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RESEARCH PAPER

Antioxidant, Cytotoxic, and Antihemolytic Activity of Greenly Synthesized Selenium Nanoparticles Using *Elettaria Cardamomum* Extract

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ABSTRACT

Elettaria cardamomum is a spice that used in treatment of several diseases in traditional medicine. Selenium nanoparticles were synthesized using the aqueous extract of *E. cardamomum* dried plant to evaluate their phytochemical constituents, antioxidant, anticancer and hemolytic activity. This research provided biologically active and cost-effective selenium nanoparticles. The synthesized nanoparticle solutions were characterized using UV-visible spectrophotometer, zeta potential analysis, and transmission electron microscope. Phenolics, flavonoids, and tannins content decreased in the prepared nanoparticles than the main extract that prove the utilization of these groups in the synthesis of nanoparticles and the decrease in antioxidant activity in the prepared nanosolution than the main extract using 2,2-diphenyl-1-picrylhydrazyl assay. The synthesized nanomaterial and *E. cardamomum* extract were tested for their anticancer activity using 6 tumor and one normal cell lines. The synthesized nano-selenium using *E. cardamomum* expressed good cytotoxic potency against tumor cell lines of HePG-2 with an IC50 of 23.33 µg/ml followed by HeLa (IC50 = 27.59 µg/ml), HeP2 (IC50 = 31.04 µg/ml), HCT-116 of moderate or less potency (IC50 = 37.36 µg/ml), PC3 of moderate or less potency (IC50 = 38.68 µg/ml) and WI-38 that expressed the lowest activity (IC50 = 52.91 µg/ml), respectively. In addition, all the samples showed weak cytotoxic activities against the normal lung fibroblast cell line. *E. cardamomum* extracts and its synthesized selenium nano-solution were non/less toxic to human erythrocytes. The results confirmed the improved biological characteristics of selenium nanoparticles formulated with *E. cardamomum*.

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INTRODUCTION

Herbal phytomedicines have attracted researchers' attention because of their potential biological activities [1]. Cardamom (*Elettaria cardamomum*) of the ginger family (Zingiberaceae) is a spice composed of small pods with dark seeds that possess a good fragrance and used in sweets, spicy food, coffee and tea [2]. It is referred to as small cardamom or green cardamom and is cultivated and grown in some Asian countries, including Nepal, Costa Rica, Guatemala, Indonesia. Sri Lanka, India, Tanzania, and Mexico [3]. It is also recognized as ''Hel" in gulf countries like Kuwait, Saudi Arabia, United Arab Emirates, Iran, Iraq, and other regions [3, 4].

The pleasant odor is attributed to the essential

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oils of cardamom that include pinene, myrcene, methyl eugenol, 1,8-cineole, a-terpinyl acetate, sabinene, phellandrene, terpinene, limonene, p-cymene, linalool, terpinen-4-ol, geraniol, and transnerolidal. Limonene, cineole, linalool, pinene, and borneol were reported to possess antioxidant scavenging activity [5]. This plant has been used for treatment of teeth and throat infections, lung congestion, tuberculosis and digestive problems [6].

They have aromatic, sweet, carminative, deodorant, diuretic, purgative, thirst reliever, and tonic characteristics. Moreover, it is used in asthma, burning sensation, cold, cough, bladder and kidney disorders, maldigestion and scanty urine [7]. Cardamom has exhibited anticancer [8] gastroprotective [9], antihypertensive [10], antiinflammatory [11] and immunomodulatory [12] antifungal and antibacterial properties [13] in numerous experimental studies.

cardamom expressed cancer chemopreventive potential against B(a)P induced forestomach papilloma genesis where it has the ability to inhibit forestomach carcinogenesis at peri-initiation stages of carcinogenesis significantly [14]. Chemo-preventive activity of cardamom has also been shown to regulate colorectal cancer [15]. D-Limonene, one of the bioactive components of cardamom, has been demonstrated to have chemopreventive action against skin, stomach, colon, liver, mammary and lung cancers in rodents [16].

This study aimed to prepare *Elettaria cardamomum* aqueous extract and to use it in synthesizing selenium nanoparticles in ecofriendly technique. It also aimed to estimate the biological activity of them as antioxidant, hemolytic, and cytotoxic materials.

MATERIALS AND METHODS

Preparation of investigated Elettaria cardamomum extract

The phytochemical components were extracted using 5 grams of *Elettaria cardamomum* in 100 ml distilled water upon shaking for 20 minutes at 65° C using water bath (Memmert WB14, Germany) then filtered using Whatman no.1 filter paper [17].

Preparation of Selenium Nanoparticles

20 mL of 1 mmol Selenium sulfate were added step wise to equal volume of plant extract upon shaking for 2 hours at 35°C [18].

Characterization of the Selenium Nanoparticles
Transmission Electron Microscope (TEM)

Particle's size, shape, crystal structure, and morphology of the prepared nano selenium were defined using TEM (JEOL TEM-2100) as described by Otunola et al., [19].

Zeta Potential technique

This technique was used for characterization of the nature of surface charge and stability of the prepared selenium nanoparticles [20] using Zeta potential analyzer (Malvern Instruments Ltd Zeta Potential Ver. 2.3) [21].

Phytochemical Analysis
Total Phenolics

Phenolics were estimated using Folin-Ciocalteu procedures adopted by Wolfe et al. [22], using gallic acid as a reference compound. Phenolics were determined as milligram gallic acid equivalent / gram dried extract using the standard curve (y = 0.0063x, r2 = 0.997).

Total Flavonoids

Flavonoids were determined by aluminum chloride method adopted by Zhishen et al. [23] using catechin as a reference compound. Flavonoids were determined as milligram catechin equivalents per gram dried extract using the standard curve (y = 0.0031x, r2 = 0.998).

Cytotoxic activity using MTT assay

MTT assay was used for assessing the cytotoxicity of the samples using cell lines of Human prostate cancer (PC3), Hepatocellular carcinoma (HePG-2), Colorectal carcinoma (HCT-116), Epithelioid cervix carcinoma (Hela), Epidermoid larynx carcinoma (HEP2), Mammary gland carcinoma (MCF-7) through estimating the cell growth according to the procedures adopted by Bondock *et al.,* [24]. Doxorubicin was used as standard anticancer drug.

Erythrocyte hemolysis Assay

5ml of whole blood were collected from healthy rats and transferred to tubes containing anticoagulation agent then centrifuged at 2000 rpm for 4 min. The obtained red blood cells were washed by phosphate buffer saline (pH 7.4) several times then resuspended in 0.5% saline [25].

0.5ml of different extract and selenium nano solution concentrations was added to 0.5ml of prepared red blood cells, then kept for 30 minutes

at 37°C then centrifuged 5 minutes at 4000 rpm. The absorbance was detected at 540 nm. Negative control was prepared by phosphate buffer saline without extract and positive control using distilled water without extract. Erythrocyte hemolytic activity was estimated according to the following equation:

$$\%\ \text{Hemolysis} = \frac{A_t - A_n}{A_c - A_n} \times 100$$

Where: A_t = tested samples absorbance, A_n = negative control absorbance, A_c = positive control absorbance.

RESULTS AND DISCUSSIONS

The phytochemical components of *Elettaria cardamomum* aqueous extract, include a broad variety of diversified secondary constituents that are used in the biosynthesis of nanoparticles [26]. This study illustrated that *Elettaria cardamomum* extract is rich with polyphenols (142.61± 1.32 milligram gallic acid equivalent/gram plant extract), flavonoids (36.76 milligram catechin equivalent/ gram plant extract), and tannins (70.84 milligram gallic acid equivalent/gram plant extract) that could be consumed for reducing and stabilizing selenium ions to produce nanoparticles. phenolics play essential role in the biological reduction of ions into nanoparticles and contribution in their stabilization [29,30]. Flavonoid compounds are stable compounds that are used in the reduction of selenium ions into nanoparticles forming novel compounds that possess very small sizes with large surface area that are chemically and biologically active [27–29]. Polyphenol's content (35.12 milligram gallic acid equivalent/gram plant extract), flavonoids (9.16 milligram catechin equivalent/ gram plant extract), and tannins (17.53 milligram gallic acid equivalent/ gram plant extract) were markedly decreased in the prepared solution of nano selenium, accordingly the results assure the utilization of these phytochemicals in the formation of nanometals.

Characterization of the synthesized Nano-selenium TEM technique

TEM was used for obtaining morphological description of the synthesized selenium particles. Fig. 1 demonstrates the micrographs, size distributions, spherical and tetragonal shapes of the synthesized selenium nanoparticles. The size of the nanoparticles ranged from 38.26 to 68.49 nm. TEM helped to assess agglomeration and/or aggregation of the prepared nano. There was little

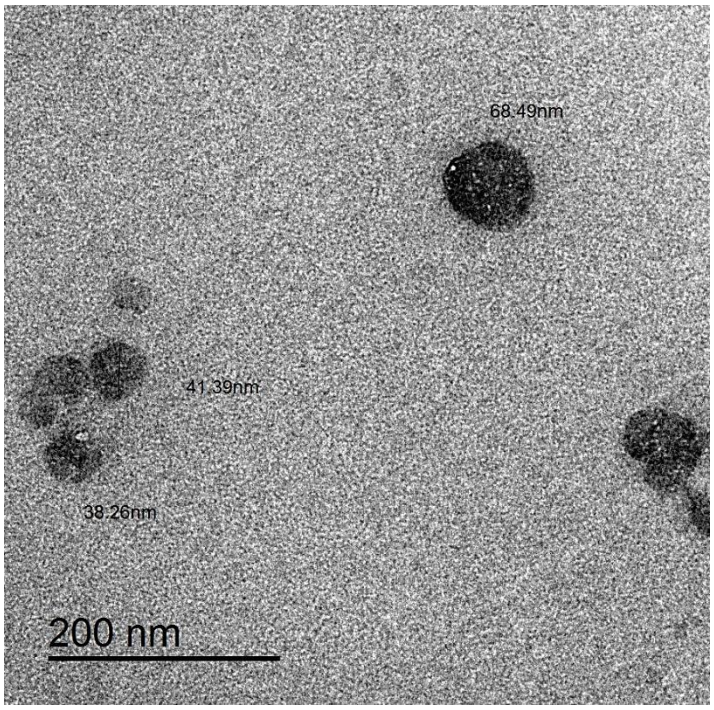

Fig. 1. Size and distributions for the synthesized selenium nanoparticles.

aggregation of the selenium nanoparticle that provides large surface area so improve efficacy as cytotoxic agents.

Zeta Potential Analysis

It is used to study the nature of the nanoparticles and to ensure their long-term stability. The nanoparticles possess double layer of ions where the electrical potentials at the borders of this layer were described as zeta potential of the nanoparticles with values in the range of +100 mV to −100 mV. Zeta potential of the synthesized selenium nanoparticles using *Elettaria cardamomum* extract found to be −11 mV (Fig. 2), that was highly stable where zeta potential with values lower than +25 mV or higher than −25 mV described to gain higher degrees of stability [20].

Biological Potentials

Antioxidant Activity

The aqueous extract of *Elettaria cardamomum* expressed an IC50 of 0.023 mg/ml that was comparable to ascorbic acid (IC50 = 0.0225 mg /ml), meanwhile the selenium nanoparticles solution expressed less antioxidant activity with IC50 value of 0.124 mg /ml. The results described the role of polyphenols, flavonoids, and tannins in the very good antioxidant activity of *Elettaria cardamomum* [30,31]. The decrease in these phytochemicals after synthesis indicates the utilizing of their active hydroxyl groups in the biosynthesis leading to the decrease in antioxidant activity.

Cytotoxic Activity

Elettaria cardamomum serve as an antitumor agent that induces less side effects than chemical drugs, such as Cyclophosphamide [8]. It was recorded that selenium nanoparticles and its zero-oxidation state express less toxic effect and more availability as well as it could easily be encapsulated [32,33]. Nano-selenium possess a

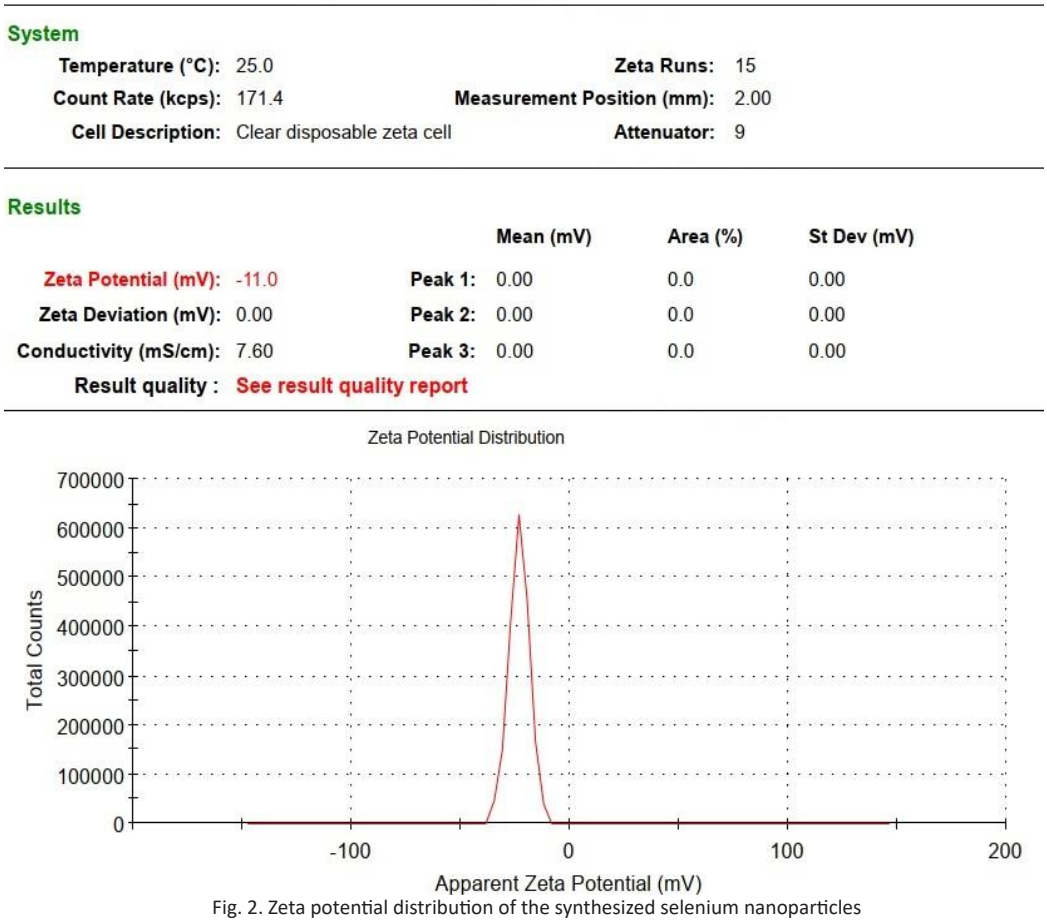

Fig. 2. Zeta potential distribution of the synthesized selenium nanoparticles

broad range of applications as antioxidants [34], potential antitumor drugs, and antimicrobial agents [35,36]. Previous studies showed that nano selenium could play a crucial role in cancer chemoprevention [37,38] and as an antitumor drug delivery carrier [39] in addition to its remarkable anticancer activity [40–42]. Treatment with selenium nanoparticles alter the mechanical characteristics of the cancer cells as they clearly diminish the adhesion forces and young's modulus [43]. In addition to the unique anticancer efficacy of selenium nanoparticles, they possess better selectivity for tumor cells [44].

In the present study, cytotoxic activity of the *Elettaria cardamomum* extract and the synthesized selenium nanoparticles were measured using MTT assay. 6 tumor cells (HepG-2, MCF-7, HCT-116, PC3, HeP2, and HeLa cell lines) and doxorubicin as a standard drug were used. IC50 is a parameter used to reflect the concentrations at which 50% of tumor cells in µg/mL were killed. IC50 is inversely proportional to the efficacy of the sample to stop

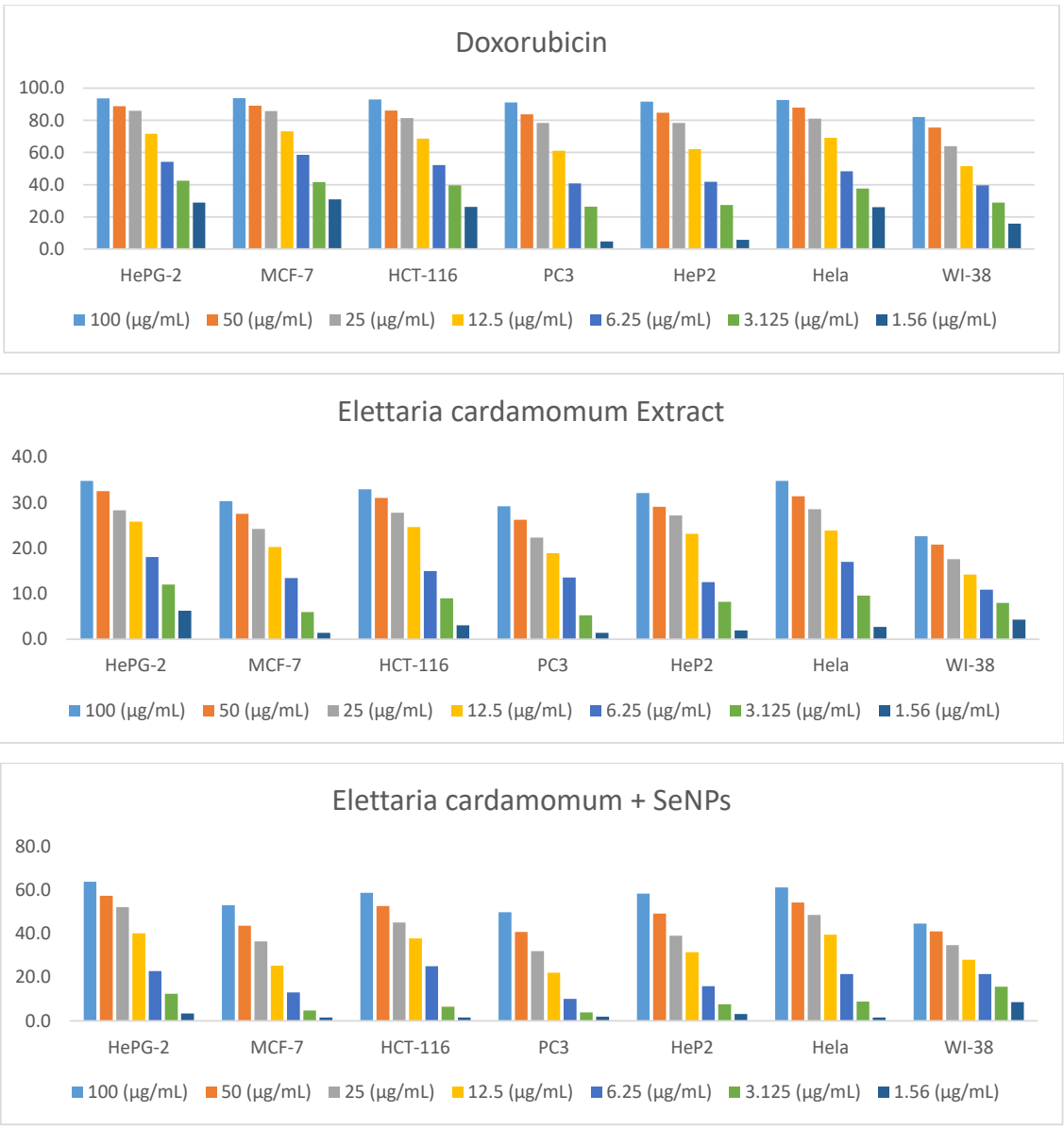

Fig. 3. Comparison scales of the inhibition percent of tumor and normal cells at varying concentrations (1.56-100 µg/ml) of the investigated standard, extract and synthesized selenium nanoparticles

Fig. 4. Comparison scales of the percent of average relative viability of tumor and normal cells at varying concentrations (1.56-100 µg/mL) of the investigated standard, extract and synthesized selenium nanoparticles

the growth of tumor cells. The results of in vitro cytotoxicity expressed as percent of inhibition, the cell's viability or potency of the samples and IC50 values are presented in Fig. 3, Fig. 4, and Fig. 5). The results revealed, that the synthesized selenium nanoparticles possessed cytotoxic potency against the tested cancer cell lines than the main extract. The nanoparticles have large surface area that increased their efficacy to inhibit tumor cells growth. The selenium nanoparticles synthesized using *Elettaria cardamomum* showed the most potent cytotoxic effect against tumor cell lines of HePG-2 with an IC50 of 23.33 µg/ml followed by HeLa with an IC50 of 27.59 µg/ml, HeP2 with an IC50 of 31.04 µg/ml, HCT-116 of moderate or less potency with an IC50 of 37.36

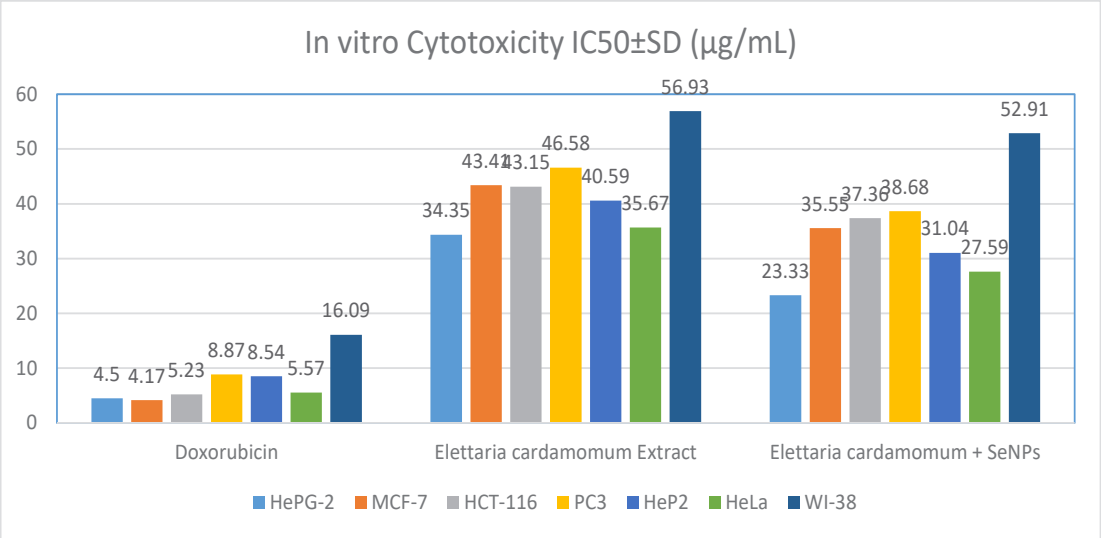

Fig. 5. A comparison of the IC50 values expressed the cytotoxic activity of the tested samples against all the examined cell lines.

µg/ml, PC3 of moderate or less potency with an IC50 of 38.68 µg/ml and WI-38 that expressed the lowest activity with an IC50 of 52.91 µg/ml, respectively. In addition, all the tested samples revealed weak cytotoxicity with the normal lung fibroblast cell line. The results illustrated the applicability of the tested *Elettaria cardamomum* and the synthesized selenium nanoparticles as antitumor drugs. The results also demonstrated that the prepared selenium nanoparticles had higher efficacy in inhibiting the growth of tumor cells more than *E. cardamomum* extract.

The results of selenium sulfate solution demonstrated that it displayed lower activities against HePG-2 cell lines (IC50 = 46.15 µg/ml), HeP2 cell lines (IC50 = 54.1 µg/ml), and HeLa cell lines (IC50 = 44.12 µg/ml). Selenium sulfate showed very low cytotoxic effect against MCF-

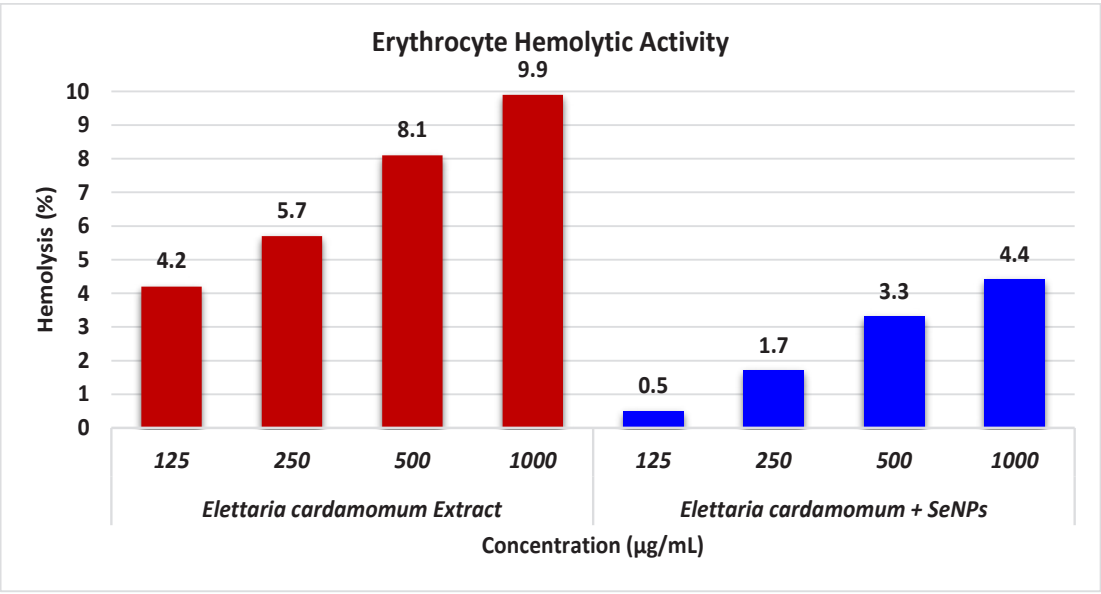

Fig. 6. Comparison scales of the erythrocyte hemolytic activity "signified as the % hemolysis" of the *Elettaria cardamomum* extract, and its synthesized selenium nanoparticles at different concentrations.

7(IC50 = 58.16 µg/ml), HCT-116(IC50 = 60.14 µg/ml), and PC3 tumor cell lines (IC50 = 64.26 µg/ml). So the salt was not effective against the tested tumor cell lines.

Erythrocyte hemolytic activity

The in vitro hemolysis assessment estimated the released hemoglobin in the plasma (as an indication for red blood cell lysis) following exposure to the tested agent. According to the US Food and Drug Administration, the in vitro hemolysis study should be implemented at the intended concentration for four administrations to evaluate the hemolytic potential of excipients intended for injectable use [45-47]. In the current study, *Elettaria cardamomum* extract and its synthesized nano selenium were screened in vitro for their hemolytic activity against human erythrocytes. The samples were prepared in serial dilutions at concentrations of 125, 250, 500, and 1000 µg/ml. The assay was implemented using an amended spectroscopic technique to investigate the hemolytic activity of the desired samples. The percentage of hemolysis was expressed as the hemolytic effect of the investigated samples at four different concentrations. Fig. 6 shows a comparison of the hemolytic activities of the Elettaria cardamomum extract and its synthesized nano selenium at different concentrations.

High concentrations revealed a high percentage of hemolysis in a proportional relationship. All tested samples displayed very low hemolytic effects on human erythrocytes. Based on these results, Elettaria cardamomum synthesized nano selenium solution showed maximum hemolytic activity [48]. The results demonstrated that the investigated samples were potent hemolytic agents. At the same time, the samples were safe for human erythrocytes. Generally, the results of this research established that aqueous extracts from Elettaria cardamomum and its nano-solutions are non/less toxic to human erythrocytes.

CONCLUSION

This study declares the possibility of formulating selenium nano-composites with aqueous extracts of *Elettaria cardamomum* to improve the natural biological efficacy of the chemical constituents. These solutions inhibited the growth of various cancer cells while not affecting the growth of normal cells. This study also proved that selenium nano-composites were less efficient than aqueous extracts as antioxidants, which confirms that the formation of selenium nanoparticles decreases the efficiency of nanomaterials to trap DPPH$^{\bullet}$ free radicals. Phytochemical analyses and antioxidant evaluations revealed similar results, showing that decreases in the phenolic and flavonoid contents impacted the DPPH$^{\bullet}$ free radical stabilization. Maximum hemolytic activities were recorded *Elettaria cardamomum* selenium nano-solutions. Because of the favorable biological properties of *Elettaria cardamomum* extract, and its selenium nano-solutions, they may be potentially useful for drug development and food processing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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