Assessment the effect of He-Ne laser treatment of *Balanites aegyptiaca* seeds on the amelioration of active constituents, antioxidant capacity, and anticancer impact *in vitro*

F.M. Mousa[a], M.M. Ali[a], A.H. Abdel-Halim[a], G. Khamis[b], M. Morsy[b], H.M. Ghanem[c]

[a]Department of Biochemistry, Biotechnology Research Institute, National Research Centre, [b]Department of Laser Applications in Metrology, Photochemistry and Agriculture (LAMPA), National Institute of Laser Enhanced Sciences, Cairo University, Giza, [c]Department of Biochemistry, Faculty of Science, Ain Shams University, Cairo, Egypt

Correspondence to Mamdouh M. Ali, (Prof. Dr.), Department of Biochemistry, Biotechnology Research Institute, National Research Centre, Dokki 12622, Giza, Egypt. Tel: +20 122 328 3749; fax: +20 233 370 931; e-mail: mmali1999@yahoo.com

Received: 11 December 2022
Revised: 26 December 2022
Accepted: 26 December 2022
Published: 31 March 2023

Egyptian Pharmaceutical Journal 2023, 22:150–163

Background and objective
Cancer is still a major health problem worldwide, with an estimated 18.1 million new cases in 2018, and it is expected to increase by 75% by 2030. Chemotherapeutic drugs have disadvantages such as toxicity to noncancerous tissues, drug resistance, and recurrence of cancer. Medicinal plants with their active components have great potential as an important source for novel drug discovery owing to their availability, efficiency, and safety. Searching for new strategies to obtain new drugs with higher efficiency and more safety represents an urgent need. Laser light treatment for seeds is known to improve germination, plant growth, and bioactive substance. The goal of this study was to investigate the effect of laser irradiation on improvement of the phytochemicals compounds and biological activities of *Balanites aegyptiaca* seeds.
Materials and methods
The effect of laser pretreatment was investigated at different powers, that is, 25, 50, 100, and 200 mW, with two-time intervals for each power (2 and 4 min), on *B. aegyptiaca* seeds to enhance the germination and antioxidant activity of the methanolic extracts of their dry plant material through different assays and select the most powerful laser pretreatment extract to evaluate the anticarcinogenic activity on different cell lines.
Results and conclusion
The results bring to light that the most efficient laser treatment for seeds of *B. aegyptiaca* was at 200 mW/4 min, which induces the highest yield percentage, total phenolic and flavonoid contents, metal chelating, reducing power, as well as free diphenylpicrylhydrazyl and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activities. Based on these outcomes, the antiproliferative screening assay of the methanolic extracts for the shoots (S) and roots (R) dry plant material of *B. aegyptiaca* after helium-neon laser treatment at 200 mW for 4 min compared with control was performed on a panel of three cancer cell lines (HepG2, HCT116, and MCF-7) using the sulphorhodamine-B assay, and cytotoxicity was determined using normal BHK fibroblast cell line. Obtained results indicated that these extracts should be regarded as potential anticarcinogenic resources against the HepG2 cell line, displayed moderate activity against MCF-7 and HCT116 cell lines, and exhibited no activity against the growth of the normal BHK cell line. Furthermore, a comparison between these laser-treated extracts, and their mixtures against their control extracts and their mixtures, using the doxorubicin as the reference drug on the HepG2 cell line was in favor of the laser-treated roots and shoots extracts, respectively.

Keywords:
anticancer, antioxidant, *Balanites aegyptiaca*, chemical constituents, helium-neon laser

Egypt Pharmaceut J 22:150–163

1687-4315

Introduction

Medicinal plants have been used in healthcare for thousands of years to treat various types of human illnesses such as diabetes, cardiovascular diseases, and cancer owing to their accessibility, low cost, affordability, and safety compared with synthetic drugs [1,2]. Many potential herbal extracts and their active components known as phytochemicals have been evaluated for cancer prevention and treatment or for decreasing the adverse effects accompanied by chemotherapy [3–5]. Anticancer drug development from natural resources is ventured throughout the world, and nowadays, ~60% of all anticancer drugs are of natural origin [6,7].

Balanites aegyptiaca (L.) Del. is a wild spiny, semideciduous or evergreen tree, classified as a member of the genus Balanites, which belongs to the zygophyllaceae family; its earlier identification was Balanitaceae or Simaroubaceae families [8–10]. It can be naturally grown in many kinds of habitats, climatic levels, and soil types [11]. It is widely distributed throughout the tropical belt of Africa, the Middle East, and South Asia [12,13]. It has antifungal, antibacterial, antivirus, antinociceptive, anticonvulsant, antioxidant, antiproliferative, and anticancer effects [14–16]. Besides its medicinal uses, it is widely used as food or confectionary, fodder, shade, charcoal, timber, fuel wood, and for keeping insects away [9,17].

The phytochemical composition of different parts of *B. aegyptiaca* showed the presence of high amounts of saponins, which represent the main chemical constituents of the *B. aegyptiaca* fruit [18]. Prior research reported the existence of saponins, steroidal sapogenins, or steroidal glycosides in all plant sections, mainly yielding diosgenin and yamogenin, which are the synthetic precursors for cortisones, oral contraceptives, and other steroidal drugs in the pharmaceutical industry [19,20]. In addition, other bioactive compounds such as steroids, alkaloids, phenolic acids, tannins, fatty acids, terpenes, flavonoids, glycosides, lipids, proteins, carbohydrates, organic acids, and vitamins were reported [21].

Searching for new techniques that are environmentally clean, safe, and enhance plant growth, yield, and biochemical and physiological parameters is interesting. Laser (light Amplification by Stimulation of Radiation) is used for this purpose regarding its distinctive characteristics [22]. Many reports revealed that laser treatments of some plant seeds, such as alfalfa [23], sunflower [24], soybean [25], *Adansonia digitata* [26], lemongrass sprouts [27], common buckwheat, and tartary buckwheat sprouts [28], at appropriate wavelength, time, and power increase shoot and root length, the yield of crop, and rate of germination, with an enhancement in protein content, enzymatic activity, and antioxidant activity. To the best of our knowledge, the effect of laser treatment on the *B. aegyptiaca* seeds to improve the bioactive compounds and biological activity of *B. aegyptiaca* seeds extract has not been investigated. From this point of view, phytochemical constituents, antioxidant capacity, as well as *in vitro* anticancer activity were estimated under helium-neon (He-Ne) laser treatment compared with the control. Our study hypothesized that laser irradiation improves the phytochemicals compounds and biological activities of *B. aegyptiaca* seeds, which leads to enhanced anticancer activity in different cell lines.

Materials and methods

Plant material and treatment conditions

The seeds of *B. aegyptiaca* were collected during the period between March and July 2019 from a local market in the New Valley Governorate, Egypt. The epicarp (outer cover) was gently removed by hand, and the mesocarp (pulp) was manually scraped with a sterile sharp blade. The hard-woody endocarp of the fruits was mechanically broken by light hammering to release the seeds, which were collected and washed with running tap water for a few minutes and were dried at room temperature for 30 min before laser pre-illumination. The light source used in this experiment was the He-Ne laser equivalent system, with a wavelength of 630 nm (equipment whitening, laser II; DMC Equipment Ltd, China) used for seed pre-illumination. The seeds were divided into nine groups (50 for each), comprising the control group without any irradiation and eight laser treatments conducted at different powers of 25, 50, 100, and 200 mW with the two-time intervals for each power at 2 and 4 min [26].

The experiment was repeated three times. Each seed was individually irradiated with laser treatment. The laser was perpendicular to the seeds, and the distance between the laser source and the seeds was 15 cm. The treated seeds and control were sterilized with 50% sodium hypochlorite (9.4% active chlorine) for 20 min, and afterward, these seeds were washed with distilled water four times.

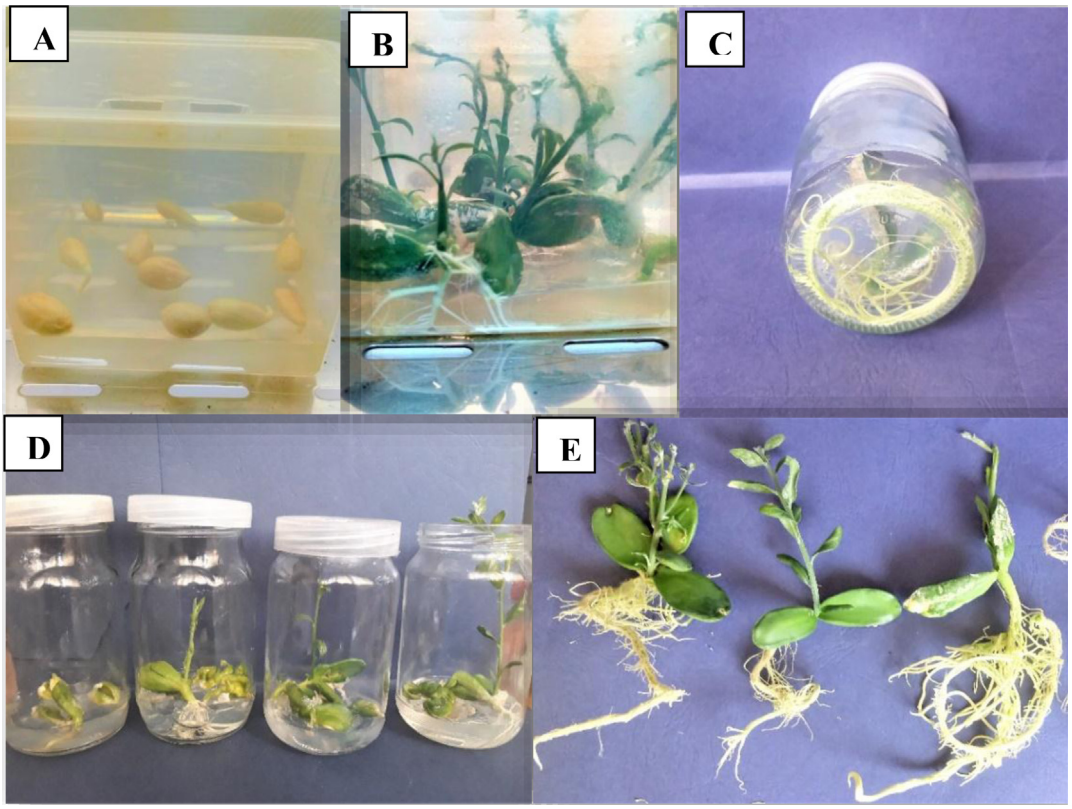
Balanites aegyptiaca seed germination

The sterilized seeds were placed on Murashige and Skoog [29] medium supplemented with 3% (w/v) sucrose and 0.4% phytagel, and the pH was adjusted to 5.7 using 1 M NaOH. The cultures were incubated at 24°C in a growth chamber under a 16 : 8 h light : dark photoperiod managed through cool white fluorescence tubes. After 4 weeks of germination (Fig. 1), the explants including shoots and roots were collected and prepared for extraction.

Plant extract preparation

The collected fresh plants (shoots and roots) of *B. aegyptiaca* were properly washed in tap water, rinsed using distilled water, cut into small pieces, and air-dried at room temperature. The air-dried plant material was pulverized into uniform powder using

Figure 1



After 4 weeks of germination, the fresh explants including shoots and roots of *Balanites aegyptiaca* were as follows: (a) the sterilized seeds placed on Murashige and Skoog (MS) medium. (b) The explant development after 4 weeks. (c) The long coiled roots of the *B. aegyptiaca* explants. (d) The different stages of the explant development. (e) During the collection of fresh explants before extract preparation.

an electric blender and was stored in a glass container in dark with avoiding exposure to sunlight to prevent the loss of active components until extraction, and analysis was done according to the method of Mohamed *et al*. [30].

The concentrated methanolic extracts were weighed, and their percentage yields were calculated based on dry weight from the following equation:

$$\text{Yield}\% = \text{W1}/\text{W2} \times 100$$

Where, W1 and W2 were the weight of the extract after the solvent evaporation and the weight of the dry plant material, respectively. A part of different dry extracts of the shoots and roots of *B. aegyptiaca*, which their seeds exposed to He-Ne laser at different time intervals and different powers before germination or those without irradiation exposure, was reconstituted with methanol to prepare different concentrations from 0.1 to 4 mg/ml, and then stored at 4°C. The other part of the extracted residues is stored in a refrigerator at 4°C for subsequent analysis. In this study, the methanolic extracts of the shoots (S) and roots (R) dry plant material of *B. aegyptiaca* after He-Ne laser treatment at different powers (25, 50, 100, and 200 mW) and different time intervals (2 and 4 min) compared with control were screened through the following phytochemical assays to elucidate the effect of different laser pretreatment on enhancing the antioxidant activity, and then the most potent laser pretreated extracts were selected to estimate the anticarcinogenic activity on different cell lines.

Phytochemical screening

Total phenolic content

The total phenolic content of the extract was estimated as gallic acid equivalents essentially according to that described by Quettier-Deleu *et al*. [31] with minor modification [7]. The results were expressed in mg of gallic acid/g dry substrate (mg_{GAE}/gds).

Total flavonoid content

The total flavonoid content was determined according to a procedure proposed by Horszwald and Andlauer [32]. The results obtained for analyzed extracts were expressed as mg of quercetin (Q) per g dry substrate of extract (mg_{Q}/gds).

Antioxidant capacity analyses

Diphenylpicrylhydrazyl free radical-scavenging assay

1,1-diphenyl-2-picryl-hydrazyl(DPPH) method was used for determination of the free radical-scavenging activity of the *B. aegyptiaca* extracts based on the method of Mahmoud *et al.* [7], Mensor *et al.* [33], and Dessalegn *et al.* [34]. The inhibitory percentage of DPPH was calculated according to the following equation:

Scavenging activity (%)=[1-(absorbance of sample/ absorbance of control)]×100%

The antioxidant activity of the extracts was expressed as IC_{50} value, which was defined as the concentration (μg/ml) of extracts that scavenges the DPPH radical by 50% and calculated by graphing the concentration against percentage inhibition.

2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) reagent was prepared and used for determination of the free radical-scavenging activity of the extracts as described by methods of Re *et al.* [35] and Abdel-Aty *et al.* [2]. The inhibitory percentage of ABTS was calculated according to the following equation:

$$\text{Scavenging activity}(\%) = [1 - (\text{absorbance of sample}/\ \text{absorbance of control})]\ \times 100\%$$

Metal (ferrous, Fe2+) ion-chelating ability assay

The chelating ability of ferrous ion in all extracts was estimated according to the original method of Decker and Welch [36] with minor modifications [37].

The percentage of inhibition of ferrozine-Fe^{2+}complex formation was calculated using the following equation:

$$\text{Chelating activity}(\%) = [1 - (\text{absorbance of sample}/\ \text{absorbance of control})] \times 100\%.$$

Reducing power assay

The reducing power of the extracts was estimated by the original method of Oyaizu [38] with minor modifications [39] with using ascorbic acid as standard at concentrations of 10, 20, 50, 100, 150, 200, and 250 μg/ml.

Determination of potential in vitro cytotoxicity of the methanolic extracts

The anticancer activity of the methanolic extracts of *B. aegyptiaca* shoots (S) and roots (R) that their seeds are exposed to He-Ne laser (L) of 200 mW for 4 min was screened against different cell lines using sulphorhodamine-B (SRB) assay according to Omar *et al.* [40] and El-Shahat *et al.* [41].

Cell lines and culturing

Human tumor carcinoma cell lines, including liver (HepG2), breast (MCF-7) and colon (HCT116) as well as normal baby hamster kidney fibroblast (BHK) cell line used in this study were obtained from the American Type Culture Collection (ATCC, Minisota, USA). All experiments carried out on the cell lines were approved by the Research Ethical Committee at the National Research Centre, Egypt (Registration Number: 92302021). The cells were maintained in DMEM supplemented with 10% HIFCS, penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C in a humidified atmosphere containing 5% CO_2. Cells at a concentration of 0.50×10^6 were grown in a $25cm^2$ flask in 5 ml of culture medium [42].

The extracts were prepared by dissolving 1 mg/1 ml of dimethylsulfoxide (DMSO) as a stock solution and diluted to the appropriate volume just before adding to the cell culture. Cells were seeded in 96-well microtiter plates at the initial concentration of 4×10^3 cell/well in a 200μl fresh medium and left for 24 h to attach to the plates. Different concentrations of the samples were used (0–500 μg/ml), where each sample concentration was added to three wells. The reference drug, doxorubicin, acts as positive control, whereas control cancer cells treated with DMSO act as negative control. The plates were incubated with the extracts for 48 h at 37°C and in an atmosphere of 5% CO_2. Following 48 h of treatment, the cells were fixed with 10 μl of cold trichloroacetic acid at 10% final concentration for 1 h at 4°C. The plates were washed five times with distilled water and stained with 50 μl of 0.2% (w/v) SRB dissolved in 1% acetic acid for 30 min in dark at room temperature. The plates were then washed four times with 1% acetic acid and air-dried. The dye was solubilized with 200 μl/well of 10 mM tris base (pH 10.5) and for 5 min on a shaker (Orbital shaker OS 20, Boeco, Germany) at 1600 rpm. The optical density (OD) of each well was measured spectrophotometrically at 570 nm with an ELISA microplate reader (Sunrise Tecan Reader, Germany), where the OD is directly proportional to the surviving fraction. The mean values of each extract concentration were calculated.

The percentage of cell survival was calculated as follows:

$$\text{Surviving fraction} = \text{OD(treated cells)}/\text{OD(control cells)}.$$

The relation between the percent of cell viability fraction and extract concentration was plotted. The IC_{50} values (the concentrations of drug required to produce 50% inhibition of cell growth or viability) were also calculated using prism version 5.

Further investigation was done to compare roots and shoots that were exposed to laser 200 mW/4 min (LR and LS) with their control shoots and roots that were not exposed to laser (CR and CS), respectively, against HepG2 cell line using SRB assay. In addition, a mixture of LR and LS was compared with a mixture of CR and CS against the HepG2 cell line.

Statistical analysis

All experiments were performed in triplicates. The data were expressed as mean±SE. Statistical analysis was carried out using the SPSS software package (version 24.0, Armonk, NY: IBM Corp, USA) and analyzed using one-way analysis of variance followed by the Tukey HSD and Games-Howell post-hoc test for multiple comparisons. *P* values less than 0.05 were considered statistically significant.

Results

To achieve the desired objective of this study, the methanolic extracts of the shoots (S) and roots (R) dry plant material of *B. aegyptiaca* after treatment of seeds with He-Ne laser (L) at different powers (25, 50, 100, and 200 mW) and different time intervals (2 and 4 min) compared with control were screened through the preceding phytochemical assays to demonstrate the effect of different laser pretreatments on boosting antioxidant activity, followed by nominating the most potent laser pretreated extract to assess the anticarcinogenic activity in different cell lines. The results are represented as follows.

Yields of different methanolic extracts

The percentage yields of the methanolic extracts of the roots (R) and shoots (S) of *B. aegyptiaca*, whose seeds were exposed to He-Ne laser (L) at different time intervals and different powers before germination, as well as the control roots (CR) and control shoots (CS) that were not exposed to irradiation, which were calculated per gram of dry plant material based on the yield (%) equation, were evaluated and are presented in Table 1. It was noticed that the methanolic extracts of the roots and shoots of *B. aegyptiaca*, whose seeds were exposed to a He-Ne laser of 200 mW for 4 min (4/200 LR and 4/200 LS), had the highest percentage yields of the concentrated extract (37.44±0.59 and 36.22±1.12%, respectively) among other methanolic extracts that were exposed to other time intervals and other powers, besides a significant increase ($P<0.001$) in percentage yields when compared with CR and CS (24.10±0.94 and 11.47±0.58%, respectively).

Table 1 The percentage yields (%) of methanolic extract of shoots (S) and roots (R) dry plant material of *Balanites aegyptiaca* after helium-neon laser treatment at different powers (25, 50, 100, and 200 mW) and different time intervals (2 and 4 min) compared with control

The *Balanites aegyptiaca* methanolic shoots extracts	Yield (W1/W2)%	The *Balanites aegyptiaca* methanolic roots extracts	Yield (W1/W2) %
CS	11.47±0.58	CR	24.10±0.94
4/200 S	36.22±1.12[a*]	4/200 R	37.44±0.59[b*]
2/200 S	28.10±1.22[a*]	2/200 R	18.21±1.19[b⁑]
4/100 S	35.28±0.61[a*]	4/100 R	10.20±0.68[b*]
2/100 S	30.19±0.59[a*]	2/100 R	24.31±0.50
4/50 S	17.61±1.08[a*]	4/50 R	9.34±0.6[b*]
2/50 S	21.52±0.64[a*]	2/50 R	10.56±0.64[b*]
4/25 S	2.48±0.04[a*]	4/25 R	20.54±1.05
2/25 S	34.52±0.68[a*]	2/25 R	24.51±1.21

The experiment was carried out in triplicate. Values are given as mean±SE of three batches. One-way analysis of variance/Tukey HSD post-hoc analyses were used for % yields comparison of means differences between groups where F ratio=156.1. [a] and [b] are significantly different from the control methanolic extracts of the shoots and roots (CS and CR, respectively) of *Balanites aegyptiaca* whose seeds were germinated without using laser exposure, at significance level. [*]*P* value less than 0.001. [⁑]*P* value less than 0.01.

The total phenolic content

The total phenolic recovery in the methanolic extracts of the roots and shoots of *B. aegyptiaca*, whose seeds were exposed to He-Ne laser at different time intervals and different powers before germination, were quantified and expressed in mg of gallic acid/g dry substrate (mg_{GAE}/gds). As presented in Table 2, the methanolic extracts of 4/200 LR and 4/200 LS (R and S of *B. aegyptiaca* whose seeds were exposed to laser at 200 mW for 4 min), showed the highest total phenolic contents (1458.2±2.34 and 890.00±2.79 mg_{GAE}/gds for 4/200 LR and 4/200 LS, respectively, using gallic acid as a standard), among other methanolic extracts of the R and S of *B. aegyptiaca* whose seeds were exposed to He-Ne laser at other time intervals and other powers before germination; especially, a significant increase ($P<0.001$) was obtained when compared with R and S that were not exposed to laser (CR and CS) (total phenolic yields of CR and CS was 262.04 ± 1.42 and 545.37 ± 3.88 mg_{GAE}/gds, respectively).

The total flavonoid content

The total flavonoid content in the methanolic extracts of the R and S of *B. aegyptiaca* whose seeds were

Table 2 The total phenolic contents (mg_{GAE}/gds) of the methanolic extracts for the shoots (S) and roots (R) dry plant material of *Balanites aegyptiaca* after helium-neon laser treatment at different powers (25, 50, 100, and 200 mW) and different time intervals (2 and 4 min) compared with control

The *Balanites aegyptiaca* methanolic shoots extracts	The total phenolic content (mg_{GAE}/gds) at 1000 μg/ml	The *Balanites aegyptiaca* methanolic roots extracts	The total phenolic content (mg_{GAE}/gds) at 1000 μg/ml
CS	545.37 ± 3.88	CR	262.04 ± 1.42
4/200 S	890.00 ± 2.79[a*]	4/200 R	1458.2 ± 2.34[b*]
2/200 S	310.96 ± 2.79[a*]	2/200 R	365.26 ± 3.76[b*]
4/100 S	288.38 ± 2.79[a*]	4/100 R	220.64 ± 1.86[b*]
2/100 S	226.02 ± 4.20[a*]	2/100 R	236.23 ± 3.76[b*]
4/50 S	242.15 ± 4.20[a*]	4/50 R	302.90 ± 3.72[b*]
2/50 S	251.82 ± 3.27[a*]	2/50 R	348.06 ± 2.79[b*]
4/25 S	229.24 ± 3.76[a*]	4/25 R	291.07 ± 3.76[b*]
2/25 S	219.57 ± 2.34[a*]	2/25 R	273.33 ± 2.34

The experiment was carried out in triplicate. Values are given as mean±SE of three batches. One-way analysis of variance/Tukey HSD post-hoc analyses were used for comparison of means where F ratio=949.6. [a] and [b] are significantly different ([*]$P<0.001$) from the control methanolic extracts of the shoots and roots (CS and CR, respectively) of *Balanites aegyptiaca* which their seeds germinated without using laser exposure.

Table 3 The total flavonoids content (mg_Q/gds) of the methanolic extracts for the shoots (S) and roots (R) dry plant material of *Balanites aegyptiaca* after helium-neon laser treatment at different powers (25, 50, 100, and 200 mW) and different time intervals (2 and 4 min) compared with control

The *Balanites aegyptiaca* methanolic shoots extracts	The total flavonoids content (mg_Q/gds) at 1000 μg/ml	The *Balanites aegyptiaca* methanolic roots extracts	The total flavonoids content (mg_Q/gds) at 1000 μg/ml
CS	37.12±2.07	CR	25.88±1.87
4/200 S	231.64±3.61[a*]	4/200 R	389.40±3.81[b*]
2/200 S	26.28±2.01	2/200 R	30.55±2.68
4/100 S	46.20±3.81	4/100 R	23.10±1.91
2/100 S	72.24±2.78[a*]	2/100 R	29.30±2.65
4/50 S	144.09±3.26[a*]	4/50 R	19.80±1.91
2/50 S	38.40±2.83	2/50 R	120.47±2.51[b*]
4/25 S	155.28±5.72[a*]	4/25 R	69.72±1.86[b*]
2/25 S	72.48±3.91[a*]	2/25 R	13.86±0.38

The experiment was carried out in triplicate. Values are given as mean±SE of three batches. One-way analysis of variance/Tukey HSD post-hoc analyses were used for the comparison of means where F ratio=1038. [a] and [b] are significantly different ([*]$P<0.001$) from the control methanolic extracts of the shoots and roots (CS and CR, respectively) of *Balanites aegyptiaca* which their seeds germinated without using laser exposure.

exposed to He-Ne laser (L) at different time intervals and different powers before germination was quantified and expressed in mg of quercetin/g dry substrate (mg_Q/gds). As shown in Table 3, the methanolic extracts of 4/200 LR and 4/200 LS showed the highest total flavonoid content (389.40 ± 3.81 and 231.64 ± 3.61 mg_Q/gds, respectively) among other methanolic extracts of the roots and shoots of *B. aegyptiaca* whose seeds were exposed to He-Ne laser at different time intervals and different powers before germination, and especially, a significant increase was obtained ($P < 0.001$) when compared with the control methanolic extracts of the roots and shoots (CR and CS) of *B. aegyptiaca* whose seeds were germinated without using laser exposure and had total flavonoid yields of 25.88 ± 1.87 and 37.12 ± 2.07 mg_Q/gds, respectively.

Antioxidant capacity analyses

Diphenylpicrylhydrazyl free radical-scavenging activity

The antioxidant activities of different concentrations (0.1–4 mg/ml) of methanolic extracts of LR and LS of *B. aegyptiaca* whose seeds were exposed to He-Ne laser (L) at different time intervals and different powers before germination, and R and S of seeds that were not exposed to irradiation (CR and CS) were measured using DPPH radical-scavenging activity assay. As demonstrated in Table 4, there was a gradual increase in the DPPH radical scavenging activity of the extracts with an increase in the concentration of the extract. The maximum radical scavenging activities were obtained at extract concentrations of 4 mg/ml for all extracts. A significant difference ($P<0.05$) was observed between LR and LS, and CR and CS. It was noticed that the methanolic extracts of LR and

Table 4 Diphenylpicrylhydrazyl free radical scavenging activity (%) of different concentrations of the methanol extracts of the shoots and roots of *Balanites aegyptiaca* after helium-neon laser treatment at different powers (25, 50, 100, and 200 mW) and different time intervals (2 and 4 min) compared with control

	Antioxidant activity					
	% DPPH free radical-scavenging at					
The *Balanites aegyptiaca* methanolic extracts	100 μg/ml	500 μg/ml	1000 μg/ml	2000 μg/ml	4000 μg/ml	IC_{50} (μg/ml)
Shoots: CS	0.14±0.01	2.74±0.03	42.41±0.04	96.31±0.10	97.56±0.09	1600.4±0.68
4/200 S	19.54±0.02^{a*}	40.20±0.02^{a*}	64.59±0.04^{a*}	97.67±0.01$^{a¥}$	98.65±0.06$^{a¥}$	794.31±1.32^{a*}
2/200 S	10.86±0.03^{a*}	21.10±0.02^{a*}	36.66±0.05^{a*}	82.52±0.54^{aX}	97.37±0.04	1531.8±2.98^{aX}
4/100 S	–5.64±0.03^{a*}	20.75±0.01^{a*}	34.35±0.25^{aX}	70.73±0.50^{aX}	96.72±0.05$^{a¥}$	1785.3±5.10^{aX}
2/100 S	7.47±0.06^{a*}	15.72±0.03^{a*}	31.52±0.06^{a*}	71.49±0.33^{a*}	96.76±0.09$^{a¥}$	1748.4±1.08^{a*}
4/50 S	18.33±0.04^{a*}	34.45±0.04^{a*}	52.70±0.61$^{a¥}$	96.60±0.18	96.15±0.05^{aX}	1041.8±7.81^{a*}
2/50 S	17.57±0.03^{a*}	14.10±0.14^{a*}	31.72±0.15^{a*}	52.19±0.17^{a*}	96.72±0.05$^{a¥}$	1870.4±3.89^{a*}
4/25 S	10.68±0.04^{a*}	13.49±0.02^{a*}	29.41±0.01^{a*}	50.54±0.01^{a*}	94.89±0.05^{a*}	1978.3±0.66^{a*}
2/25 S	6.62±0.02^{a*}	10.98±0.16^{aX}	21.67±0.02^{a*}	41.55±0.01^{a*}	88.63±0.05^{a*}	2270.3±1.16^{a*}
Roots: CR	5.73±0.07	32.38±0.05	53.92±0.09	96.19±0.12	96.82±0.11	1206.2±2.32
4/200 R	43.80±0.04^{b*}	95.39±0.02^{b*}	96.74±0.09^{b*}	100.4±0.02^{bX}	104.53±0.05^{b*}	148.08±0.31^{b*}
2/200 R	13.57±0.02^{b*}	32.37±0.04	56.66±0.30	96.08±0.09	96.40±0.12	1091.1±5.03^{bX}
4/100 R	16.44±0.06^{b*}	28.15±0.02^{b*}	48.82±0.80	94.17±0.14^{bX}	96.08±0.09	1204.2±9.98
2/100 R	13.57±0.02^{b*}	34.03±0.01^{bX}	53.73±0.21	95.40±0.15	96.85±0.09	1104.7±1.06^{b*}
4/50 R	16.22±0.04^{b*}	30.77±0.06^{b*}	56.46±0.16^{bX}	96.17±0.37	95.28±0.55	1081.3±6.35^{bX}
2/50 R	10.20±0.06^{b*}	27.70±0.03^{b*}	48.62±0.64	96.80±0.28	95.47±0.11$^{b¥}$	1260.3±8.91
4/25 R	13.31±0.02^{b*}	25.11±0.01^{b*}	46.18±0.03^{b*}	93.13±0.02^{bX}	97.40±0.11	1295.7±0.97^{b*}
2/25 R	9.13±0.04^{b*}	22.79±0.13^{b*}	36.27±0.03^{b*}	80.51±0.01^{b*}	85.07±0.11^{b*}	1682.3±1.88^{b*}

DPPH, diphenylpicrylhydrazyl. The experiment was carried out in triplicate. Values are given as mean±SE of three batches. One-way analysis of variance/Tukey HSD post-hoc analyses were used for % DPPH free radical-scavenging at 100 μg/ml (% $DPPH_{100}$) comparison of mean differences between groups where F ratio=6.347×10 ^{4}, whereas one-way Welch analysis of variance/Games-Howell post-hoc analyses were used for % DPPH free radical-scavenging at 500, 1000, 2000, and 4000 μg/ml and IC_{50} (μg/ml) comparison of mean differences between groups where F ratio=5.434×10^5, 7.269×10^4, 5.253×10^5, 1.817×10^3, and 3.975×10^5, respectively. Means bearing ^{a} and ^{b} are significantly different from the control methanolic extracts of the shoots and roots (CS and CR, respectively) of *Balanites aegyptiaca* which their seeds germinated without using laser exposure, at significance level. $^{*}P$ value less than 0.001. ^{X}P value less than 0.01.$^{¥}P$ value less than 0.05.

LS of *B. aegyptiaca* whose seeds were exposed to He-Ne laser of 200 mW for 4 min (4/200 LR and 4/200 LS) had the highest antioxidant activities (104.53±0.05 and 98.65±0.06% inhibition of radical formation) at concentration of 4 mg/ml with IC_{50} values (148.08 ±0.31 and 794.31±1.32 μg/ml, respectively). However, CR and CS (antioxidant activities were 96.82±0.11 and 97.56±0.09% inhibition of radical formation) with IC_{50} values of 1206.2±2.32 and 1600.4±0.68 μg/ml, respectively, showed a significant difference ($P<0.001$) as compared with LR and LS.

2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

The methanolic extracts of LR and LS germination showed antioxidant activity by quenching $ABTS^{\bullet+}$ cation radicals. As presented in Table 5, it was noticed that the methanolic extracts of the roots and shoots of *B. aegyptiaca* whose seeds were exposed to the He-Ne laser of 200 Mw for 4 min (4/200 LR and 4/200 LS) were the most effective scavengers of the $ABTS^{+}$ cation radicals with ~130.93±3.39 and 114.79±2.22% scavenging of radicals formation, respectively, among other methanolic extracts that were exposed to other time intervals and other powers. Besides, there was a significant increase ($P<0.05$) in scavenging activity when compared with CR and CS.

Metal (ferrous) ion-chelating ability

The chelating activities for the ferrous ion of the extracts were carried out by the inhibition of the formation of blue-colored ferrous ion-ferrozine complex. As presented in Table 6, the methanolic extracts of 4/200 LR and 4/200 LS had the most iron chelating activities with ~136.32±0.52 and 119.13±0.65%, respectively, among other methanolic extracts, particularly with a significant increase ($P<0.001$) in iron chelating activities when compared with CR and CS.

Reducing power assay

The reducing power of the methanolic extracts of the shoots and roots of *B. aegyptiaca*, whose seeds were exposed to He-Ne laser at different time intervals and different powers before germination were quantified and expressed in mg of vitamin C/L of extracts (mg_{VitC}/L). As presented in Table 7, the methanolic extracts 4/200 LR and 4/200 LS showed the highest

Table 5 2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity (%) of the methanolic extracts for the shoots (S) and roots (R) dry plant material of *Balanites aegyptiaca* after helium-neon laser treatment at different powers (25, 50, 100, and 200 mW) and different time intervals (2 and 4 min) compared with control

The *Balanites aegyptiaca* methanolic shoots extracts	% ABTS free radical-scavenging at 1000 μg/ml	The *Balanites aegyptiaca* methanolic roots extracts	% ABTS free radical-scavenging at 1000 μg/ml
CS	87.45±1.06	CR	91.80±1.58
4/200 S	114.79±2.22$^{a¥}$	4/200 R	130.93±3.39$^{b¥}$
2/200 S	85.58±0.14	2/200 R	89.39±0.18
4/100 S	90.91±0.12	4/100 R	92.81±0.13
2/100 S	94.16±0.14	2/100 R	99.48±0.18
4/50 S	89.57±0.30	4/50 R	86.42±0.54
2/50 S	78.87±2.22	2/50 R	86.95±0.22
4/25 S	85.91±0.22	4/25 R	86.78±0.23
2/25 S	77.92±0.11	2/25 R	83.68±0.32

ABTS, 2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). The experiment was carried out in triplicate. Values are given as mean±SE of three batches. One-way Welch analysis of variance/Games-Howell post-hoc analyses were used for ABTS comparison of means where F ratio=566.8. [a] and [b] are significantly different ($^{¥}P<0.05$) from the control methanolic extracts of the shoots and roots (CS and CR, respectively) of *Balanites aegyptiaca* which their seeds germinated without using laser exposure.

Table 6 Metal ion-chelating activity (%) of the methanolic extracts for the shoots (S) and roots (R) dry plant material of *Balanites aegyptiaca* after helium-neon laser treatment at different powers (25, 50, 100, and 200 mW) and different time intervals (2 and 4 min) compared with control

The *Balanites aegyptiaca* methanolic shoots extracts	% Metal ion-chelating activity at 1000 μg/ml	The *Balanites aegyptiaca* methanolic roots extracts	% Metal ion-chelating activity at 1000 μg/ml
CS	97.01±0.40	CR	96.86±0.26
4/200 S	119.13±0.65^{a*}	4/200 R	136.32±0.52 ^{b*}
2/200 S	10.46±0.65^{a*}	2/200 R	74.89±0.52^{b*}
4/100 S	91.78±0.54^{a*}	4/100 R	97.31±0.69
2/100 S	96.71±0.83	2/100 R	99.55±0.26$^{b¥}$
4/50 S	79.82±0.52^{a*}	4/50 R	98.65±0.26
2/50 S	84.75±0.26^{a*}	2/50 R	16.89±0.40 ^{b*}
4/25 S	2.24±0.26^{a*}	4/25 R	84.75±0.26 ^{b*}
2/25 S	75.93±0.65^{a*}	2/25 R	89.24±0.26 ^{b*}

The experiment was carried out in triplicate. Values are given as mean±SE of three batches. One-way analysis of variance/Tukey HSD post-hoc analyses were used for % FIC activity comparison of means differences between groups where F ratio=533.4. [a] and [b] are significantly different from the control methanolic extracts of the shoots and roots (CS and CR, respectively) of *Balanites aegyptiaca* which their seeds germinated without using laser exposure, at significance level. $^{*}P$ value less than 0.001. $^{¥}P$ value less than 0.05.

values of reducing power (2.11±0.067 and 1.84±0.035 mg_{VitC}/L for 4/200 R and 4/200 S, respectively, using vitamin C as a standard) among other methanolic extracts with a significant increase ($P<0.05$) as compared with CR and CS, which had total phenolic yields of 1.39±0.040 and 1.50±0.022 mg_{VitC}/l, respectively.

In vitro cytotoxicity

Upon the previous phytochemical screening, the most efficient laser treatment for seeds of *B. aegyptiaca* was at 200 mW/4 min. Therefore, the antiproliferative activity of the methanolic extracts of 4/200 LR and 4/200 LS was evaluated using SRB assay against HepG2, MCF-7, and HCT116 cell lines as well as the normal BHK, as shown in Table 8 and Figs. 2 and 3. It was evident that 4/200 LR and 4/200 LS displayed potent anticancer activity against HepG2 (IC_{50} values of 3.32±0.02 and 3.52±0.02 μg/ml, respectively), and moderate activity against MCF-7 (IC_{50} was 45.20 ±0.60 and 46.73±0.25 μg/ml, respectively) and HCT116 (IC_{50} was 58.14±0.39 and 78.33±0.25 μg/ml, respectively) cell lines. In addition, 4/200 LR and 4/200 LS exhibited no activity against the growth of the normal BHK cell line (IC_{50} was 171.87±1.62 and 161.41±0.56 μg/ml, respectively).

In addition, the HepG2 cell line was used to assess LR and LS methanolic extracts as compared with their control methanolic extracts of the roots and shoots (CR and CS) of *B. aegyptiaca* whose seeds were germinated without using laser exposure. Another comparison was done between a mixture of LR and LS methanolic extracts L(R+S) and a mixture of CR and CS methanolic extracts C(R+S) against the HepG2 cell line. For comparison, treatment with doxorubicin was

Table 7 The reducing power value (mg_{VitC}/L) of the methanolic extracts for the shoots (S) and roots (R) dry plant material of *Balanites aegyptiaca* after helium-neon laser treatment at different powers (25, 50, 100, and 200 mW) and different time intervals (2 and 4 min) compared with control

The *Balanites aegyptiaca* methanolic shoots extracts	The reducing power value (mg_{VitC}/l) at 1000 µg/ml	The *Balanites aegyptiaca* methanolic roots extracts	The reducing power value (mg_{VitC}/l) at 1000 µg/ml
CS	1.50±0.022	CR	1.39±0.040
4/200 S	$1.84±0.035^{a¥}$	4/200 R	$2.11±0.067^{b¥}$
2/200 S	$1.18±0.002^{a¥}$	2/200 R	1.14±0.002
4/100 S	$1.08±0.002^{a¥}$	4/100 R	1.29±0.005
2/100 S	1.37±0.002	2/100 R	1.24±0.005
4/50 S	$1.25±0.004^{a¥}$	4/50 R	1.22±0.005
2/50 S	$1.20±0.002^{a¥}$	2/50 R	1.41±0.005
4/25 S	$0.97±0.003^{a¥}$	4/25 R	0.97±0.005
2/25 S	1.32±0.005	2/25 R	0.96±0.004

The experiment was carried out in triplicate. Values are given as mean±SE of three batches. One-way Welch analysis of variance/Games-Howell post-hoc analyses were used for comparison of means where F ratio=$1.049×10^3$. [a] and [b] are significantly different ($^{¥}P<0.05$) from the control methanolic extracts of the shoots and roots (CS and CR, respectively) of *Balanites aegyptiaca* which their seeds germinated without using laser exposure.

Table 8 Cytotoxicity (IC_{50}, µg/ml) of the methanolic extracts of roots and shoots of *Balanites aegyptiaca* which their seeds were exposed to helium-neon laser of 200 Mw for 4 min against different human tumor HepG2, MCF-7 and HCT116, and normal BHK cell lines as measured with sulphorhodamine-B assay method

	IC_{50} (µg/ml)			
	HepG2	MCF-7	HCT116	BHK
LR	$3.32±0.02^{h}$	$45.20±0.60^{f}$	$58.14±0.39^{d}$	$171.87±1.62^{a}$
LS	$3.52±0.02^{g}$	$46.73±0.25^{e}$	$78.33±0.25^{c}$	$161.41±0.56^{b}$

Values are given as mean±SE of three batches. One-way analysis of variance/Tukey HSD post-hoc analyses were used for IC_{50} comparison of means differences between different cell lines where F ratio=$6.62×10^3$ and $4.05×10^4$ for LR and LS, respectively. Whereas, an independent sample *t* test was used for IC_{50} comparison of means differences between R_L and S_L on each of different cell lines with F ratio=0.470, 0.39, 1.221 and 2.018 for HepG2, MCF-7, HCT116 and BHK respectively. Means are ranked in descending order (a:h) and the largest mean take the first alphabetical superscripts. Means bearing different superscripts are significantly different ($P<0.05$).

used as a positive control, and treatment with DMSO was used as a negative control. The results showed that both LR and LS methanolic extracts had the most potent anticancer activity against the HepG2 cell line (IC_{50} 3.31±0.02 and 3.52±0.02 µg/ml, respectively), which was near to the reference drug, doxorubicin (IC_{50}: 3.03±0.07 µg/ml), as compared with those not exposed to laser (IC_{50} was 9.02±0.08 and 7.76±0.01 µg/ml for CR and CS, respectively). However, IC_{50} was 5.25±0.10 and 11.43±0.06 µg/ml for mixtures of L (R+S) and C (R+S), respectively. It was clear that the methanolic extracts of plants that were exposed to a He-Ne laser of 200 mW for 4 min had more anticarcinogenic effect than those methanolic extracts without laser exposure in all its forms, whether root, shoot, or their mixture (Table 9, Fig. 4).

Discussion

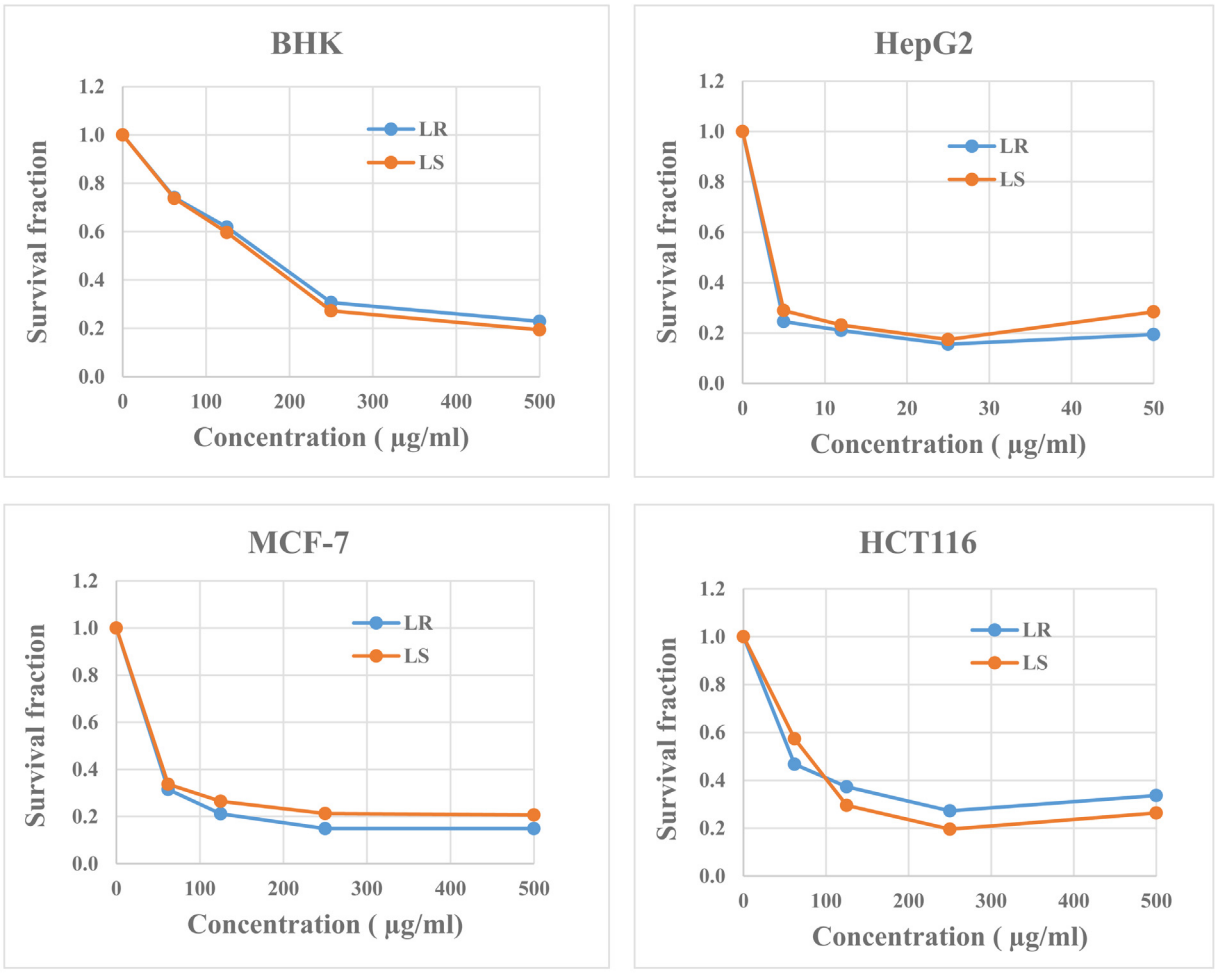
Plant-derived compounds have gained more attention owing to their anticancer activities and their ability to improve the body's defense system [43]. Searching for new techniques that increase their efficiency with the lowest side effects is of great interest [44]. Treatment of plants with a laser results in an increase in their bioenergetic potential, which results in the stimulation of their biological and biochemical processes [45].

Induction of helium-neon laser exposure of shoots and roots yield of *Balanites aegyptiaca*

The plant characteristics changes based on the effects of laser light, electromagnetism, and temperature [46]. The plant macromolecules absorb light at a specific wavelength and trigger photosynthetic activity, leading to increase growth rate, crop yield, and generally the plant quality, as well as the accumulation of bioactive compounds such as antioxidants [25,26]. Moreover, the growth rate was related to the temperature of laser light treatment; these external factors are not efficient until the induction of internal factors such as enzymes and hormones is done [47].

The present work explained that there was a correlation between the intensity of the He-Ne laser and the percent yield of plant extract. The methanolic extracts of the roots and shoots of *B. aegyptiaca* whose seeds were treated with He-Ne laser at power 200 mW and 4 min (4/200 LR, 4/200 LS) showed the highest percentage yields of concentrated extract among other methanolic extracts of *B. aegyptiaca* that were exposed (other time intervals and other powers) or not exposed (CR and CS) to He-Ne laser before germination. In agreement, Perveen *et al.* [24] showed that laser treatment of seeds

Figure 2



The cytotoxicity (IC_{50}, µg/ml) *of* the methanolic extracts of roots and shoots of *Balanites aegyptiaca* whose seeds were exposed to He-Ne laser of 200 mW for 4 min against different human cancer HepG2, MCF-7, and HCT116, as well as normal BHK cell lines as measured with the SRB assay method. He-Ne, helium-neon; SRB, sulphorhodamine-B.

increases shoot and root length and crop yields. In addition, Chen *et al.* [46] reported that the photosynthesis mechanism started when the plant absorbs the light through the macromolecules at a specific wavelength, which induced the yield percentage.

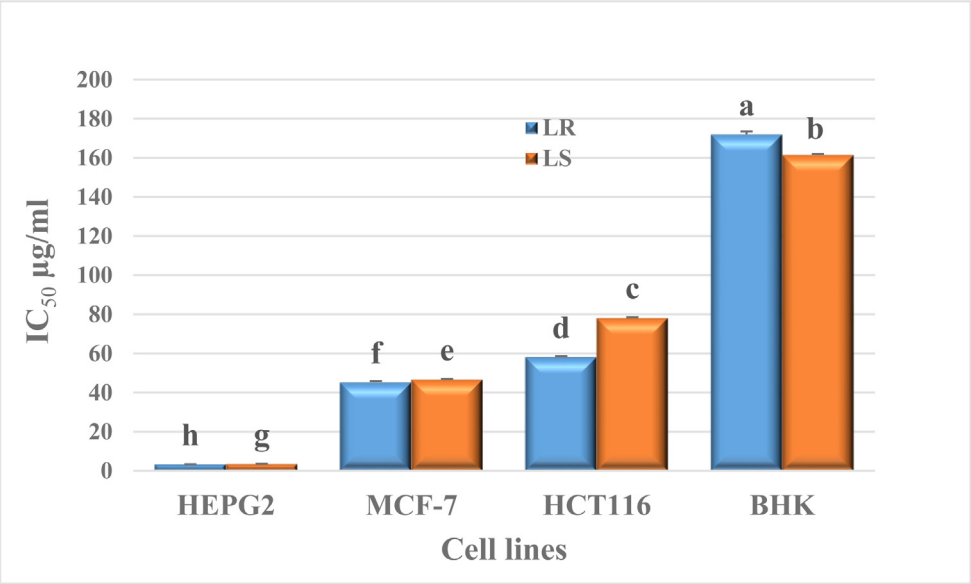
Induction of helium-neon laser exposure of total phenolic and flavonoid contents of shoots and roots of *Balanites aegyptiaca*

As mentioned before, laser exposure enhances the total phenolic and flavonoid contents of some plants such as sunflowers [24], soybean [25], buckwheat sprouts [28], and lemongrass sprouts [27], where plant macromolecules able absorb light that enhances the photosynthetic activity, which increases the secondary metabolites [26]. In agreement with the previous findings, the present work showed that the methanolic extracts of the roots and shoots of *B. aegyptiaca* whose seeds were treated with He-Ne laser at power 200 mW and 4 min (4/200 LR, 4/200 LS) showed the highest total phenolic and flavonoid contents among other methanolic extracts of the roots and shoots of *B. aegyptiaca* whose seeds were exposed (other time intervals and other powers) or not exposed to He-Ne laser before germination. There was a significant increase in total phenolic and flavonoid contents ($P<0.001$) as compared with CR and CS.

Induction of helium-neon laser exposure of antioxidant capacity of shoots and roots of *Balanites aegyptiaca*

As mentioned before, there was a positive correlation between total phenolic and flavonoid contents and antioxidant capacity, whereas an increase in concentration led to increased antioxidant activity [48]. In the present study, it was found that there was a gradual increase in the DPPH radical scavenging activity of the extracts with an increase in the concentration of the extract. The maximum radical scavenging activities were obtained at extract

Figure 3



Cytotoxicity (IC_{50}, µg/ml) of the methanolic extracts of roots and shoots of *Balanites aegyptiaca* whose seeds were exposed to He-Ne laser of 200 mW for 4 min against different human tumor HepG2, MCF-7, and HCT116, and normal fibroblast BHK cell lines as measured with the SRB assay method. Values are given as mean±SE of three batches. One-way analysis of variance/Tukey HSD post-hoc analyses were used for IC_{50} comparison of means differences between different cell lines where F ratio=6.6210^3 and 4.0510^4 for LR and LS, respectively. However, an independent sample t test was used for IC_{50} comparison of mean differences between LR and LS on each of different cell lines with F ratio=0.470, 0.39, 1.221, and 2.018 for, HepG2, MCF-7, HCT116, and BHK, respectively. Means are ranked in descending order and the largest mean takes the first alphabetical superscripts. Means bearing different superscripts are significantly different ($P<0.05$). He-Ne, helium-neon; SRB, sulphorhodamine-B.

Table 9 Cytotoxicity (IC_{50}, µg/ml) of anticancer effect of the methanolic extracts for the shoots (S) and roots (R) dry plant material of *Balanites aegyptiaca* after helium-neon laser treatment at 200 mW for 4 min compared with control against liver cancer cell line HepG2 as measured with sulphorhodamine-B assay method

	IC_{50} (µg/ml)						
	Dox	LR	CR	LS	CS	L(R+S)	C(R+S)
HepG2	3.03±0.07	3.32±0.02	9.02±0.08[a*]	3.52±0.02	7.76±0.01[a*]	5.25±0.10[a*]	11.43±0.06[a*]

The experiment was carried out in triplicate. Values are given as mean±SE of three batches. One-way Welch analysis of variance/Games-Howell post-hoc analyses were used for IC_{50} comparison of means where F ratio=1.109×10^4. [a] is significantly different from the reference drug, doxorubicin, at significance level.[*] *P* value less than 0.001.

concentrations of 4 mg/ml for all extracts. In addition, there was a significant increase ($P<0.05$) in the DPPH radical scavenging activity of the extracts of roots and shoots of *B. aegyptiaca* whose seeds were treated with the laser before germination compared with those not treated with laser, indicating the increase in antioxidant activity after treatment with laser.

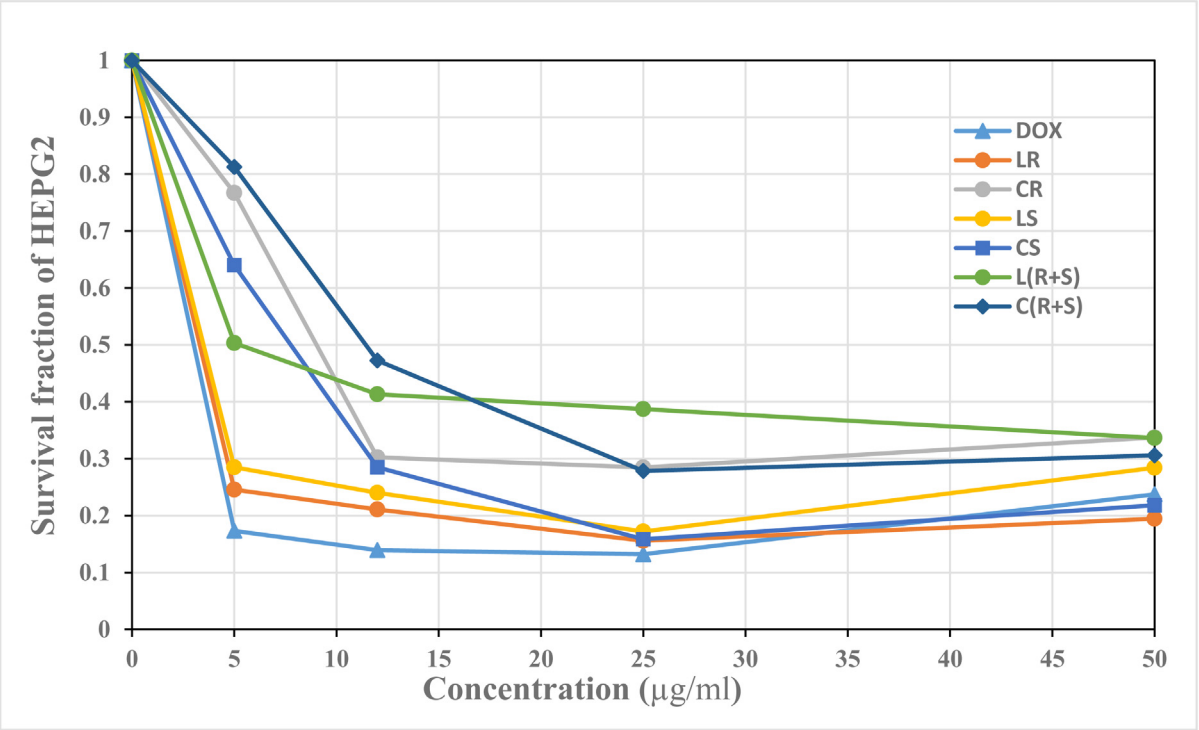
Our results showed that the methanolic extracts of 4/200 LR and 4/200 LS were the most effective scavengers of the $ABTS^+$ cation radicals among other methanolic extracts (exposed to other time intervals and other powers) with a significant increase ($P<0.05$) in scavenging activity when compared with CR and CS of seeds that were not exposed to irradiation.

The present study revealed that the methanolic extracts of 4/200 LR and 4/200 LS had the most iron-chelating activities among other methanolic extracts, especially with a significant increase ($P<0.001$) in iron-chelating activities when compared with CR and CS.

Our results showed that the methanolic extracts of 4/200 LR and 4/200 LS had showed the highest values of reducing power among other methanolic extracts with a significant increase ($P<0.05$) as compared with CR and CS.

In conclusion, laser light improved antioxidant activity through increasing levels of ferric reducing antioxidant power, iron-chelating activity, and free radical scavenging activity of DPPH and ABTS. In agreement with our results, previous studies revealed

Figure 4



The effect of different concentrations of different methanolic extracts for the shoots (S) and roots (R) dry plant material of *Balanites aegyptiaca* after He-Ne laser treatment at 200 mW for 4 min compared with control against liver cancer cell line HepG2 measured by the SRB assay. He-Ne, helium-neon; SRB, sulphorhodamine-B.

that laser treatment increased the antioxidant activity of seeds and sprouts where light energy is converted to chemical energy, which enhances the physiological and biochemical processes and increases enzymatic activities [26,28]. In addition, Khamis *et al.* [49] reported that *B. aegyptiaca* varieties contain high levels of enzymatic and nonenzymatic antioxidants such as tocopherol, ascorbate peroxidase, glutathione peroxidase, as well as glutathione-s-transferase and catalase. Other studies explained that phenolic compounds possessed antioxidant and anti-inflammatory effects and they represent the first line of defense against stressors [50,51].

Induction of helium-neon laser exposure of anticancer activity of shoots and roots of *Balanites aegyptiaca* on different cell lines

The anticancer effect of phytochemicals was mostly through regulating molecular mechanisms that are involved in cancer growth and progression such as regulation of the immune system, increasing antioxidant capacity, induction of apoptosis, inhibiting proliferation, and inactivation of carcinogen [4].

The present study revealed that both LR and LS methanolic extracts treated with 200 mW for 4 min exerted the most potent anticarcinogenic effect against HepG2 than the other human tumor cell lines (MCF-7 and HCT116). However, they exhibited no activity against the growth of the normal BHK cell line. In addition, the methanolic extracts LR and LS whose seeds were exposed to a He-Ne laser of 200 mW for 4 min had more anticarcinogenic effects than those methanolic extracts without laser exposure (CR and CS), as well as both mixtures of methanolic extracts of roots and shoots whose seeds were treated or not with laser L(R+S) and C(R+S) against HepG2 cell line, respectively. In agreement with our results, it was mentioned that phenols are a group of antioxidants with anticancer effect where plant-derived phenolic compounds have an important role in cancer prevention and treatment through induction of apoptosis, suppression of cell proliferation, and blocking of signaling pathways that trigger cancer [28,52]. A plethora of studies indicated the effect of phyto-constituents as flavonoids against cancer through increasing antioxidant activities and reducing inflammation [53–55].

Conclusion

He-Ne Laser treatment of *B. aegyptiaca* seeds improved the yield percentage of shoots and roots gradually with increase in the power of laser light. The antioxidant and

anticancer effects of *B. aegyptiaca* were increased through increasing phenolic and flavonoid contents of germinated shoots and roots. The most effective methanolic extract of *B. aegyptiaca* with the highest yield percentage, total phenolic and flavonoid contents, antioxidant capacity, and anticarcinogenic effect in vitro was that treated with 200 mW for 4 min. As the power of the laser increased, its improvement effect increased. Further preclinical investigation should be carried out on the anticancer effect of He-Ne Laser treatment of *B. aegyptiaca* seeds on chemically induced hepatocellular carcinoma in experimental animals.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med 2013; 10:210–229.

2 Abdel-Aty AM, Hamed MB, Salama WH, Ali MM, Fahmy AS, Mohamed SA. *Ficus carica, Ficus sycomorus* and *Euphorbia tirucalli* latex extracts: Phytochemical screening, antioxidant and cytotoxic properties. Biocatalysis Agri Biotechnol 2019; 20:101199.

3 Chen CY, Chen CC, Shieh TM, Hsueh C, Wang SH, Leu YL, *et al.* Corylin suppresses hepatocellular carcinoma progression via the inhibition of epithelial-mesenchymal transition, mediated by long noncoding RNA GAS5. Int J Mol Sci 2018; 19:380.

4 Choudhari AS, Mandave PC, Deshpande M, Ranjekar P, Parkash O. Phytochemicals in cancer treatment: from preclinical studies to clinical practice. Front Pharmacol 2020; 10:1614.

5 Samuel SM, Kubatka P, Büsselberg D. Treating cancers using nature's medicine: significance and challenges. Biomolecules 2021; 11:1698.

6 Hassan LEA, Dahham SS, Saghir SAM, Mohammed AMA, Eltayeb NM, Abdul Majid AMS, Abdul Majid AS. Chemotherapeutic potentials of the stem bark of *Balanites aegyptiaca* (L.) Delile: an antiangiogenic, antitumor and antioxidant agent. BMC Complement Altern Med 2016; 16:396.

7 Mahmoud AE, Fathy SA, Ali MM, Ezz MK, Mohammed AT. Antioxidant and anticancer efficacy of therapeutic bioactive compounds from fermented olive waste. Grass Y Asceites 2018; 69:e266.

8 Khamis G, Papenbrock J. Newly established drought-tolerant plants as renewable primary products as source of bioenergy. Emirates J Food Agric 2014; 26:1067–1080.

9 Al Thobaiti SA, Zeid A. Medicinal properties of desert date plants (*Balanites aegyptiaca*): an overview. Glob J Pharmacol 2018; 12:01–12.

10 Abdelaziz SM, Lemine FMM, Tfeil HO, Filali-Maltouf A. Aoms boukhary phytochemicals, antioxidant activity and ethnobotanical uses of *Balanites aegyptiaca* (L.) Del. fruits from the Arid Zone of Mauritania, Northwest Africa. Plants (Basel) 2020; 9:1–15.

11 Hwida MF, Metwally SA, Lobna ST. In vitro growth behavior and leaf anatomical structure of *Balanites aegyptiaca* and *Cotoneoster horizontalis* affected by different types of laser radiation. J Appl Sci Res 2012; 8:2386–2396.

12 Chothani DL, Vaghasiya HU. A review on *Balanites aegyptiaca* Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity. Phcog Rev 2011; 5:55–62.

13 Ezzat SM, Motaal AA, El Awdan SA. In vitro and in vivo antidiabetic potential of extracts and a furostanol saponin from *Balanites Aegyptiac*. Pharm Biol 2017; 55:1931–1936.

14 Elfeel AA, Warrag EI. Uses and conservation status of *Balanites aegyptiaca* (L.) Del. (Hegleig Tree) in Sudan: local people perspective. AJAS 2011; 3:386–390.

15 Zaahkouk SAM, Aboul-Ela EI, Ramadan MA, Bakry S, Mhany ABM. Anti carcinogenic activity of methanolic extract of *Balanites aegyptiaca* against breast, colon, and liver cancer cells. Int J Adv Res 2015; 3:255–266.

16 Khamis G, Saleh AM, Habeeb TH, Hozzein WN, Wadaan MAM, Papenbrock J, AbdElgawad H. Provenance effect on bioactive phytochemicals and nutritional and health benefits of the desert date *Balanites aegyptiaca*. J Food Biochem 2020; 44:e13229.

17 Gideon PK. Verinumber. The contribution of agroforestry tree product to rural farmers in Karim Lamido local government. JFEWR 2013; 5:1–27.

18 Yadav JP, Panghal M. *Balanites aegyptiaca* (L.) Del. (Hingot): a review of its traditional uses, phytochemistry and pharmacological properties. Int J Green Pharm 2010; 4:140–146.

19 Bashir NAO, Elhussein SAA. Variation in the levels of steroidal sapogenins within the mature fruit of *Balanites aegyptiaca* and among kernels of *Balanites* fruit accessions collected from different geographical localities in Sudan. RJPBCS 2010; 8:768–780.

20 Wakawa AI, Sambo AB, Yusuf S. Phytochemistry and proximate composition of root, stem bark, leaf and fruit of desert date, *Balanites aegyptiaca*. Int J Phytopharm 2018; 7:464–470.

21 Hosakatte N, Guggalada G, Dewir Y, Ibrahim A. Phytochemicals and biological activity of desert date (*Balanites aegyptiaca* (L.) Delile). Plants 2020; 10:1–32.

22 Taha RA, Taha LS, Metwally SA. In vitro cultures of jojoba (*Simmondsia chinensis* L.) affecting by laser irradiation. JCBPS 2012; 5:3906–3913.

23 Dziwulska A, Wilczek M, Wintal M. Effect of laser stimulation on crop yield of alfalfa and hybrid alfalfa studied in years of full land use. Acta Geophys 2006; 7:327–336.

24 Perveen R, Jamil Y, Ashraf M, Ali Q, Iqbal M, Ahmad MR. He-Ne laser-induced improvement in biochemical, physiological, growth and yield characteristics in sunflower (*Helianthus annuus* L.). Photochem Photobiol 2011; 87:1453–1463.

25 Asghar T, Jamil Y, Iqbal M, Abbas M. Laser light and magnetic field stimulation effect on biochemical, enzymes activities and chlorophyll contents in soybean seeds and seedlings during early growth stages. J Photochem Photobiol B Biol 2016; 165:283–290.

26 Khamis G, Hassan M, Morsy M, Ibrahim MA, Abd-Elsalam RM, El Badawy SH, *et al.* Innovative application of helium-neon laser: enhancing the germination of *Adansonia digitata* and evaluatingthe hepatoprotective activities in mice. Environ Sci Pollution Res 2020; 27:26520–26531.

27 Okla MK, El-Tayeb MA, Qahtan AA, Abdel-Maksoud MA, Elbadawi YB, Alaskary MK, *et al.* Laser light treatment of seeds for improving the biomass photosynthesis, chemical composition and biological activities of lemongrass sprouts. Agronomy 2021; 11:478–485.

28 Almuhayawi MS, Hassan AHA, Abdel-Mawgoud M, Khamis G, Selim S, Al Jaouni SK, AbdElgawad H. Laser light as a promising approach to improve the nutritional value, antioxidant capacity and anti-inflammatory activity of flavonoid-rich buckwheat sprouts. Food Chem 2021; 345:128788.

29 Murashige T, Skoog FA. revised medium for rapid growth and bioassays with tobacco tissue culture. Plant Physiol 1962; 15:473–497.

30 Mohamed FZ, Fahmi AA, Saad A, Mehaya FM, Badawy EA. Biochemical and phytochemical studies on *Balanities aegyptiaca* fruits. Biochem Lett 2015; 10:13–26.

31 Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M, *et al.* Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J Ethnopharmacol 2000; 72:35–42.

32 Horszwald A, Andlauer W. Characterisation of bioactive compounds in berry juices by traditional photometric and modern microplate methods. J Berry Res 2011; 1:189–199.

33 Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS, Leitao SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 2001; 15:127–130.

34 Dessalegn E, Bultosa G, Haki G, Chen F, Vasantha Rupasinghe H. Antioxidant and cytotoxicity to liver cancer HepG2 cells in-vitro of Korarima (*Aframomumcorrorima* (Braun) P.C.M. Jansen) seed extracts. Int J Food Prop 2022; 25:1–10.

35 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999; 26:1231–1237.

36 Decker EA, Welch B. Role of ferritin as a lipid oxidation catalyst in muscle food. J Agric Food Chem 1990; 38:674–677.

37 Salama BM, Helmy WA, Ragab TIM, Ali MM, Taie H, Esawy MA. Characterization of a new efficient low molecular weight Bacillus subtilis NRC16 levansucrase and its levan. J Basic Microbiol 2019; 59:1004–1015.

38 Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986; 44:307–315.

39 Gaber TM, El-Hamamsy SMA, Ahmed NS, Ali MM. Amelioration effect of Carica papaya Fruit extracts on doxorubicin – induced cardiotoxicity in rats. Inter J Vet Sci 2020; 9:349–354.

40 Omar WM, Ahmed AE, Raslan M, El-Nesr K, Ali MM, Diaa el –Deen M, El Dahshan D. Effect of lutein-rich extract on human cancer cells. Middle East J Cancer 2021; 12:147–150.

41 El-Shahat M, Salama MAM, El-Farargy AF, Ali MM, Ahmed DM. Effective pharmacophore for CDC25 phosphatass enzyme inhibitors: newly synthesized bromothiazolopyrimidine derivatives. Mini Rev Med Chem 2021; 21:118–131.

42 Magd-El-Din AA, Mousa HA, Labib AA, Hassan AS, Abd El-All AS, Ali MM, *et al.* Benzimidazole – schiff bases and their complexes: cynthesis, cnticancer activity and molecular modeling as aurora kinase inhibitor. Zeit Für Naturforschung c 2018; 73:465–478.

43 Hussien AG, Borai IH, Said MM, Mahmoud K, Ali MM. Chemotherapeutic effect of Ulmus pumila leaves methanolic extract against N-methyl-N-nitrosourea-induced mammary carcinoma in female rats: An in vitro and in vivo study. J Appl Pharmaceut Sci 2019; 9:057–068.

44 Al-Malki AL, Barbour EK, Abulnaja KO, Moselhy SS, Kumosani TA, Choudhry H. *Balanites aegyptiaca* protection against proliferation of different cancer cell line. Afr J Tradit Complement Altern Med 2016; 13:25–30.

45 Sevostyanova N, Danilovskikh M, Vinnik L, Trezorova O, Pchelina EA. The use of laser radiation to stimulate the growth of kohlrabi cabbage. IOP Conf Ser Earth Environ Sci 2021; 640:022081.

46 Chen YP, Yuea M, Wang XL. Influence of He-Ne laser irradiation on seeds thermodynamic parameters and seedlings growth of Isatis indogotica. Plant Sci 2005; 168:601–606.

47 Muszynski S, Gladyszewska B. Representation of He- Ne laser irradiation effect on radish seeds with selected germination indices. Int Agrophys 2008; 22:151–157.

48 Konan Y, Witabouna KM, Bassirou B, Kagoyire K. Antioxidant activity and total phenolic content of nine plants from Côte d'Ivoire (West Africa). J Appl Pharm Sci 2014; 4:36.

49 Khamis G, AbdElgawad H, Schaarschmidt F, Beemster GTS, Asard H, Jutta Papenbrock J. Morphological and biochemical responses of *Balanites aegyptiaca* to drought stress and recovery are provenance-dependent. J Agron Crop Sci 2019; 205:490–507.

50 Abdel-Halim AH, Fyiad AA, Aboulthana WM, El-Sammad NM, Youssef AM, Ali MM. Assessment of the anti-diabetic effect of *Bauhinia variegata* gold nano-extract against streptozotocin induced diabetes mellitus in rats. J Appl Pharm Sci 2020; 10:077–091.

51 Mahmoud AE, Abdel-Halim AH, El-Sammad NM, Ali MM. The prophylactic effect of an extract from fermented egyptian Mango (*Mangifera indica* L.) peel against cisplatin-induced cardiotoxicity and nephrotoxicity in rats. Int J Pharma Res 2021; 13:2601–2611.

52 Hassan SK, Mousa AM, El-Sammad NM, Abdel-Halim AH, Khalil WKB, Elsayed EA, *et al.* Antitumor activity of *Cuphea ignea* extract against benzo (a)pyrene-induced lung tumorigenesis in Swiss albino mice. Toxicol Rep 2019; 6:1071–1085.

53 Olejnik A, Tomczyk J, Kowalska K, Grajek W. The role of natural dietary compounds in colorectal cancer chemoprevention. Postepy Higieny Medycyny Doswiadczinej (Online) 2010; 64:175–187.

54 Tahanian E, Sanchez LA, Shiao TC, Roy R, Annabi B. Flavonoids targeting of IκB phosphorylation abrogates carcinogen-induced MMP-9 and COX-2 expression in human brain endothelial cells. Drug Design Dev Thear 2011; 5:299–309.

55 Abdel-Halim AH, Fyiad AA, Aboulthana WM, Youssef AM, Sabry NM, Khalil WKB, Ali MM. Evaluation of the therapeutic effect of nano-gold Bauhinea-variegata leaves extract against breast cancer- induced rats. Int J Pharm Res 2021; 13:2467–2477.