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Effect of Laser Light on Growth, Physiology, Accumulation of Phytochemicals, and Biological Activities of Sprouts of Three *Brassica* Cultivars

Saad M. Almuhayawi,* Mohammed S. Almuhayawi, Soad K. Al Jaouni, Samy Selim, and Abdelrahim H.A. Hassan*

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ABSTRACT: *Brassica* sprouts are known as a good source of antimicrobial bioactive compounds such as phenolics and glucosinolates (GLs). We aim at understanding how He−Ne laser light treatment (632 nm, 5 mW) improves sprout growth and physiology and stimulates the accumulation of bioactive metabolites in three *Brassica* spp., i.e., mustard, cauliflower, and turnip. Moreover, how these changes consequently promote their biological activities. Laser light improved growth, photosynthesis, and respiration, which induced the accumulation of primary and secondary metabolites. Laser light boosted the levels of pigments, phenolics, and indole and aromatic precursors of GLs, which resulted in increased total GLs and glucoraphanin contents. Moreover, laser light induced the myrosinase activity to provoke GLs hydrolysis to bioactive sulforaphane. Interestingly, laser light also reduced the anti-nutrient content and enhanced the overall biological activities of treated sprouts including antioxidant, antibacterial, anti-inflammatory, and anticancer activities. Accordingly, laser light is a promising approach for boosting the accumulation of beneficial metabolites in *Brassica* sprouts and, subsequently, their biological activities.

KEYWORDS: *Brassica sprouts, He−Ne laser light irradiation, glucosinolates metabolism, anti-nutrient antibacterial, anticancer, antioxidant, anti-inflammatory*

INTRODUCTION

The growing demand for plant-based foods has been greatly associated with their variability in providing health benefits besides their basic function as nutrient suppliers.[1,2] Among the highly nutritive plant sources, sprouts have received great attention due to their valuable content of health-promoting phytochemicals. For instance, they are rich in fatty acids, amino acids, vitamins, and antioxidants, which support their biological role as antioxidants and anticancer agents.[3] Besides, sprouts have relatively low amounts of anti-nutrient compared with their mature plants.[4]

Brassica sprouts, particularly broccoli, mustard, cauliflower, and turnip, have been known as good sources of minerals, vitamins, phenolic compounds, and glucosinolates (GLs).[5−7] In this context, GLs, especially glucoraphanin, have been reported to exist in higher concentrations in some *Brassica* sprouts, such as broccoli, than in the mature plants.[5] In addition to their health-promoting properties, like antioxidant, anti-inflammatory, and anti-carcinogenic properties, thus, they significantly reduce the risk of many diseases.[8] The health-promoting effects of *Brassica* sprouts are mainly ascribed to the presence of GLs, a special group of secondary metabolites that are released during cutting or chewing the plant tissues. As a result, GLs are enzymatically hydrolyzed by myrosinase into multiple bioactive byproducts, including isothiocyanates (ITCs), thiocyanates, and nitriles, along with epithionitriles.[9,10] Several factors have been recognized to influence the GLs hydrolysis process, i.e., the activity of myrosinase enzyme, growth factors,[11] and enzymolysis conditions including temperature, reaction time, pH, and epithiospecifier protein.[12,13] ITCs play a key role in reducing cancer risks by triggering the activity of NAD(P) H: quinoneoxidoreductase 1 (NQO1) and glutathione S-transferase (GST) enzymes.[14] Being the principal hydrolysis product of ITC, sulforaphane is produced from its precursor glucoraphanin and is considered as one of the most potent inducers of phase II detoxification enzyme quinone reductase (QR).[15] Thus, sulforaphane can reduce DNA damage by capturing the activated carcinogens.[15]

Several species of *Brassica* have been investigated for their bioactive phytochemicals and health-promoting values that support their use in traditional medicine. For instance, turnip (*Brassica rapa*) has been traditionally used for the treatment of human diseases.[16,17] Turnip is rich in GLs, especially gluconapin, which is responsible for the bitter taste, which differentiates them from other *Brassica* spp.[18] In addition, the bioactive gluconasturtin in turnip root is degraded into phenethyl isothiocyanate, which plays a vital role as an

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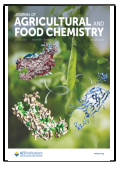

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anticancer.[19,20] Cauliflower (*Brassica oleracea* var. *botrytis*), another member of Brassicaceae, showed higher levels of sulphoraphane, indole-3-carbinol, and 2-propenyl isothiocyanates, which result from the hydrolysis of glucoraphanin, glucobrassicin, and sinigrin, respectively.[21] Another important example of Brassicaceae is black mustard (*Brassica nigra*), which has been used in traditional medicine for the curing of carcinomas, snakebite, and toothache.[22] Mustard has been previously screened for its wide array of bioactivities such as an antioxidant.[23]

Therefore, improving the phytochemical contents in *Brassica* plants and their sprouts to boost their nutritional and health-promoting effects has arisen many concerns. In this regard, several plant growth and/or bioactivity-promoting techniques have been widely applied, on the basis of the positive impacts of some environmental stressors and elicitors on the production of bioactive secondary metabolites in sprouts.[24,25] For instance, magnetic field and laser irradiation have recently been used for increasing plant nutritive value and productivity.[26] According to their uses and applications, laser light is categorized into two types, pulsed and continued lasers. The first, such as Nd:YAG and XeCl lasers, has been used in medicine, while the second one, such as He−Ne and CO_2, has been applied in agriculture for improving crop production.[27] The plant macromolecules, such as chlorophylls, are responsible for light absorption at certain wavelengths to drive photosynthesis, which in turn enhances plant growth and tissue quality.[27] For instance, the seeds of sunflower subjected to a low-power laser at 100 mJ showed increased antioxidant capacity.[28] Moreover, laser treatment-induced phenolic levels (10 mW) in seedling of baobab (*Adansonia digitata*) improved its hepato-protective activity in mice.[29] However, to our knowledge, the efficacy of laser light in boosting the nutritional values and health-promoting effects of plant sprouts has not been widely investigated. Here, we hypothesized that He−Ne laser light treatment will impact the biomass accumulation, physiology, and metabolism of *Brassica* sprouts and will consequently improve their biological activities. Thus, the objectives of the current study were to appraise the He−Ne laser light-induced effects on the level of the bioactive molecules in three species of *Brassica* sprouts: mustard, cauliflower, and turnip. Principally, GLs content, their hydrolysis products, and phenolic compounds, along with amino acids-derived GLs were determined in sprouts grown from laser light-treated seeds and control ones. In addition, the antioxidant, anti-inflammatory, anti-carcinogenic, and anti-bacterial activities of *Brassica* sprouts grown from laser-irradiated seeds were also evaluated. Thus, the novelty of this study is to mechanistically understand how He−Ne laser light treatment positively impacts the growth and metabolism of different *Brassica* sprouts.

MATERIALS AND METHODS

Experimental Setup. The seeds of mustard, cauliflower, and turnip species were soaked in distilled water for 2 h. About 100 seeds were irradiated with a helium−neon (He−Ne) laser (632 nm at a power of 5 mW for 5 min and 500 mJ energy, laser II, DMC Equipment Ltd.), and the control (100 seeds) was not irradiated. The light source of beam diameter was 1 mm, and the distance from the embryonic area side used in this experiment was 12 cm.

The treated and untreated seeds were transferred to trays filled with vermiculite and watered daily with Milli-Q water. The seeds were germinated in a growth cabinet at 25 °C air temperature, photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1}, 16 h light/8 h dark cycle, and relative humidity (60%). The sprouts at 9 days old from each tray (a biological replicate) were harvested and weighed to calculate their fresh weight. The experiment of seed irradiation with a laser and germination was repeated three times. Then, we stored frozen sprouts in liquid N at −80 °C for further biochemical analyses. Five to six biological replicates across the three experiments (each biological replicate was a pool of 10−15 plants from the same tray) were used for biochemical and biological activity measurements.

Physiological Analyses. The photosynthesis and dark respiration of treated and untreated *Brassica* sprouts were measured by utilizing an EGM-4 infrared gas analyzer (PP Systems, Hitchin, UK) and calculated as μmol CO_2 m^{-2} s^{-1}. Whole sprout photosynthetic and dark respiration were determined from the measurements of net CO_2 exchange.

Pigment Contents. The contents of chlorophyll and carotenoid were assayed according to the method reported by AbdElgawad et al.[30] About 200 mg of fresh sprout samples were homogenized in 80% acetone. After centrifugation at 14 000*g* at 4 °C for 15 min, the supernatant was read at 470, 663, and 645 nm wavelengths to measure the pigment contents.

Determination of Phenolic Compounds. The levels of phenolic compounds were extracted in HPLC grade methanol l and measured (Shimadzu HPLC system,SCL-10 AVP, Tokyo, Japan) through their separation on a column (a Lichrosorb Si-60, 7 μm, 3 mm × 150 mm) and detected by a diode array detector (SPDM10AVP).[31] Samples were eluted by water/formic acid (90:10, v/v) and acetonitrile/water/formic acid (85:10:5, v/v/v), and the flow rate was 0.8 mL/min. We used baicalein (100 μg/mL) as an internal standard, and the phenolic concentrations of compounds were determined by using a corresponding standard.

Glucosinolates Analyses. *Extraction and Determination of Glucosinolates.* Glucosinolates were measured according to Almuhayawi et al.[32] Sprouts were steamed for 2 min over boiling water to inactivate the activity of myrosinase. Then, sprouts were homogenized in MeOH/water (70:30; v/v) containing 1.5 g/L trifluoroacetic acid (TFA). The extracts were incubated under constant agitation in a thermostatic bath. This extraction lasted 30 min at 70 °C, and then, the extracts were centrifuged (8000*g* for 20 min) and the supernatants were filtered through 0.5 mm filter papers (Whatman). After evaporation at 40 °C, the dry precipitate was resuspended in 0.2 mM, pH 7.0 of HEPES−KOH.

Total glucosinolate concentrations were measured in 10 μL of the extract after mixing at 37 °C for 24 h with 0.12 U thioglucosidase (myrosinase enzyme in HEPES−KOH[33]). Eighteen millimolar Perchloric acid solution was added to stop the reaction. The product of glucosinolates hydrolysis (glucose) was measured and determined by applying the stoichiometry (1 mol of glucose is equivalent to 1 mol of total glucosinolate).

Extraction and Determination of Glucoraphanin. Fresh sprouts were steamed rapidly to inactivate myrosinase activity. Steamed sprouts were extracted from in 5 mL of methanol (70%) according to Almuhayawi et al.[32] Glucoraphanin content was countified according to Celik et al.[34] About 20 μL of the filtrate was eluted by a mixture of acetonitrile/water/formic acid (1:99:0.1v/v/v) (the flow rate was 1 mL/min) and separated on a Zorbax Eclipse SB- aq column (150 mm × 4.6 mm i.d., 5 mm), and glucoraphanin was detected using a DAD detector (235 nm).

Extraction and Determination of Sulforaphane and Sulforaphane Nitrile Content. Sulforaphane and sulforaphane nitrile were extracted from *Brassica* sprouts.[35] Sprouts were mixed with deionized water and after being autolysed at room temperature to allow for a complete conversion of glucosinolates to sulforaphane and sulforaphane nitrile (reaction mixture: myrosinase catalyzed in 0.01 M and pH 7.4, Na-phosphate buffer, at 37 °C), and after NaCl and Na_2SO_4 (1:0.75:1, w/w/w) addition, the paste was extracted in methylene chloride. Then, the filter was dried using a rotatory evaporator, and the residue was redissolved in acetonitrile (5% v/v).

We quantified the sulforaphane content according to Celik et al.,[34] after intruding it to an Oasis HLB, 3 cc cartridge. After filtration, the

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Table 1. Effect of He−Ne Laser Light Treatment on the Growth, Physiology, and Pigment Contents of Three Cultivars of *Brassica* Sprouts As Compared with Control Conditions[a]

treatment	control			laser-treated		
Brassica spp.	turnip	cauliflower	mustard	turnip	cauliflower	mustard
fresh weight (g)	2.09 ± 0.8[a]	2.091 ± 0.1a	2.45 ± 0.001[a]	3.75 ± 0.011[b]	3.44 ± 0.22[b]	3.75 ± 0.15[b]
dry weight (g)	0.139 ± 0.008[e]	0.195 ± 0.007[de]	0.285 ± 0.008[bc]	0.235 ± 0.011[cd]	0.35 ± 0.012[ab]	0.415 ± 0.005[a]
			physiology (μmol $CO_2m^{-2}S^{-1}$)			
photosynthesis	5.57 ± 0.81[a]	5.01 ± 0.7[a]	4.59 ± 0.48[a]	7.15 ± 0.74[b]	7.52 ± 1.3[b]	6.64 ± 0.07[b]
respiration	0.007 ± 0.0[a]	0.009 ± 0.0[a]	0.011 ± 0.0[a]	0.015 ± 0.001[b]	0.023 ± 0.001[b]	0.014 ± 0.001[b]
			pigments (mg/g FW)			
chlorophyll a	1.475 ± 0.776[a]	1.443 ± 0.437[a]	1.477 ± 1.128[a]	2.334 ± 0.703[b]	2.229 ± 0.484[b]	2.601 ± 0.91[b]
chlorophyll b	0.576 ± 0.102[a]	0.532 ± 0.048[a]	0.608 ± 0.153[a]	0.618 ± 0.089[a]	0.664 ± 0.052[ab]	0.711 ± 0.193[ab]
chlorophyll a+b	2.050 ± 0.850[a]	1.975 ± 0.48[a]	2.085 ± 1.226[a]	2.951 ± 0.755[b]	2.84 ± 0.454[b]	3.412 ± 0.828[b]
carotene	0.075 ± 0.001[a]	0.07 ± 0.002[a]	0.080 ± 0.0[a]	0.208 ± 0.096[b]	0.162 ± 0.059[b]	0.25 ± 0.131[b]

[a]Data are represented by mean ± standard deviations of at least three replicates. Different small letter superscripts (a, b, c, and d) within a row indicate significant differences between means at $p < 0.05$.

filtrate was separated on a Zorbax Extend-C18 column (250 mm × 4.6 mm i.d., 5 mm) and by a mixture of acetonitrile/water (30:70, v/v) at a flow rate of 0.8 mL/min. A DAD detection at 202 nm was performed to measure SF.

Extractions containing sulforaphane and sulforaphane nitrile were analyzed[32] by LC−ESI-MS/MS and using LC−MS analysis (Q-Tof Premier mass spectrometer), respectively. The separation was conducted by using an Atlantis T3 C18 column (100 mm × 2.1 mm; 3 μm) at 40 °C. The eluent was 10 mM ammonium acetate buffer (pH = 4.5) and formic acid (0.1%) in acetonitrile (0.2 mL/min). We used leucine-enkephalin as an internal reference compound.

Determination of Amino Acids Contents. Amino acids were extracted in 80% ethanol (v/v) according to Zinta et al.[36] Spouts were spiked with norvaline to estimate the loss of amino acids during extraction and centrifuged at 1400*g* for 25 min. After centrifugation for 30 min (14 000 rpm), the aqueous phase was filtered (0.2 μm Millipore microfilters). Amino acids were measured (Waters Acquity UPLC-tqd system (Milford, MA) after separation on BEH amide (2.1 mm × 50 mm column) through elution by a gradient of solvent A (0.1% formic acid in H_2O) and solvent B (0.1% FA in acetonitrile) over 10 min at 0.3 mL/min. Amino acids were quantified using a calibration curve obtained with the corresponding standards.

Myrosinase Assay. Sprouts were extracted in 0.1 M phosphate buffer (pH 6.0), which contains 5 mM benzamidine. After stirring at 4 °C for 30 min, extracts were centrifuged at 13 000 rpm and filtered through a 0.45 μm filter. After protein purification through its precipitation in saturated ammonium sulfate (70%), the precipitate was dissolved in phosphate buffer (10 mM, pH 6). The activity of myrosinase was evaluated by measuring the sinigrin hydrolysis at 227 nm.[32] The reaction buffer contained sodium phosphate (3 mM, pH 6.0), 20 μg of protein extract, and 0.24 mM sinigrin at 27 °C.

Determination of Total Antioxidant Capacity. The antioxidant capacities of the sprout extracts were found by applying the assays of DPPH (diphenylpicrylhydrazyl), FRAP (ferric reducing antioxidant power), and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).[37] The expression unit of antioxidant capacity was mg trolox/g FW or μmol trolox/g FW.

Determination of Anti-Nutrient Content. The tannin content was determined[38] by homogenizing 0.2 g of FW tissue in 2 mL of 0.1 M acetate buffer pH 5, containing 2 mg of bovine serum albumin, incubating the mixture for 15 min at room temperature and then centrifuging it (14 000*g*, 4 °C, 15 min). The pellet was dissolved in 4 mL of 1% (w/v) SDS and 5% (v/v) triethanolamine solution. One millilliter of 10 mM $FeCl_3$ in 0.01 N HCl was added, and the absorbance was determined at 510 nm. Tannic acid was used as the standard. Oxalates were measured according to Munro and Bassir;[39] the oxalic acid was extracted in ethanol and precipitated as a calcium salt. The oxalate was then dissolved in sulfuric acid, and the concentrations of oxalate in the solution were determined by titration with K_2MnO_4 for a faint pink end point. Phytate was measured spectrophotometrically as described by Onwuka.[40] The sample was extracted in 0.2 M HCl and shaken for 30 min. After boiling in a water bath for 30 min, it was cooled down in ice water for 15 min. Extracted phytate was mixed with a 2,2-biphydine solution, and the respective absorbances were measured at 520 nm in the mixture of pyridine addition. Hydrocyanic acid was measured by the alkaline picrate colorimeter method. The sample was dissolved in distilled water, and a piece of picrate paper (yellow) was suspended over the mixture, which was incubated at room temperature for 18 h. The picrate paper was diluted in 60 mL of distilled water, and the diluents were measured at 540 nm.

Biological Activities: Extraction Methods. Brassica sprouts were extracted by homogenizing 0.1 g of lyophilized sprouts in aqueous ethanol (1 mL, 80%, v/v). Extracts were centrifuged for 25 min at 4°C, and a clear supernatant was used.

Anti-Inflammatory Activity. *Lipoxygenase (LOX) Assay.* Ethanol sprouts (10 mg/mL) were extracted and measured according to Almuhayawi et al.,[32] The extract was mixed with LOX (400 U/mL) for 5 min at 25 °C in dark conditions, and then, linoleic acid was added as a substrate. The reaction was maintained for 20 min at 25 °C, and then, we added ferrous orange xylenol reagent containing 90% methanol, 100 μM xylenol orange, 30 mM H_2SO_4, and 10 μM $FeSO_4$. After incubation for 30 min at 25 °C, the absorbance was measured at 560.

Cyclooxygenase-2 Assay. The experiment was conducted according to the manufacturer's instructions of a COX assay kit (Cayman chemical company, Ann Arbor, MI). A microtiter plate was incubated for 90 min at 25 °C, and the reading was performed at 420 nm.

Inhibition of Micellar Solubility of Cholesterol (anti-cholesterolemic activity). The micellar solubility of cholesterol was measured according to Lin et al.[41] Sprout extract was added to micellar solution containing cholesterol (2 mM), sodium taurocholate (10 mM), NaCl (132 mM), oleic acid (5 mM), and sodium phosphate (15 mM, pH 7.4). The mixture was incubated at 37 °C for 24 h, and the micellar solution was then ultracentrifuged (40 000 rpm for 60 min at 20 °C). The spectrophotometric determination of cholesterol content was at 500 nm by using a cholesterol analysis kit (Pointe Scientific, C7510).The inhibition activity of the micellar solubility of cholesterol for each sample was calculated.

Antibacterial Activities. Ethanolic extracts of treated and untreated sprouts were used to measure antibacterial activities according to the disc diffusion method (bacterial suspension containing 10^6 CFU/mL of the bacterial strains). Sterilized filter paper discs containing 5 μg extract/disc were used. Ethanol was used as a negative control. These discs were placed on agar plates preinoculated with bacteria and incubated at 37 °C for 24 h. Vernier caliper was used to measure the inhibition zones.

Statistical Analyses. Statistical analyses (SPSS statistical package, SPSS Inc., Chicago, IL) were used. One-way analysis of variance

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Table 2. Effect of He−Ne Laser Light Treatment on the Aliphatic, Indole, and Aromatic Glucosinolate Precursor Contents of Three Cultivars of *Brassica* Sprouts As Compared with Control Conditions[a]

treatment	control			laser-treated		
Brassica spp.	turnip	cauliflower	mustard	turnip	cauliflower	mustard
	aliphatic glucosinolate precursors (pmol/g)					
alanine	2.5 ± 0.14^{a}	0.96 ± 0.05^{b}	1.17 ± 0.07^{b}	2.44 ± 0.14^{a}	0.86 ± 0.25^{b}	1.11 ± 0.09^{b}
leucine	14.3 ± 0.58^{b}	11.2 ± 0.45^{b}	9.38 ± 0.38^{b}	22.6 ± 0.6^{a}	21.4 ± 0.8^{a}	22.73 ± 0.92^{a}
isoleucine	2.51 ± 0.17^{b}	1.71 ± 0.1^{b}	2.26 ± 0.13^{b}	2.6 ± 0.21^{b}	2.36 ± 0.14^{b}	4.42 ± 0.17^{a}
valine	16.3 ± 0.66^{bc}	13.12 ± 0.68^{bc}	12.5 ± 0.6^{c}	21 ± 0.5^{ab}	26.9 ± 1.09^{a}	9.57 ± 1.87^{c}
methionine	11.5 ± 0.34^{bc}	14.53 ± 0.57^{b}	7.66 ± 0.56^{c}	20 ± 0.83^{a}	22.9 ± 0.48^{a}	13.9 ± 0.91^{b}
	indole and aromatic glucosinolate precursors (pmol/g)					
phenylalanine	8.57 ± 0.84^{b}	12.01 ± 0.81^{ab}	8.59 ± 0.48^{b}	13.1 ± 0.7^{ab}	20.52 ± 2.3^{a}	17.6 ± 0.57^{ab}
tyrosine	9.69 ± 0.39^{c}	10.14 ± 0.41^{c}	8.29 ± 0.34^{c}	14.2 ± 0.7^{bc}	25.05 ± 1.9^{a}	18.78 ± 1.14^{ab}
tryptophan	7.38 ± 0.4^{c}	6.36 ± 0.3^{c}	6.75 ± 0.02^{c}	12.4 ± 0.6^{ab}	8.61 ± 0.33^{bc}	14.75 ± 0.8^{a}

[a]Data are represented by mean ± standard deviations of at least three replicates. Different small letter superscripts (a, b, c, and d) within a row indicate significant differences between means at $p < 0.05$.

(ANOVA) was used, and Tukey's test ($P \leq 0.05$) was carried out. Principle component analysis (PCA) analysis was performed (XLSTAT software, 2011). Each experiment was replicated at least three times.

3. Results and Discussion. *Increased Photosynthesis and Respiration by Enhancing the Growth of Brassica Sprouts.* Laser light has been proven to enhance the growth and nutritive values of plants.[42] When applied at a certain dose, laser light can successfully increase the internal energy of the seeds by converting light energy into chemical energy,[27] which subsequently could be utilized to activate the physiological processes of plants like germination, photosynthesis, and respiration.[43] Much of the produced energy could be then used for the stimulation of plant growth. In this context, several reports have ascribed the positive influence of laser irradiation on seed germination and plant growth to the exerted electromagnetic field and energy, which make laser light act as an engine that could accelerate cell metabolism, improve growth, and eventually increase the plant yields.[27,44] Supporting such a hypothesis, the present study reported the increased growth of sprouts from laser light-treated seeds *Brassica* sprouts, where the laser-treated sprouts showed faster growth and hence accumulated more biomass than the untreated sprouts (Table 1). The mustard species displayed the highest increase in biomass, followed by the cauliflower species, while turnip presented the lowest biomass, in comparison to untreated He−Ne laser sprouts. Similar to our observations, the laser-treated seeds of some plants, such as *Isatis indogotica*[27] and sunflower[28] had improved growth compared to untreated ones. These laser-provoked effects on plant growth have been also reflected on several growth factors such as indole acetic acid, amylase, and protease enzymes, which play a key role in plant growth.[27,44,45] Consequently, the observed induction in plant growth after laser light treatment in the current study could be partially attributed to the increased chlorophyll contents and consequently the increased net photosynthetic activity after laser light application. Additionally, respiration improvement has been observed, as indicated from the increased respiratory rate by about 35% in the He−Ne laser light-treated group in comparison to that of the control group (Table 1). In this regard, significant increases in photosynthetic activities have been reported in sunflower exposed to laser irradiation.[28] This increase in the respiratory rate may be attributed to the enhancing effect of the laser on the metabolically important enzymes that play a central role in respiration.[43] Accordingly, the elevated photosynthetic and respiration rates in response to laser treatment is speculated to enhance more accumulation of bioactive primary and secondary metabolites.[46]

He−Ne Laser light Treatment Raised Amino Acid-Derived GLs in Brassica Sprouts. The amino acid content in plants is a further reflection of their nutritional and medicinal significances.[47] In addition to their nutritive value, free amino acids could act as precursors for other bioactive secondary metabolites such as GLs.[32] Therefore, enriching the amino acid contents in *Brassica* sprouts by using He−Ne laser light is probable to boost their nutraceutical and pharmaceutical properties.

The current investigation of the amino acid profile in the He−Ne laser light-induced *Brassica* sprouts led to identifying five aliphatic GLs precursors (e.g., alanine, leucine, isoleucine, valine, and methionine) and three indole and aromatic GLs precursors (e.g., phenylalanine, tyrosine, and tryptophan) (Table 2). Obviously, the majority of the amino acids in almost all the studied species were significantly increased under the effect of He−Ne laser light, except for isoleucine and valine in turnip and mustard, respectively. There was no significant increase in alanine content in the three species. Most importantly, essential amino acids were significantly improved in all species, whereby the highest concentrations were reached for leucine and methionine, followed by tryptophan. Considerable amounts of alanine, threonine, glutamic acid, glutamine, and valine were previously found in the turnip species.[48] It has also been previously reported that other *Brassica* sprouts, such as broccoli sprouts, contain higher levels of essential amino acids in comparison to broccoli seeds.[49] On exposure to physical stress factors such as laser irradiation, plants tend to accumulate metabolites with a low molecular weight, such as amino acids, as a kind of adaptive responses.[50] More interesting, laser light might have an indirect effect on amino acid accumulation, through affecting protease enzyme that converts protein into amino acids, the enzyme that plays a beneficial role in seed germination.[43,51] The high levels observed generally in methionine and tryptophane were positively correlated with the increments in GLs content reported herein, since tryptophan and methionine serve as precursors for indolic and alphatic GLs.[32,52]

Laser Light Provoked GLs Production and Hydrolysis in Brassica Sprouts. Myrosinase (thioglucosidase, EC 3.2.1.147) is the enzyme responsible for the hydrolysis of GLs to volatile products, mainly nitriles and isothiocyanates. Light conditions have been shown to manipulate the GLs content and myrosinase activity in some *Brassica* sprouts.[53] Accordingly, being a special kind of light, the laser is expected to induce pronounced effects on GLs content, like other physical methods such as red and blue LED light treatment.[54−56]

These results clearly revealed that the total GLs levels were much more enhanced in all the three species that received He−Ne laser light treatment versus the control sprouts (Figure 1). Concomitantly, the dominant GLs (glucoraphanin) and its hydrolyzed product sulforaphane were further increased in all He−Ne laser-treated sprouts, where the cauliflower and mustard species showed the maximum levels of sulforaphane and glucoraphanin, respectively. It has been revealed that aliphatic GLs (e.g., glucoraphanin) are the predominant GLs in broccoli sprouts rather than indole GLs, which are mainly found in mature plants.[5,57]

Our laser light treatments triggered increases in the activity of myrosinase in all the studied species, as the enzyme that is responsible for the hydrolysis of GLs into (ITC) products including inactive GLs (sulforaphane nitrile) and active GLs (sulforaphane).[13] Improved

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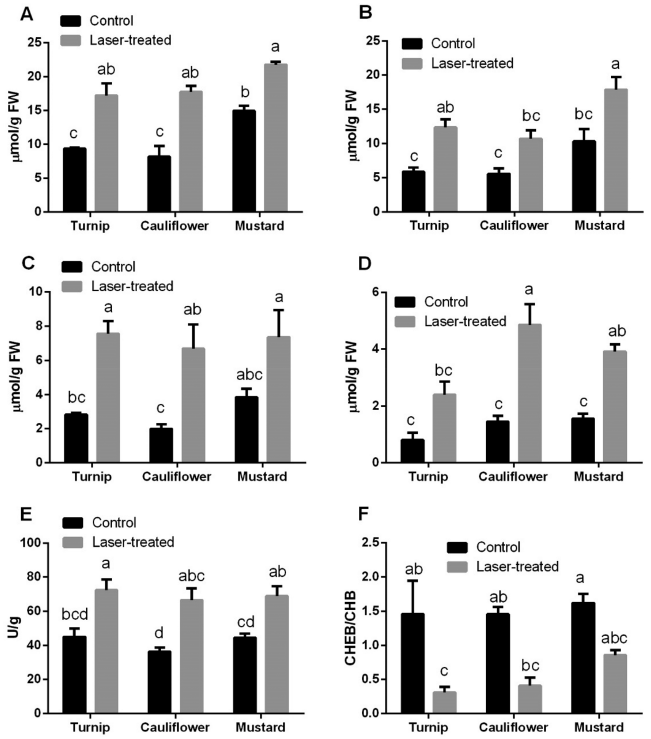

Figure 1. Effect of He−Ne laser light treatment on the levels of (A) total glucosinolates, (B) glucoraphanin, (C) sulforaphane nitrile, (D) sulforaphane, (E) myrosinase, and (F) epithiospecifier protein activity of three cultivars of *Brassica* sprouts as compared with the control conditions. Data are represented by mean ± standard deviations of at least three replicates. Different small letters (a, b, c, d) above the bars indicate significant differences between means at $p < 0.05$.

myrosinase enzyme activity paralleled the higher levels of SF and glucoraphanin, which normally indicates higher rates of GLs degradation. In agreement with our results, SF was recognized to be the most prominent ITC, and also, glucoraphanin has been shown to account for the majority of the total GLs, described as the major compound in some *Brassica* sprouts such as broccoli.[53,58]

On the contrary, in the presence of epithiospecifier protein, glucoraphanin is most likely to be converted into sulforaphane nitrile instead of sulforaphane;[15] thus, the lower the activity of epithiospecifier protein, the higher the production of the health-promoting phytochemical sulforaphane.[59] Moreover, *Brassica* cultivars with a reduced level of epithiospecifier protein revealed their enhanced possibility as an anticancer food item.[59] According to this study, there was a decrease in epithiospecifier protein activity in all laser-exposed sprouts compared with that of untreated sprouts. However, a different situation was reported here, whereby the levels of sulforaphane nitrile were notably increased in all species examined under laser light treatment.

Overall, the laser-treated *Brassica* sprouts presented elevated levels of GLs accompanied by higher myrosinase activity and hydrolyzed SF, along with decreased epithiospecifier protein activity. To keep the balance between GLs biosynthesis and hydrolysis, it is necessary to optimize the sprouting and hydrolysis conditions under biophysical treatments such as laser light, which might compensate for high GLs losses caused by high myrosinase activity, hence improving the nutritional values of the sprouts of *Brassica* cultivars.

Laser Light Reduced the Anti-Nutrient Contents in Brassica Sprouts. To test the effect of laser light on the anti-nutrient contents of target *Brassica* spp., we measured tannins, oxalate, phytate, and hydrogen cyanide (Table 3). Tannins in food impose an astringent taste and affect food palatability. Tannins are commonly found in metabolites that possess anti-nutritional and anti-feed properties.[60] They have been reported to possess anti-nutrient effects by forming complexes with essential nutrients including enzymes of the digestive tract, thereby suppressing the availability and utilization of essential nutrients.[61] Similarly, phytate and oxalate are anti-nutritional factors, which are present in various fruits and vegetables, with high concentrations discovered to cause great effects on mineral bioavailability in foods.[62] Interestingly, treatment with laser light significantly ($p < 0.05$) reduced the four measured anti-nutrient compounds in mustard cultivar. Besides, it significantly ($p < 0.05$) lowered cyanide and oxalate in cauliflower cultivar. Additionally, laser treatment significantly diminished ($p < 0.05$) phytate, cyanide, and oxalate in turnip cultivar. Overall, the levels of anti-nutrients were decreased by laser light treatment, and their concentrations were lower than the lethal dose, which hence may not elicit a toxic effect when consumed.[63]

Laser Light Enhanced the Phenolic Profile of Brassica Sprouts. In addition to their richness in GLs, *Brassica* sprouts have been shown to be abundant sources of phenolic compounds, which are well-characterized as important contributing factors to the total antioxidant capacity, besides their significant role in the chemo-preventive effects of *Brassica* sprouts.[64,65]

Several phenolic compounds were previously detected in the turnip species, particularly kaempferol, isorhamnetin, quercetin, and their glycosides, along with sinapic acid, which was identified in large quantities.[66,67] Cauliflower is also known to be rich in many phenolics such as ferulic, gallic acids, chlorogenic, and catechin.[68−71] Meanwhile, the most abundant phenolic compounds reported in black mustard were gallic acid, quercetin, ferulic acid, caffeic acid, and rutin.[72]

The present findings showed that galic acid was the most abundant phenolic, while rutin is the dominant flavonoid in the three species, treated or untreated with laser light (Table 4). Apparently, the laser light treatment caused significantly increased contents of most phenolic acids and flavonoids quantified in all species. The laser-treated turnip species exhibited a greater diversity in the phenolic profile, containing almost all the detected phenolic compounds with the exception of resorcinol, in comparison to mustard and cauliflower. Interestingly, laser light also promoted the induction of some phenolic compounds, like luteolin, genistein, ferulic acid, and chlorogenic acid, which were absent in the untreated sprouts. These compounds might be developed in response to laser irradiation as a stress factor. On the contrary, laser light did not have such an effect on other undetected phenolic compounds such as resorcinol, velutin, daidzein, fisetin, and *O*-hydroxydaidzein. At the same time, the latter was observed to disappear under the effect of the laser.

Table 3. Anti-Nutrient Contents (µg/g FW) of Control and Laser-Treated Sprouts of Three Cultivars of *Brassica*[a]

	control			laser-treated		
anti-nutrients	turnip	cauliflower	mustard	turnip	cauliflower	mustard
tannins	20.1 ± 1.5^a	19.3 ± 1.2^a	23.3 ± 2.4^a	19.3 ± 1.9^a	18.8 ± 1.9^a	19.4 ± 3.2^b
phytates	172.4 ± 5.1^a	151.4 ± 4.7^a	126.8 ± 11.2^a	134.5 ± 7^b	144.1 ± 1.1^a	91.8 ± 9^b
cyanides	62.1 ± 0.5^a	70.8 ± 4.1^a	55.4 ± 2.7^a	47.4 ± 3.2^b	55.1 ± 2.4^b	41.1 ± 2.5^b
oxalates	115.9 ± 5.6^a	100.2 ± 2.7^a	131.5 ± 1.5^a	96.2 ± 8.8^b	77.5 ± 7.1^b	111.6 ± 5.1^b

[a]Data are represented by the mean of cytotoxic activity (%) ± standard deviations. Different superscript letters (a, b, and c) within a row indicate significant differences between the control and laser treatment of each cultivar at $p < 0.05$.

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J. Agric. Food Chem. 2021, 69, 6240−6250

Table 4. Effect of He−Ne Laser Light Treatment on the Phenolic Profile and Levels of Total Phenols and Total Flavonoids of Three Cultivars of *Brassica* Sprouts As Compared with Control Conditions[a]

treatment	control			laser-treated		
Brassica spp.	turnip	cauliflower	mustard	turnip	cauliflower	mustard
caffeic acid	1.86 ± 0.01^{a}	1.054 ± 0.013^{a}	1.73 ± 0.01^{a}	1.137 ± 0.016^{a}	1.86 ± 0.021^{a}	1.1 ± 0.02^{a}
ferulic acid	0.097 ± 0.011^{a}	nd	0.083 ± 0.01^{a}	0.155 ± 0.018^{a}	0.065 ± 0.03^{a}	0.13 ± 0.02^{a}
protocatechuic acid	0.22 ± 0.003^{a}	0.14 ± 0.003^{a}	0.19 ± 0.003^{a}	0.35 ± 0.004^{a}	0.22 ± 0.005^{a}	0.31 ± 0.00^{a}
catechin	0.131 ± 0.015^{a}	0.063 ± 0.015^{a}	0.086 ± 0.012^{a}	0.208 ± 0.024^{a}	0.102 ± 0.02^{a}	0.14 ± 0.01^{a}
galic acid	2.869 ± 0.101^{a}	2.54 ± 0.132^{a}	2.74 ± 0.102^{a}	3.39 ± 0.161^{a}	2.859 ± 0.21^{a}	3.1 ± 0.16^{a}
p-coumaric acid	0.9 ± 0.01^{a}	0.56 ± 0.014^{a}	0.77 ± 0.011^{a}	0.144 ± 0.017^{a}	0.87 ± 0.021^{a}	1.12 ± 0.02^{a}
resorcinol	ND	0.0005 ± 0.0^{ab}	0.0007 ± 0.0^{ab}	ND	0.001 ± 0.0^{ab}	0.001 ± 0.0^{a}
chlorogenic acid	0.006 ± 0.001^{ab}	0.003 ± 0.001^{ab}	ND	0.009 ± 0.001^{a}	0.005 ± 0.00^{ab}	0.006 ± 0.0^{ab}
sinapic acid	ND	0.032 ± 0.01^{ab}	0.044 ± 0.01^{ab}	0.018 ± 0.001^{ab}	0.05 ± 0.01^{ab}	0.07 ± 0.01^{a}
quercetin	0.29 ± 0.003^{a}	0.18 ± 0.004^{a}	0.25 ± 0.003^{a}	0.72 ± 0.008^{a}	0.45 ± 0.01^{a}	0.67 ± 0.01^{a}
quercetrin	0.003 ± 0.0^{a}	0.002 ± 0.0^{a}	0.003 ± 0.0^{a}	0.005 ± 0.001^{a}	0.005 ± 0.0^{a}	0.004 ± 0.0^{a}
luteolin	0.004 ± 0.001^{ab}	ND	0.004 ± 0.0^{ab}	0.01 ± 0.001^{a}	0.006 ± 0.^{ab}	0.009 ± 0.0^{a}
apigenin	0.49 ± 0.006^{a}	0.31 ± 0.008^{a}	0.42 ± 0.006^{a}	1.12 ± 0.014^{a}	0.7 ± 0.017^{a}	1.1 ± 0.015^{a}
isoquercetrin	0.005 ± 0.001^{a}	0.003 ± 0.001^{a}	0.004 ± 0.0^{a}	0.01 ± 0.001^{a}	0.007 ± 0.0^{a}	0.01 ± 0.0^{a}
rutin	0.333 ± 0.039^{a}	0.207 ± 0.051^{a}	0.283 ± 0.04^{a}	0.868 ± 0.101^{a}	0.49 ± 0.1^{a}	0.7 ± 0.1^{a}
ellagic acid	0.18 ± 0.001^{b}	0.15 ± 0.001^{b}	0.016 ± 0.0^{b}	0.21 ± 0.002^{a}	0.11 ± 0.0^{ab}	0.18 ± 0.0^{ab}
velutin	0.009 ± 0.001^{b}	ND	ND	0.022 ± 0.002^{a}	ND	ND
naringenin	0.004 ± 0.0^{a}	0.002 ± 0.001^{a}	0.003 ± 0.0^{a}	0.009 ± 0.001^{a}	0.006 ± 0.0^{a}	0.007 ± 0.0^{a}
genistein	ND	0.03 ± 0.007^{ab}	0.04 ± 0.005^{ab}	0.084 ± 0.011^{a}	0.07 ± 0.02^{ab}	0.08 ± 0.01^{a}
didzein	0.02 ± 0.0^{bc}	ND	0.02 ± 0.0^{bc}	0.05 ± 0.001^{ab}	ND	0.04 ± 0.0^{a}
fisetin	0.001 ± 0.0^{b}	ND	ND	0.003 ± 0.0^{a}	ND	ND
O-hydroxydaidzein	0.0010 ± 0.0^{a}	ND	ND	0.003 ± 0.0^{a}	ND	ND
total phenols	5.824 ± 0.09^{bcd}	3.959 ± 0.11d	5.43 ± 0.08^{cd}	9.26 ± 0.03^{ab}	7.67 ± 0.2^{abc}	9.55 ± 0.42^{a}
total flavonoids	1.60 ± 0.104^{b}	2.047 ± 0.28^{b}	1.911 ± 0.49^{b}	2.19 ± 0.16^{b}	3.32 ± 0.2^{ab}	4.02 ± 0.8^{a}

[a]Data are represented by mean ± standard deviations of at least three replicates. Different small letter superscripts (a, b, c, and d) within a row indicate significant differences between means at $p < 0.05$.

In line with our findings, previous reports have clearly indicated that laser light caused significant rises in the total phenolic content of soybean[43] and sunflower.[28] Likewise, the phenolic compounds of broccoli sprouts have been shown to increase under light conditions.[73] Such light-induced increment might be due to the ability of light to promote photosynthesis and the malonyl-CoA pathway, which linked with the biosynthesis of phenolic compounds in sprouts.[74] Moreover, laser light increased photosynthetic activity, which would result in more synthesis of carbohydrates, which serve as precursors for various classes of carbon-based secondary metabolites like polyphenols.[75]

Induced GLs and Phenolic Levels in Laser Light-Treated Brassica Sprouts Improved Their Antioxidant and Anticancer Activities. Due to their higher levels of GLs and phenolic compounds, *Brassica* sprouts exhibited high antioxidant properties.[76−78] In the current study, antioxidant activities as indicated by the FRAP and DPPH assay of laser-treated *Brassica* sprouts were markedly enhanced as relative compared to their corresponding controls, where the mustard species showed a higher antioxidant capacity than cauliflower and turnip (Figure 2A−C). The significant antioxidant activities of some *Brassica* spp., such as broccoli sprouts, support our results.[32] In this regard, plant extracts possessed a high total antioxidant capacity, which was positively correlated with the elevation in phenolic compounds content.[79] Moreover, it was found that turnip sprouts displayed a higher DPPH free radical scavenging activity in comparison to cauliflower and mustard sprouts.[78] Such activities have been mainly ascribed to the presence of glucobrassicin, besides glucoiberin and gluconapin. Some mustard varieties have also reportedly shown elevated antioxidant activities.[72]

The laser enhanced effects on the antioxidant activities of *Brassica* sprouts have been confirmed in this study, which could be explained by high levels of phenolic compounds. On the contreary, laser light has been proven to enhance enzymatic antioxidants,[43] which are basically in the front line of the plant antioxidative defense system to limit the reactive oxygen species (ROS) harmful effects.[31]

Brassica spp. have been used to prohibit the risk of numerous types of tumors, in relation to their higher contents of bioactive phytochemicals.[7] According to our results, it is clear that laser light treatment decreased the levels of the detoxification enzymes, which are considered as anticancer indicators, i.e., glutathione S-transferase (GST) and quinone reductase (QR),[14,32] in all *Brassica* sprouts, where the mustard species displayed the maximum levels of both anticancer indicators (Figure 2D,E). Additionally, we confirmed such enhancement in the anticancer activities of laser-treated *Brassica* sprouts through *in vitro* testing their cytotoxic activities (%) against four different human tumor cell lines, namely, hepatocellular carcinoma (HepG2), colon carcinoma (Colo205), embryonic kidney adenocarcinoma (Emb293), and urinary bladder carcinoma (T24P) as outlined in Table 5. We found significant increases ($p < 0.05$) in the anticancer cytotoxic activities of the three cultivars of *Brassica* sprouts by laser treatment against Colo205, Emb293, and T24P cell lines. However, against HepG2 cell lines, the elevation in the cytotoxic activities was found significant only in the mustard cultivar, while others showed slight improvements (Table 5). The anti-carcinogenic activity of some *Brassica* sprouts, such as broccoli, has been attributed to the presence of ITC, which were reported in higher concentrations in our study. ITC has been assumed to promote the activity of QR and GST, which consequently reduces the threat of urinary bladder cancer in humans.[14] Besides, sulforaphane is supposed to play a role in the chemo-protective effects against several types of cancer cells through two dual actions: the first is inhibiting phase I enzymes, which are accountable for the conversion of procarcinogens to carcinogens, and the second promotes the up-regulation of phase II detoxification enzyme (QR).[80]

Similar to our results, turnip extracts have been reported to exhibit hepatoprotective, nephroprotective, and anticancer activities.[81−83] Regarding the mustard species, it has been found that mustard

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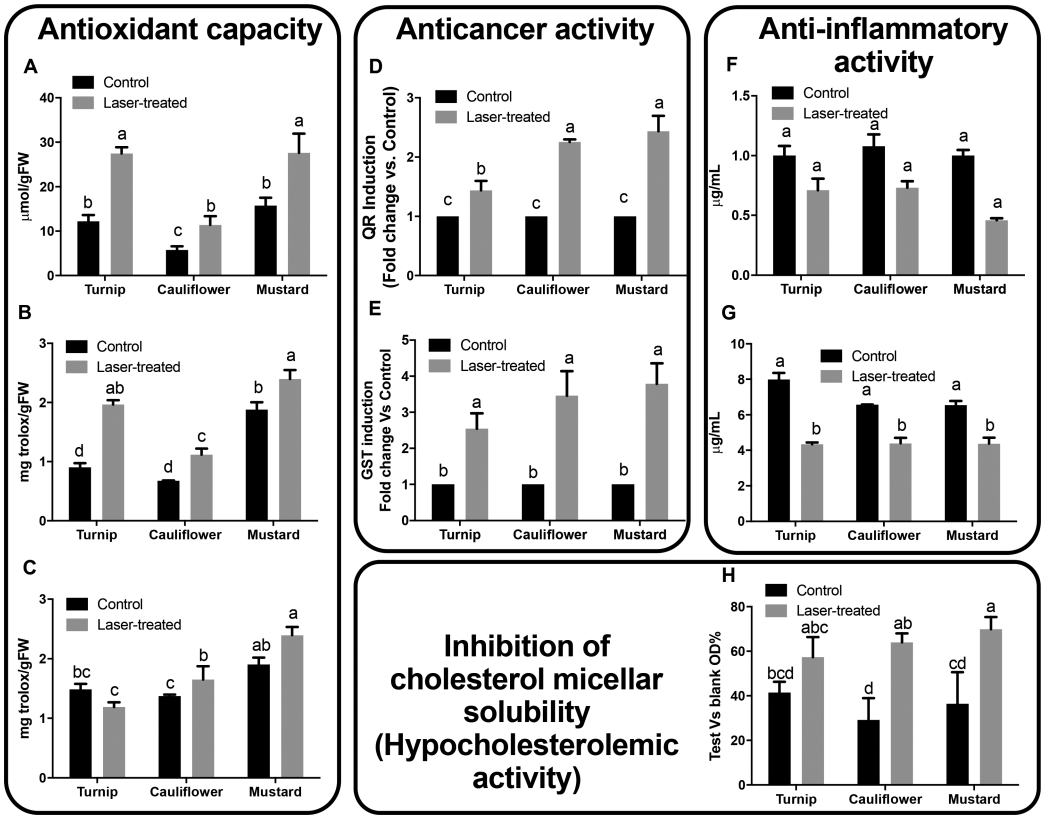

Figure 2. Effect of laser light treatment on the antioxidant capacity in terms of (A) FRAP, (B) DPPH, and (C) ABTS, the anticancer activity represented by (D) quinone reductase induction and (E) glutathione-S-transferase, the anti-inflammatory activity in terms of (F) cyclooxygenase-2 and (G) lipoxygenase, and the hypocholesterolemic properties represented by (H) the inhibition of cholesterol micellar solubility of three cultivars of *Brassica* sprouts as compared with control conditions. Data are represented by mean ± standard deviations of at least three replicates. Different small letters (a, b, c, and d) above the bars indicate significant differences between means at $p < 0.05$.

Table 5. Anticancer Activity of Control and He−Ne Laser-Treated Sprouts of Three Cultivars of *Brassica* against Human Cancer Cell Lines Including Hepatocellular Carcinoma (HepG2), Colon Carcinoma (Colo205), Embryonic Kidney Adenocarcinoma (Emb293), and Urinary Bladder Carcinoma (T24P)[a]

	control			laser-treated		
human tumor cell lines	turnip	cauliflower	mustard	turnip	cauliflower	mustard
HepG2	44.6 ± 2^{a}	47.3 ± 3^{a}	51.3 ± 1.4^{a}	49.3 ± 1.9^{a}	55.8 ± 1.9^{a}	69.8 ± 2.1^{b}
Colo205	57.8 ± 7.1^{a}	53.6 ± 3.7^{a}	46.2 ± 3.1^{a}	64.5 ± 7^{b}	74.7 ± 1.1^{b}	66.8 ± 8^{b}
Emb293	42.4 ± 0.3^{a}	60.8 ± 8.1^{a}	65.4 ± 4.4^{a}	67.4 ± 1.7^{b}	75.1 ± 6.7^{b}	71.4 ± 2.5^{b}
T24P	55.9 ± 0.6^{a}	50.2 ± 2.7^{a}	49.5 ± 4.5^{a}	86.2 ± 5.2^{b}	76.5 ± 3.5^{b}	82.6 ± 1.5^{b}

[a]Data are represented by the means of cytotoxic activity (%) ± standard deviations. Different superscript letters (a, b, c) within a row indicate significant differences between means at $p < 0.05$.

extracts displayed antiproliferative effects against several types of cancer such as hepatocellular (HepG2), cervical (HeLa), colorectal (HCT), and breast (MCF-7) carcinoma.[84] As a comparison, it has been indicated that turnip sprouts presented a higher antiproliferative effect than those of cauliflower and mustard sprouts.[78] The various anticancer properties of *Brassica* spp. could be also ascribed to the existence of phenolics, which has been reported to have a role in the inhibition of tumor growth.[83] In addition to phenolics, the presence of the glucosinolate sinigrin was reported to take part in the inhibition of hepatocarcinogenesis.[85] Moreover, mustard extracts rich in allyl isothiocyanate were reported to possess cytotoxic effects against bladder cancer cells.[86,87] The presence of allyl isothiocyanate, together with phenyl isothiocyanate, in mustard extracts has been found to exhibit anti-inflammatory effects by inhibiting some inflammation-promoting cytokines such as interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α).[88]

Overall, laser light treatment induced the antioxidant activity and the anticancer activity as indicated by increased QR and GST enzymes activities in all the studied *Brassica* sprouts. This was a consequence of the increased levels of GLs and phenolic compounds laser light-treated brassica sprouts.

Laser Light Treatment Improved the Anti-Inflammatory Efficacy of Brassica Sprouts. Numerous studies have investigated the anti-inflammatory properties of *Brassica* sprouts. COX-2 and LOX are well-known as inflammatory markers. Given this, COX-2 was associated with the inflammatory tissues as an inducible isoform.[89] Also, many skin inflammations and other inflammatory conditions were associated with LOX.[90] Herein, we assessed the anti-inflammatory activity of untreated and laser light-treated *Brassica* sprouts through LOX and COX-2 assays to investigate the effect of laser treatment on this property (Figure 2F,G). The COX-2 marker was inhibited in the three cultivars of *Brassica* sprouts as a result of laser light treatment; however, the reduction was not significant in any of them. On the contrary, the LOX marker was significantly reduced

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Table 6. Effect of Laser Light Treatment on the Antibacterial Properties of Three Cultivars of *Brassica* Sprouts As Compared with the Control Conditions[a]

	control sprouts			laser-treated sprouts		
bacterial spp.	turnip	cauliflower	mustard	turnip	cauliflower	mustard
Staphylococcus aureus	10.1 ± 0.7^{b}	12.8 ± 1.0^{b}	11.5 ± 2.0^{b}	21.7 ± 2.6^{a}	24.0 ± 4.6^{a}	25.7 ± 0.8^{a}
Escherichia coli	19.4 ± 0.5^{b}	20.9 ± 3.0^{ab}	17.4 ± 2.8^{b}	22.6 ± 1.0^{ab}	19.9 ± 1.9^{b}	28.2 ± 3.4^{a}
Bacillus cereus	11.1 ± 0.9^{b}	12.1 ± 0.6^{a}	11.4 ± 1.1^{a}	17.2 ± 2.6^{b}	21.0 ± 1.3^{c}	15.3 ± 0.9^{b}
Listeria monocytogenes	7.3 ± 1.0^{a}	8.3 ± 0.8^{a}	12.0 ± 0.5^{b}	13.0 ± 0.7^{b}	11.3 ± 0.3^{b}	16.4 ± 0.6^{c}
Salmonella spp.	12.3 ± 1.0^{a}	11.3 ± 0.7^{a}	14.0 ± 1.2^{ab}	13.4 ± 1.5^{a}	17.8 ± 1.3^{c}	15.4 ± 0.8^{b}
Pseudomonas aeruginosa	14 ± 1.5^{b}	16.5 ± 1.3^{ab}	13.1 ± 1.4^{b}	20.2 ± 2.7^{ab}	19.8 ± 2.4^{ab}	22.9 ± 3.0^{a}
Bacillus subtilis	13.8 ± 1.8^{b}	16.9 ± 1.4^{b}	15.2 ± 2.6^{b}	16.5 ± 3.5^{b}	18.5 ± 2.7^{ab}	26.6 ± 4.1^{a}
Sarcina lutea	15.3 ± 2.0^{b}	22.3 ± 1.8^{ab}	20.0 ± 3.5^{ab}	27.0 ± 1.8^{a}	24.8 ± 4.3^{ab}	27.4 ± 3.6^{a}

[a]Data are represented by the means of inhibition zone diameters (mm) ± standard deviations of at least three replicates. Different small letter superscripts (a, b, c, and d) within a row indicate significant differences between the control and laser treatment of each cultivar at $p < 0.05$.

in all laser light-treated cultivars ($P < 0.05$). That improvement in the anti-inflammatory properties of *Brassica* sprouts as a result of laser light treatment could be attributed to the enhancement in the total antioxidant capacity due to the increased accumulation of glucosinolates, phenols, flavonoids, and pigments.[32]

Laser Light Treatment Enhanced the Hypocholesterolemic Effect of Brassica Sprouts. The efficacy of laser light on the hypocholesterolemic properties of *Brassica* sprouts was evaluated by determining the inhibition in the micellar solubility of cholesterol caused by extracts of *Brassica* sprouts grown from laser light-treated seeds versus the control ones (Figure 2H). The rates of inhibition of the micellar solubility of cholesterol by turnip, cauliflower, and mustard cultivars extracts under control conditions were 41.48, 29.26, and 36.44%, respectively, whereas the exposure to laser light significantly increased ($p < 0.05$) the rates of inhibitions in all cultivars to be 57.39, 63.99, and 69.80%, respectively, with no significant differences among treated cultivars. The decline was in the micellar solubility of cholesterol aids in hindering the absorption of cholesterol by the small intestine,[91] which helps in dropping the cholesterol level in blood. Incidentally, phenolics, saponins, and alkaloids of plants were found to decrease the micellar solubility of cholesterol.[92] Consequently, the improvement in the levels of phenolics, flavonoids, and other antioxidant metabolites due to laser treatment could explain the enhanced reduction of the micellar solubility of cholesterol.[93]

Laser Light-Treated *Brassica* Sprouts Showed Enhanced Antibacterial Activities. The antibacterial potentials of *Brassica* sprouts were evaluated under both control and laser light treatment conditions in terms of the diameters of inhibition zones of bacterial growth using the disc diffusion method (Table 6). We evaluated the antibacterial activities of *Brassica* sprout extracts against foodborne bacterial pathogens included *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* spp. The laser treatment significantly ($p < 0.05$) enhanced the antibacterial properties of all examined cultivar sprouts against *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*. Additionally, it significantly ($p < 0.05$) improved the antibacterial activity of mustard cultivar sprouts against *E. coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. Furthermore, laser light raised the antimicrobial properties of cauliflower cultivar sprouts and turnip cultivar sprouts against *Salmonella* spp. and *Sarcina lutea*, respectively. The enhancement in the antibacterial properties of *Brassica* spouts could be ascribed to the high levels of bioactive metabolites. These increases positively affected all the biological activities of *Brassica* sprouts including, as well, the antioxidant, anti-inflammatory, and hypocholestrolemic activities.[94]

Cultivar-Specific Responses to Laser Light Treatment. To summarize the differences between the three cultivars in response to laser light treatment, principal component analysis (PCA) was conducted for all obtained results (Figure 3). The PCA clearly indicated that there were clear sprout cultivar-specific responses to laser light treatment. The first two components (PC1 and PC2) altogether show 94% of the variability. A clear separation according to laser light treatment was recorded across PC1. PC1 was high in

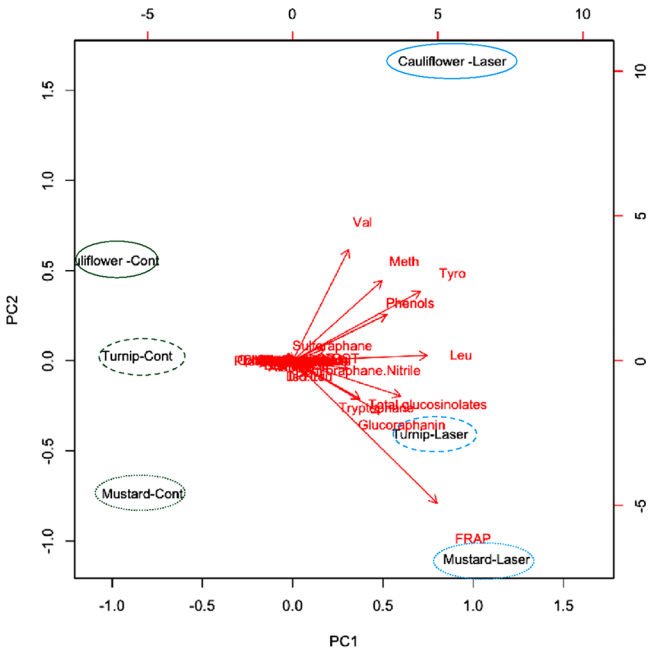

Figure 3. Principle component analysis of all measured physiological and biochemical parameters of the three cultivars of *Brassica* sprouts either untreated or treated with laser light.

parameters related to total antioxidant, phenolics, and amino acids (e.g., methionine, tyrosine, valine, leucine), as well as glucosinolates and their products (Figure 3). It was also indicated that the maximum separation was recorded for cauliflower and mustard sprouts across PC2. Turnip sprouts had more obvious responses to the positive effect of laser light than cauliflower and black mustard sprouts. The differences among the three *Brassica* spp. might be attributed to the ontogeny and species diversity. In agreement, the turnip species have shown a higher diversity in GLs content as well as a higher total phenolic content than those of other *Brassica* spp.[95,96] Turnip sprouts have also exhibited higher antioxidant and antiproliferative properties in comparison to cauliflower and yellow mustard sprouts.[78]

AUTHOR INFORMATION

Corresponding Authors

Saad M. Almuhayawi − *Department of Otolaryngology-Head and Neck Surgery, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia*; Email: dr.muhayawi@kau.edu.sa

Abdelrahim H.A. Hassan − *Department of Food Safety & Technology, Faculty of Veterinary Medicine, Beni-Suef*

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University, Beni-Suef 62511, Egypt; orcid.org/0000-0001-7905-6821; Email: abdelrahim@vet.bsu.edu.eg

Authors

Mohammed S. Almuhayawi − *Department of Medical Microbiology and Parasitology, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia*

Soad K. Al Jaouni − *Department of Hematology/Pediatric Oncology, Yousef Abdulatif Jameel Scientific Chair of Prophetic Medicine Application, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia*

Samy Selim − *Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Sakaka, Saudi Arabia*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.1c01550

Notes

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■ ABBREVIATIONS

ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; Colo205, colon carcinoma cell line; COX-2, cyclooxygenase-2; DPPH, diphenylpicrylhydrazyl; Emb293, embryonic kidney adenocarcinoma cell line; ESP, epithiospecifier protein; FRAP, ferric reducing antioxidant power; GLs, glucosinolates; GST, glutathione S-transferase; He−Ne, helium−neon; HepG2, hepatocellular carcinoma cell line; ITC, isothiocyanates; LOX, lipoxygenase; NQO1, NAD(P) H/quinoneoxidoreductase 1; QR, quinone reductase; T24P, urinary bladder carcinoma cell line

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