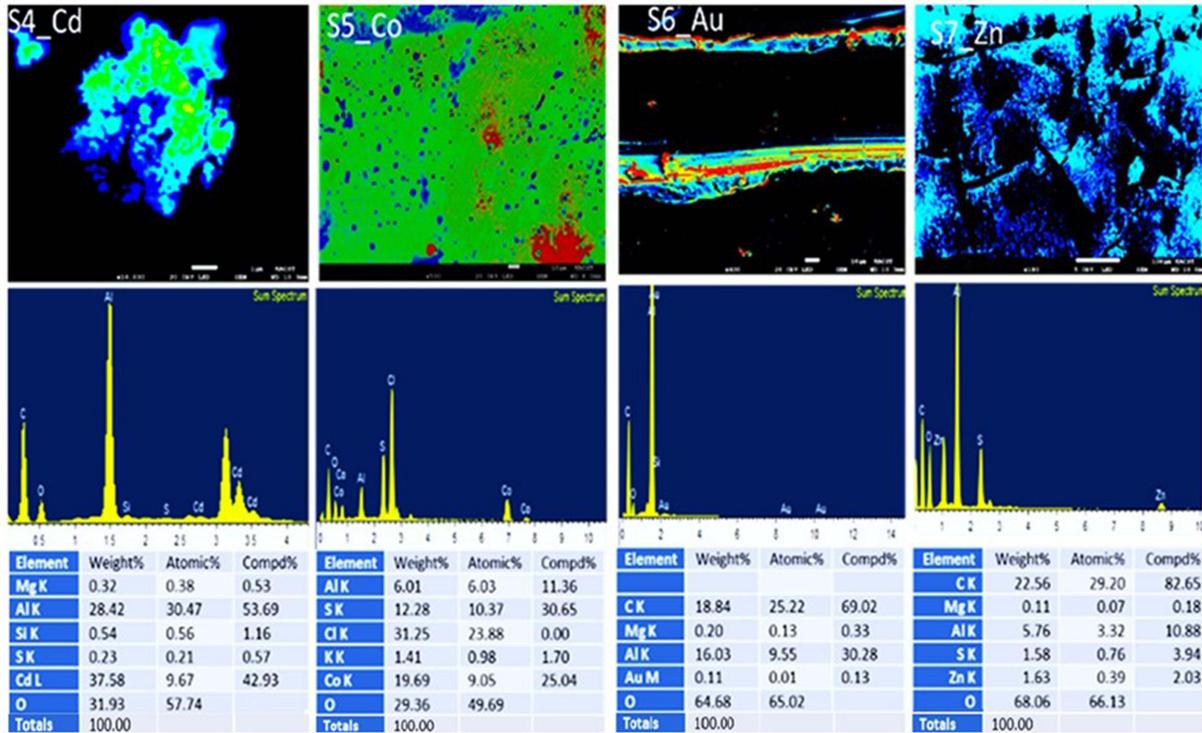
### In-vitro Antitumorigenic Effect on Colon Cancer Cell Lines

The antineoplastic activity of the gingerol-metals complexes was experimented with by examining its cytotoxic effect on colorectal cancer cell lines HCT116 and HT-29 using MTS assay. A wide range from 2  $\mu$ M to 20  $\mu$ M

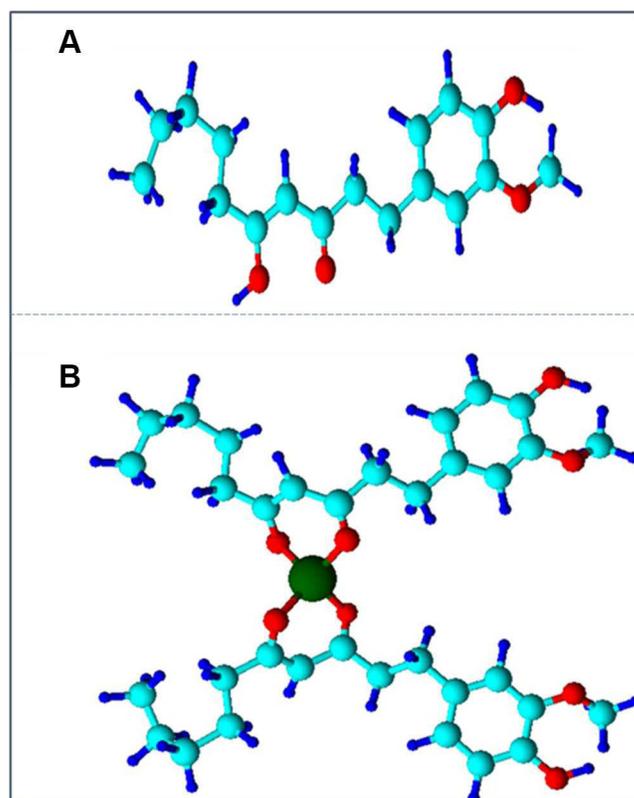
**A**



**B**

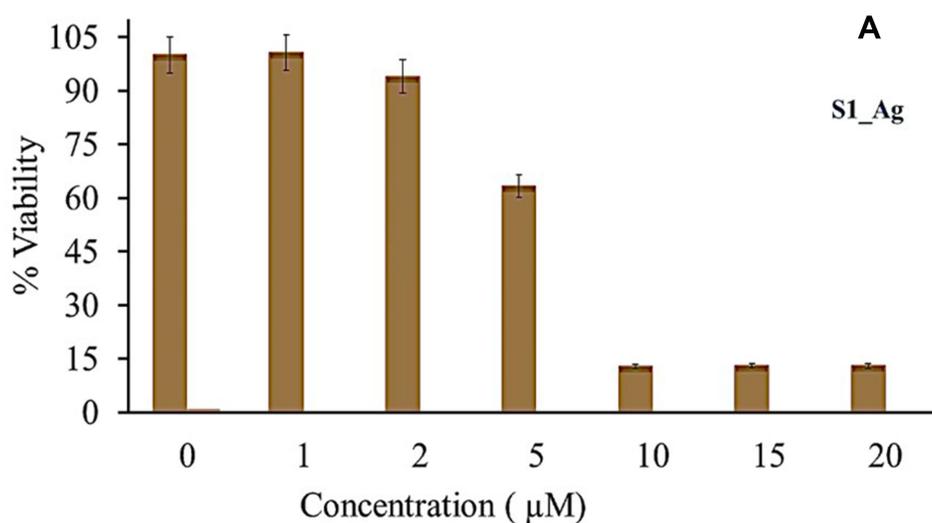


**Figure 4 (A)** SEM, EDX for Control sample (CT), S1, S2, S3. **(B)** SEM, EDX for S4, S5, S6 and S7.

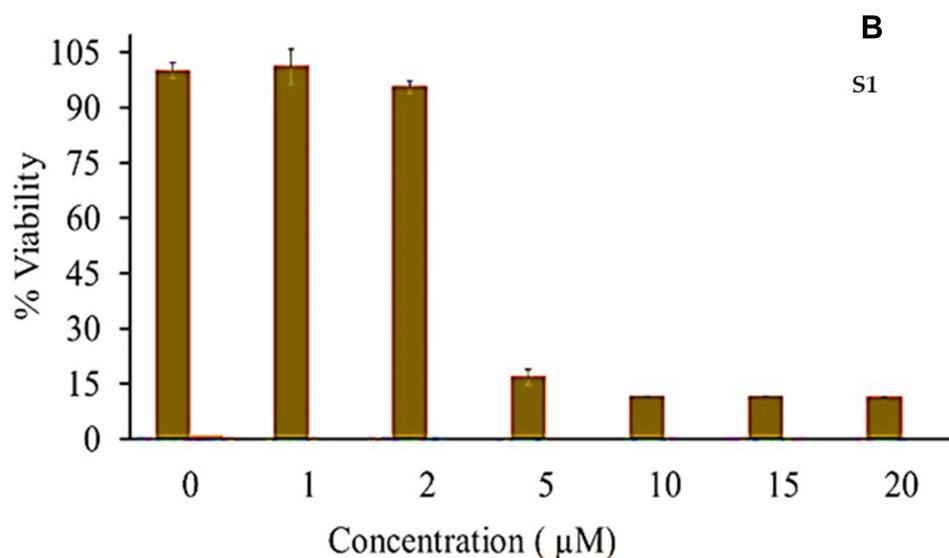


**Figure 5** Suggested structure of the complex formation between gingerol and metal ions; (A) gingerol structure, and (B) the structure of the gingerol-metals complex.

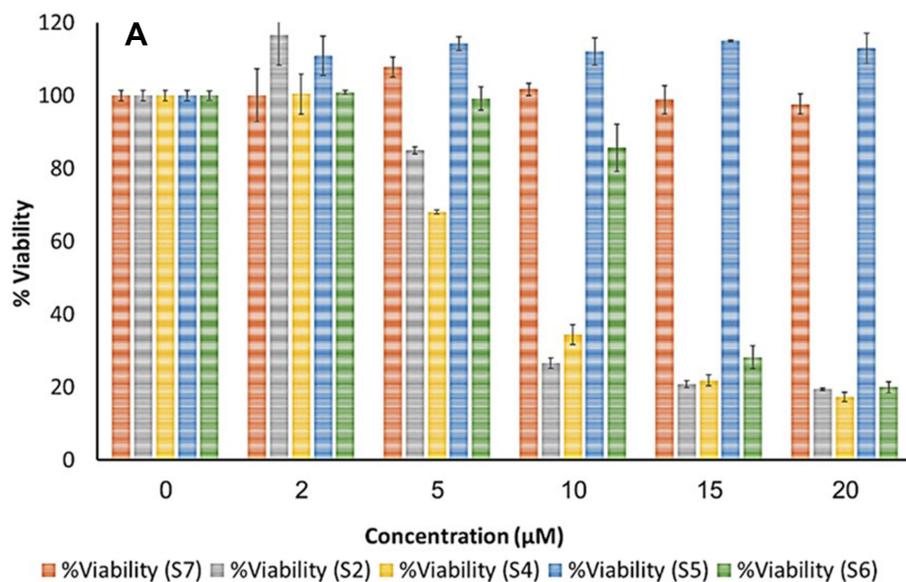
concentrations of test compounds were used on these colorectal cancer cells. The compound S1 reveals a considerable decrease in cell viability with the increase in the concentration of the drug after 24 h treatment period in both the cell lines as shown in Figure 6. While S2 and S4 show a significant decrease in cell viability with increasing concentrations of the drugs after 48 h and the S6 complex showed cytotoxic activity at a concentration above 10  $\mu\text{M}$  as shown in Figure 7. The highest tested concentration of 20  $\mu\text{M}$  shows a decrease of 23% in the viability in both the cell lines. The IC<sub>50</sub> for the metal complexes on HCT116 and HT-29 cell lines are displayed in



**Figure 6** Continued.

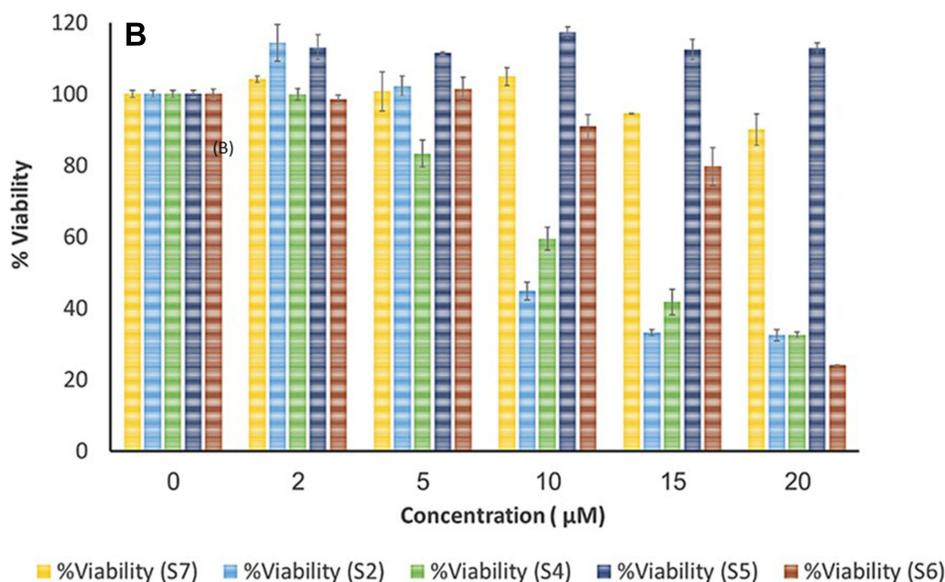


**Figure 6 (A)** Decreased cell viability by S1 for HCT 116. **(B)** Decreased cell viability by S1 for HT-29 (HCT 116 **(A)** and HT-29 **(B)**) were treated with the reported concentration of the compound for 24h. The MTS assay results are expressed as mean  $\pm$  S.D. from experiments performed in triplicate.



**Figure 7 Continued.**

**Table 3.** Compared to HCT116, the HT-29 cells showed higher sensitivity to the complex. Pranzini et al indicated that 5-Fluorouracil (5-FU) is a primary standard chemotherapy drug for the management of multiple solid malignancies, comprising advanced and metastatic colorectal cancer (CRC). 5-FU shows its toxicity on cancer cells by interfering with nucleotide biosynthesis and nucleotide pool composition, primarily by inhibiting thymidylate synthase. Their data show that after 48 hours, the 5-FU IC<sub>50</sub> for HCT-116 and HT-29 is around 3.2 μM and 1.6 μM, respectively.<sup>32</sup> While our results for S1, after 24 hours show 5.24 μM for HCT-116 and 3.185 μM for HT-29, respectively, for the half-maximal inhibitory concentration (IC<sub>50</sub>). The effect of complexing *N*-carbene ligand with silver(I) was studied by Huque et al revealed that the Ag-complex increase the efficiency against the HCT 116 and HT29 cell lines.<sup>33</sup> In another study by Mohamed et al, silver(I)-NHC complexes show far less effect compared to



**Figure 7** (A) Decreased cell viability of various metal complexes HCT 116. (B) Decreased cell viability of various metal complexes HT-29. HCT 116 (A) and HT-29 (B) were treated with the reported concentration of the compound for 48h. The MTS assay results are expressed as mean  $\pm$  S.D. from experiments performed in triplicate.

our data against HCT 116 and HT29 cell lines.<sup>34</sup> The S3, S5 as well as S7 gingerol-metals complexes in our data did not demonstrate cytotoxic activity in the colorectal cancer cells.

The antitumor properties of Ag(I) ions are continuously explored in various types of human cancer cells including pancreatic cancer cell PANC-1, lung cancer cells H1299 and breast cancer. It has been noticed that the antitumor characteristics of Ag(I) ions are implicated in a wide range of mechanisms including DNA damage<sup>35</sup> stimulation of cell apoptosis, necrosis, and autophagy<sup>36,37</sup> and the generation of reactive oxygen species (ROS)<sup>38</sup> and in the inhibition of cell migration and invasion.<sup>39</sup> Several studies indicated that silver(I) complexes show better cytotoxicity against tumor cells including myeloid leukemia cells K562, MCF-7 breast cancer, colorectal Adenocarcinoma Cells Caco-2 cells, and human Hepatoma HepG2.<sup>40,41</sup> Our study suggested that gingerol-metal complex-Ag has the potential to be developed as chemotherapeutic agent against colorectal cancers.

## Conclusions

Developing an efficient material with a valid target for the cancer cells is critical. In the recent past, gingerol-metals complexes have been synthesized with natural products which possess great potency in the treatment of cancer treatments. In the current study, a novel series of gingerol-metals complexes were investigated and the complexation is confirmed by applying different techniques. The results demonstrated that gingerols' efficiency was improved when it integrated with metal ions. The activity depends on the metal type (Ag, Au, Cd, Co, Cu, Ni, and Zn). For example, the gingerol-metals

**Table 3** IC<sub>50</sub>, the Measure of the Effectiveness of a Substance in Inhibiting a Specific Biological or Biochemical Function

Batch	IC <sub>50</sub> (µM) For Colorectal Cell Lines	
	HCT116	HT-29
CT	97.202	98.498
S1	5.246	3.185
S2	7.729	6.106
S4	10.150	6.103
S6	17.637	11.887

complex-Ag showed the most activity among the other complexes. The in-vitro anticancer activity was evaluated using HCT116 and HT29 human colorectal cancer cell lines. The gingerol-metals complexes (S1) showed significant inhibition in cell viability after 24 h of the treatment period in HT-29 cells and HCT116 cell lines with half-maximal inhibitory focus (IC50). The effect of the prepared materials is based on unique chemical composition background with an environment friendly for cancer therapy. The results suggest that S1 has the potential to be applied as a chemotherapeutic synthetic drug for colorectal cancer. Finally, the prepared materials can be progressively developed for improvement in cancer treatment.

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## Disclosure

The authors report no conflicts of interest in this work.

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