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Laser Light Treatment of Seeds for Improving the Biomass Photosynthesis, Chemical Composition and Biological Activities of Lemongrass Sprouts

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Abstract: Compared to seeds and mature plants, sprouts are well characterized based on their nutritive values and biological properties. Moreover, laser light application is known to be a promising approach to improving plant growth, photosynthesis, and nutraceutical values. However, no studies have investigated the phytochemicals and biological activity of lemongrass (*Cymbopogon proximus* (Hochst. ex A.Rich.) Chiov.) sprouts or the further improvement of their quality by applying laser light treatment. We carried out a preliminary experiment for the optimization of laser treatment conditions, finding that a helium neon (He–Ne) laser at 632 nm and 5 mW for 5 min provided the most favorable conditions. We then investigated fresh weight, photosynthetic reactions, and primary and secondary metabolites, including sugars, amino acids, organic acids, essential oils, and phenolic compounds. Moreover, we studied the effect of laser light-induced changes in chemical compositions on the antioxidant, anti-diabetic, and anti-cholesterol activities of *Cymbopogon proximus* sprouts grown from laser-treated seeds. Laser light treatment increased the photosynthesis and respiration and hence the fresh weight of *Cymbopogon proximus* sprouts. Overall, sprouting increased most bioactive primary and secondary metabolites as compared to seeds. Increased photosynthesis by laser light improved carbon allocation and raised non-structural carbohydrates, which in turn led to improved synthesis of amino acids, organic acids, and essential oils, as well as phenolic and flavonoid compounds. As a result, laser light significantly improved the antioxidant capacity in terms of increasing the levels of ferric reducing antioxidant power (FRAP) (from 9.5 to 21 µmole trolox/g fresh weight (FW)), oxygen radical absorbance (ORAC) (from 400 to 1100 µmole trolox/100 g FW), and DPPH (from 5% to 25% of inhibation) and enhanced the hypocholesterolemic and antidiabetic activity through increasing the percentage of cholesterol micellar solubility (CMS) inhibition (from 42% to 62%) and glycemic index (from 33 to 17 µmole/g) over sprouts and seeds. In conclusion, the synergism of seed laser treatment and sprouting induced the health-promoting bioactive compounds in *Cymbopogon proximus* as compared to seeds, which can be applied at a large scale to improve the biochemical, physiological, and nutraceutical values of medicinal and crop sprouts.

Keywords: sprouts; laser irradiation; *Cymbopogon proximus*; photosynthesis; bioactive compounds; anti-cholesterol activity; anti-diabetic activity

1. Introduction

Natural product research is now rapidly growing and attracting many research groups all over the world. Sprouts have been considered rich sources of health-promoting natural products such as amino acids, essential oils, polyphenols, minerals, and vitamins, which give them an advantage of being important food additives with various biological properties [1]. As compared to seeds and mature plants and in addition to their high nutritive values, sprouts are also well characterized by their lower content of anti-nutritional factors [2]. *Cymbopogon* is a widely distributed plant genus and has been used in food additives [3]. It has pharmacological importance due to its highly enriched content of significant compounds like essential oils [4]. In this regard, this plant genus was traditionally associated with the treatment of several human diseases [5,6], and many studies have revealed its anticancer, anti-inflammatory, antioxidant, antidiabetic and antimicrobial bioactivities [6–12]. Among the well-known lemongrass members are West Indian lemon grass (*Cymbopogon citratus* (DC.)) and Halfabar (*Cymbopogon proximus* (Hochst. ex A.Rich.) Chiov.), which have a characteristic lemon flavor. *Cymbopogon proximus* has many biological properties, including antioxidant, antimicrobial, and antiemetic activities [13–16]. Interestingly, it was also introduced as an antihypertensive agent after its observed hypotensive effect in experimentally induced hypertension in rats [17,18]. The essential oils from *Cymbopogon proximus* were proven to protect rats against isoproterenol-induced cardiac hypertrophy and fibrosis [19]. Thus, improving the phytochemical content of these plants, particularly their sprouts, is expected to enhance their health-promoting and functional food values. To enhance the nutritional and health-promoting effects of plants and their sprouts, several techniques that make use of environmental conditions have been applied to improve the production of nutritive phytochemicals [20–22]. In this regard, the application of laser light as the light source for plant production is a newly growing area of research and the possible positive effects of laser light on growing plants have been investigated. Laser light is classified into pulsed and continuous lasers. The continuous laser (e.g., He-Ne) and elevated CO_2 have been employed in improving plant and sprout mass and bioactivity [20,21]. For example, He-Ne laser light improved fresh weight, increased minerals and antioxidant metabolites, and boosted the antioxidant capacity and the anti-inflammatory activities of buckwheat sprouts [21]. Laser light has induced a significant increase in the antioxidant capacity and biomass accumulation of sunflowers [23]. This growth and metabolism improvement by laser light is based on the ability of plant macromolecules to absorb light at a specific wavelength to trigger photosynthetic activity, resulting in an increased fresh weight [24]. Moreover, conversely, the use of specified lasers for indoor horticulture is a good solution to overcome the obstacles of using artificial lighting, where plants grown under laser illumination have completed their full growth cycle with phenotypes resembling those of plants grown under LEDs but with lower energy and cost [25]. As a consequence, in plant factories, the application of lasers for growing vegetables has become the first choice among other lighting options due to its energy-saving advantages [26]. Indeed, laser light application in plant production was found to enhance different growth stages, as well as physiological, biochemical, and yield attributes of plants, cereal crops, and vegetables [27]. In light of these recent findings, our current study was conducted, for the first time, to study the possible positive effects of sprouting and laser light application on phytochemicals and the bioactivity of *C. proximus*. We hypothesized that laser light could improve the sprouting impact on the bioactive phytochemicals, antioxidant capacity, hypocholesterolemic activity, biological activities, and health-promoting values of *Cymbopogon proximus* as compared to their seeds.

2. Materials and Methods

2.1. Plant Material and Experimental Conditions

Cymbopogon proximus seeds were collected from Field Crops Research Institute, Agricultural Research Centre, Giza, Egypt. Before laser re-illumination, seeds were soaked in distilled water for two hours, then they were categorized into two groups (with each group containing 100 seeds), i.e., a control group (non-irradiated) and a laser-irradiated

group. The light source (a helium-neon (He-Ne) laser, equipment whitening, laser II, DMC Equipment Ltd., São Carlos, SP, Brasil) was used to treat seeds. Seeds were irradiated for 5 min under the following conditions—632 nm, a power of 5 mW and 500 mJ energy, beam diameter 1 mm and with a distance of 12 cm between the seeds and the laser source. These laser treatment conditions were optimized based on a preliminary experiment (Table 1), in which we tested the effectiveness of three laser types—helium neon (632 nm, 5 mW), helium cadmium (442 nm, 16 mW) and argon (514 nm, 8 mW)—at different exposure times (0, 2, 5, and 10 min) on the plant fresh weight and the overall antioxidant capacity in terms of ferric reducing antioxidant power (FRAP). The pretreated seeds were kept and rinsed in distilled water and spread on trays lined with vermiculite and watered every two days with Milli-Q water, and 150 mL of aquaponic water was poured evenly in each tray. The trays were maintained in a controlled growth chamber (25 °C, 16 h light/8 h dark cycle) managed through cool white fluorescent tubes with photosynthetically active radiation (PAR) of 400 µmol m^{-2} s^{-1} and a relative humidity of 60% per day. Ten-day-old sprouts from each tray were taken and weighed as fresh mass and stored at −80 °C for further biochemical analyses. Experiments were repeated 3 times. Approximately 15 plants were pooled from each tray and treated as biological replicates (each biological replicate was pooled from the same tray) and were used for each measurement.

Table 1. Effect of laser treatment on responses of sprouts in terms of plant fresh weight and antioxidant capacity (ferric reducing antioxidant power, FRAP). Helium-neon (632 nm, 5 mW), helium cadmium (442 nm, 16 mW), and argon (514 nm, 8 mW) were applied at different exposure times (0, 2, 5 and 10 min). Data are represented by means ± standard errors. Different small letters (a, b . . .) above bars indicate significant differences between means at $p < 0.05$.

Time (min)	He-Ne_Laser	He-Ca_Laser	Ar_Laser
		FW (g/sprout)	
0	1.4 ± 0.12 a	1.4 ± 0.11 a	1.4 ± 0.12 a
2	1.8 ± 0.06 b	1.4 ± 0.12 a	1.7 ± 0.34
5	2.1 ± 0.05 b	1.5 ± 0.1 a	1.7 ± 0.11 a
10	2.1 ± 0.06 b	1.8 ± 0.13 b	1.8 ± 0.1 b
		FRAP (µmol trolox/gFW)	
0	13.1 ± 0.5 a	13.1 ± 0.5 a	13.1 ± 0.5 a
2	17.5 ± 0.17 b	14.1 ± 0.19 a	13.9 ± 0.74 a
5	20.9 ± 1.1 b	18.3 ± 0.25 b	15.7 ± 0.87 a
10	19.8 ± 2.74 b	17.3 ± 0.9 ab	17.8 ± 0.4 b

2.2. Photosynthesis Analysis

Photosynthesis and dark respiration of treated lemongrass sprouts (µmol CO_2 m^{-2} s^{-1}) were measured by utilizing an EGM-4 infrared gas analyzer (PP Systems, Hitchin, UK). Whole-sprout photosynthesis and dark respiration were determined from 180 s measurements of net carbon dioxide exchange (NE).

2.3. Metabolite Analyses

2.3.1. Primary Metabolism

Sugar Analysis

The estimation of sugar levels in plant samples was carried out as previously described by Abdelgawad et al. [28], using high-performance liquid chromatography (HPLC), with a UPLC TQD device (Milford, Worcester County, MA, USA). Sugars were extracted from *Cymbopogon proximus* seeds or sprouts in 2 mL of the acetonitrile/water (1:1, *v/v*). After boiling at 55–60 °C for 15 min, then filtered through a Whatman No. 541 filter paper. Column temperature and injection volume were set at 30 °C and 20 µL, respectively. The

mobile phase was acetonitrile and HPLC-grade water, 75:25 (*v/v*) at 1 mL·min^{-1}. Identified sugars were quantified using peak areas and a comparison with a calibration curve obtained with the corresponding standards.

Amino Acid Analysis

Extraction: Amino acids were extracted by homogenizing 100 mg of lemongrass sprouts or seeds in 1 mL of aqueous ethanol (80%, *v/v*) using norvaline as an internal standard to increase the accuracy of quantitation, as well as to correct for different mass spectrometry responses.

Deamination: Amino acid analysis was undertaken using the method described by Zinta et al. [29]. The supernatant was centrifuged for 10 min at 8000 g using Millipore microfilters (0.2 µm), the aqueous phase. The levels of amino acids were estimated using a Waters Acquity UPLC TQD device (Milford, Worcester County, MA, USA) coupled to a BEH amide column.

Organic Acid Analysis

Extraction: Organic acids were extracted in 0.1% phosphoric acid, performed using 500 mg of sprout or seed powder, followed by the centrifugation of the mixture at 14,000 g for 30 min at 4 °C.

Deamination: Clear supernatants were filtered through Millipore microfilters (0.2 µm) and used for organic acid analyses using HPLC (Shimadzu HPLC system, SCL-10 AVP, Tokyo, Japan, reversed-phase at 4 °C) coupled with a SUPELCOGELC-610H column and UV detector (LaChrom L-7455 diode series, Tokyo, Japan). The mobile phase used was phosphoric acid (0.1%, *v/v*), which was run at a flow rate of 0.45 mL min^{-1}.

Essential Oil Analysis

Extraction: Seeds or sprout samples were extracted with petroleum ether for 48 h at room temperature. The extract was evaporated to dryness using rotary evaporation at reduced pressure. The essential oil was passed over dark anhydrous sodium sulfate to remove moisture.

Deamination: The essential oils were analyzed through GC/MS, following the method described by Hassanpour et al. [30]. Seeds or sprouts were air-dried, then 10 g of the dried sample was used for the extraction of essential oils. The dried parts were subjected to steam distillation for 3 h using a Clevenger-type instrument and the essential oil content was then calculated as mg/g fresh weight (FW).

2.3.2. Secondary Metabolites

Determination of Phenolic Profile

Phenolic compounds were quantified through UHPLC-MS/MS analysis [31]. A known weight of the dried powdered sprouts or seeds was extracted (80%, *v/v*) using ethanol at 70 °C for 30 min. After centrifugation (12,000 rpm for 30 min), it was concentrated using a rotary evaporator (IKA-WERKE-RV06ML; Staufen, Germany). The obtained residue was dissolved in HPLC-grade methanol and analyzed by means of an Acquity UPLC System (Waters, Milford, CT, USA), equipped with an Acquity BEH C18 column (100 mm × 2.1 mm, with a 1.7-µm particle size). The mobile phase (A: ultrapure water containing 0.1% formic acid and eluent B: acetonitrile) was applied at a flow rate of 0.2 mL/min. The linear gradient of the mobile phase was started at 3% B, increased to 100% B in 10 min. The internal standard was 3,5-dichloro-4-hydroxybenzoic acid.

2.4. Biological Activity

Extraction: Samples were separately ground and extracted with ethanol at room temperature for 12 h. The extract was centrifuged at 8000 g for 25 min and the supernatant was filtered using Whatmann No. 1 filter paper. After concentrating the extract using a rotary evaporator, the samples were stored at −20 °C until use.

2.5. Antioxidant Capacity

We used the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), oxygen radical absorbance (ORAC) and ferric reducing antioxidant power (FRAP) assays to measure total antioxidant power in *Cymbopogon proximus* seeds, as described before [32]. Briefly, 0.25 g of the seed powder was extracted in ethanol (80%) and centrifuged at 14,000 rpm for 30 min. The antioxidant capacity was measured by mixing 0.1 mL of the diluted seed extracts with 0.25 mL of the DPPH solution, ORAC (in the presence of Cu^{2+} and H_2O_2) or FRAP reagent, mixing TPTZ (10 mM) and $FeCl_3$ (20 mM) in acetate buffer (0.25 M, pH 3.6). After incubation at room temperature, the absorbance was measured at 517 nm and 600 nm using the spectrometric method, respectively. The copper-initiated prooxidant activity was calculated using (Area Blank − Area Sample)/Area Blank × 100 and expressed as prooxidant units; one unit equaled the prooxidant activity that reduced the area under the fluorescein decay curve by 1% in the ORAC assay.

2.6. Hypocholesterolemic Activity

2.6.1. Inhibition of Micellar Solubility of Cholesterol

The effect of *Cymbopogon proximus* sprout and seed extracts on the micellar solubility of cholesterol was measured as described in Hozzien et al. [33]. Micellar solution (2 mM cholesterol, 10 mM sodium taurocholate, 132 mM NaCl, 5 mM oleic acid, 15 mM sodium phosphate (pH 7.4) at the rate of 10 mg/mL) was mixed with the extract. The mixture was vortexed, then sonicated for 2 min and incubated at 37 °C for 24 h. The micellar solution was ultra-centrifuged at 40,000 rpm for 50 min at 22 °C. The supernatant was used for spectrophotometric determination of cholesterol content at 500 nm by means of an enzymatic method using a cholesterol analysis kit (C7510—Pointe Scientific, Inc., Canton, MI, USA). Inhibition activity of cholesterol micellar solubility was calculated.

2.6.2. Pancreatic α-Amylase Inhibition Assay

To measure the inhibition of the pancreatic α-amylase inhibition, the extract was mixed with a reaction solution (starch (1 g/L) and phosphate buffer (pH 6.9). The reaction was started by adding 3 U/mL of amylase enzyme. After 10 min of incubation, 500 µL dinitro salicylic (DNS) reagent was added to stop the reaction. The reaction mixture was heated for 10 min at 100 °C. In the end, 500 µL of 40% potassium sodium tartrate solution was added to the mixtures. The absorbance was recorded at 540 nm.

2.6.3. Pancreatic Lipase Inhibition Assay

The inhibitory activity of *Cymbopogon proximus* sprout and seed extracts against pancreatic lipase was measured using 4-MUO, as described in Hozzien et al. [33]. Sprout extracts were mixed with 0.5 mL of the freshly prepared lipase (1 mg/mL; lipase from porcine pancreas, Sigma-Aldrich). After stirring, the reaction mixtures were centrifuged at 4000 rpm for 10 min, and then 2 mL of the 4-MUO (0.1 mM) solutions was added. The reaction mixture was incubated at 37 °C and 0.2 mL aliquots were taken at different time points. The 4-MUO hydrolysis by lipase was measured fluorometrically (excitation wavelength of 350 nm and an emission wavelength of 450 nm). We calculated IC50 values (mg/mL) to estimate the concentration of the sprout or seed extract that inhibited 50% of the pancreatic lipase activity.

2.7. Anti-Diabetic Activity

2.7.1. Determination of In Vitro Glycemic Index

The GI was determined by means of in vitro starch hydrolysis [34]. Seeds and sprouts were ground and incubated with pepsin (100 mg/mL) in a reaction buffer of HCl-KCl buffer (pH 1.5). After incubation for one hour at 40 °C under shaking conditions, the mixture was diluted in phosphate buffer (pH 6.9) and then α-amylase was added and incubated at 37 °C. Approximately 1-mL aliquots were taken every 30 min and boiled for 20 min to inactivate the amylase enzyme. Residual starch was converted to glucose by

adding 0.4 M pf sodium acetate buffer (pH 4.75) and 60 µL amylo glucosidase, and the reaction mixture was incubated at 60 °C for 50 min. Approximately 0.6-mL aliquots were taken and incubated with 1.2 mL glucose oxidase/peroxidase at 37 °C for 35 min. The absorbance of the mixture was measured at 500 nm. Starch digestion rate was expressed as the percentage of hydrolyzed starch at different times (0, 30, 60, 90, 120, and 180 min). The hydrolysis and the area under the hydrolysis curve (AUC, 0–180 min) were calculated. Then, the hydrolysis index was calculated as the relation between the AUC for a sample and the AUC for a standard multiplied by 100.

2.7.2. α-Glucosidase Inhibition Assay

The inhibition of α-Glucosidase inhibition was determined [34]. The seed and sprout hydroethanolic extract was mixed with α-glucosidase (2 U/mL) and incubated at 37 °C for 5 min. Then, 1 mM para-nitrophenyl glucopyranoside dissolved in 50 mM phosphate buffer (pH 6.8) was added to the reaction buffer and incubated for 20 min at 37 °C. The reaction was stopped by adding sodium carbonate (1 M). α-Glucosidase activity was determined at 405 nm to quantify the amount of para-nitrophenolate released by para-nitrophenyl glucopyranoside and the α-glucosidase inhibitory activity was calculated.

2.7.3. α-Amylase Inhibition Assay

α-Amylase inhibition was determined with the method described in [34]. Starch (2 mg) was mixed with 0.5 M Tris-HCl buffer (pH 6.9) and 0.01 M $CaCl_2$. After boiling for 5 min, samples were cooled at room temperature and incubated for 5 min at 37 °C. Then, we added α-amylase (U/mL) and incubated it at 37 °C for 10 min. Subsequently, 500 µL 0.1% 3,5-dinitro salicylic acid was added and incubated for 10 min at 100 °C. After cooling, the absorbance was determined at 540 nm. The α-amylase inhibition was calculated.

2.8. Statistical Analyses

Statistical analyses were performed using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was applied to all data. Tukey's Test ($p \leq 0.05$) was carried out as the post-hoc test for mean separations. Each experiment was replicated at least three times (n = 3–5). Hierarchical clustering (Pearson correlation) was generated using the Multi-Experimental Viewer (TM4 software package).

3. Results and Discussion

3.1. Increased Photosynthesis and Respiration by Laser Light Improved Cymbopogon proximus Growth

Laser light has been proven to enhance the fresh weight and nutritive values of plants [34] and sprouts [21]. When applied at a certain dose, laser light can successfully increase the internal energy of the seeds by converting light energy into chemical energy [24], which subsequently could be utilized to activate the physiological processes of plants, such as germination, photosynthesis, and respiration [27]. In this context, laser light could enhance chlorophyll content [23,24]. On the molecular level, transcriptome analysis has also revealed an upregulation in the genes responsible for photosynthesis in He-Ne laser-pretreated wheat seedlings [35]. In the current study, the laser-treated group of *Cymbopogon proximus* showed a perceived increase in the fresh weight (43%) and the dry weight (64%) in comparison with the control group, respectively (Table 2). This is in agreement with previous studies that have confirmed that laser light can exert an enhancing effect on growth and biomass accumulation in plants [23,24]. It was reported that laser pre-sowing seed treatments have enhanced the chlorophyll content and metabolically important enzymes of soybeans [27]. Consequently, the observed induction in plant growth after laser light treatment in the current study could be partially attributed to the increased chlorophyll content and consequently to the increased net photosynthetic activity after laser light application. Additionally, respiration improvement was observed, as indicated by the respiratory rate, which increased by about 20% in the laser-treated group in comparison to the control group (Table 2). 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 [27].

Table 2. Effect of He-Ne laser light treatment on fresh weight, dry weight, photosynthesis, and respiration levels of *Cymbopogon proximus* sprouts. DW: dry weight; FW: fresh weight. Data are represented by means ± standard errors. Different small letters (a, b, ...) above bars indicate significant differences between means at $p < 0.05$.

	Control	Laser
FW (g/sprout)	1.45 ± 0.2 a	2.08 ± 0.26 b
DW (g/sprout)	0.15 ± 0.05 a	0.25 ± 0.03 b
Photosynthesis (μmol CO_2 m^{-2} s^{-1})	2.9 ± 0.35 a	4.08 ± 0.15 b
Respiration (μmol CO_2 m^{-2} s^{-1})	0.018 ± 0.003 a	0.025 ± 0.005 b

3.2. Improved Photosynthetic Reactions in Laser Light-Treated Cymbopogon proximus Sprouts Induced the Assumption of Bioactive Primary Metabolites

The tissue metabolites were improved after laser treatment, as indicated by the increased tissue levels of sugar, amino acid, and organic acids in *Cymbopogon proximus* sprouts from laser-treated seeds when compared with control sprouts, which also showed significantly higher results than control seeds. There was an increase in the levels of soluble sugars (90%) and total sugars (48%) in the laser-treated sprouts compared to the control sprouts. In addition, control sprouts revealed significantly ($p < 0.05$) higher values of sucrose, fructose, and total carbohydrates as compared to seeds (Table 3). It was reported that sugars are directly involved in plant development and growth [36,37], so the increased photosynthetic rate observed in the current study may point to increased sugar production. As a result, high sugar availability could induce the biosynthesis of other classes of primary and secondary metabolites, in which they are degraded by dark respiration to provide the needed C-skeleton and energy [38]. Similarly, most of the measured organic acids and amino acids were improved by laser treatment in sprouts grown from treated seeds compared to control sprouting. In addition, control sprouting presented higher levels of these acids than seeds (Table 3). The levels of many amino acids were increased significantly ($p < 0.05$) in laser-treated sprouts. The levels of lysine (21%), isoleucine (40%), glycine (42%), phenylalanine (50%), cystine (88%), glutamine (14%), and glutamic acid (26%) were significantly increased in laser light-treated sprouts in comparison to the control sprouts, which displayed significantly higher contents than seeds as well (Table 3). The incapability of human bodies to synthesize certain amino acids is generating interest in increasing the concentrations of these essential amino acids in plants [39], so the observed increase in the amino acid levels after laser application in the current study could be considered a crucial advantage of applying this technique. In this regard, the accumulation of essential amino acids is an important factor that affects the plant's nutritional value, besides being involved in protein synthesis [39]. Moreover, amino acids are globally considered natural plant growth stimulators [40] and are currently used for improving plant productivity [41]. In the current study, the levels of malic, succinic, and citric acids were significantly ($p < 0.05$) increased in laser-treated sprouts compared to control sprouts, and these acids were significantly higher in control sprouts than in seeds (Table 3). The high sugars and organic acids reported in the treated *Cymbopogon proximus* sprouts could support their nutritional value because they are directly related to taste and flavor [42]. It has been clearly reported that laser light can effectively activate the production of plant primary and secondary metabolites [43]. The observed increase in the levels of organic acids in the laser-treated group of *Cymbopogon proximus* may be attributed to the decrease in the nitrate content. It is well documented that the nitrate content in plants is inversely correlated with the concentration of sugars and organic acids [44]. As laser treatment increases carbohydrate synthesis, this leads to an increase in the supply of ferredoxin and NADPH, which are used for the reduction of nitrate in leaves [45].

Table 3. Effect of He-Ne laser light treatment on sugar, amino acid, and organic acid contents in *Cymbopogon proximus* sprouts and seeds. Data are represented by means ± standard error.

Parameters	Seed-Control	Sprout-Control	Sprout-Laser
Sugars			
Glucose	1.2 ± 0.1 a	1.3 ± 0 a	2.2 ± 0.23 b
Fructose	0.32 ± 0.04 a	0.49 ± 0.05 b	0.9 ± 0.01 c
Sucrose	1.1 ± 0.06 a	1.9 ± 0.06 b	2.7 ± 0.09 b
Soluble sugars	4.9 ± 0.7 a	5.4 ± 0.2 a	10.3 ± 2.6 a
Starch	48.7 ± 5.3 a	52.7 ± 2.4 a	49.3 ± 5.7 a
Total Carbohydrates	83.7 ± 3.8 a	92.7 ± 2.1 b	137 ± 9.7 c
Amino Acids			
Lysine	2.1 ± 0.1 a	3.9 ± 0.2 b	4.9 ± 0.03 c
Histidine	2.1 ± 0.12 a	2.8 ± 0.22 a	2.7 ± 0.17 a
Alanine	16.9 ± 2.1 a	17.3 ± 1 a	18.9 ± 1.7 a
Arginine	1.1 ± 0.61 a	2 ± 0.01 b	2.3 ± 0.1 b
Isoleucine	0.02 ± 0.0 a	0.2 ± 0.01 b	0.3 ± 0.0 c
Asparagine	0.4 ± 0.06 a	1 ± 0.06 b	1.8 ± 0.1 c
Ornithine	0.15 ± 0.01 a	0.2 ± 0.03 a	0.2 ± 0.02 a
Glycine	1.1 ± 0.1 a	1.2 ± 0.07 a	2 ± 0.13 b
Phenylalanine	0.1 ± 0.01 a	0.3 ± 0.02 b	0.7 ± 0 c
Serine	0.15 ± 0.01 a	0.3 ± 0.02 b	0.6 ± 0.04 b
Proline	1.1 ± 0.1 a	1.1 ± 0.07 a	2.4 ± 0.1 c
Valine	0.5 ± 0.1 a	0.5 ± 0.03 a	0.6 ± 0.04 a
Aspartate	0.01 ± 0 a	0.03 ± 0 b	0.05 ± 0 c
Cystine	0.02 ± 0 a	0.03 ± 0 a	0.2 ± 0.01 b
Leucine	0.03 ± 0 a	0.02 ± 0 a	0.03 ± 0.1 a
Methionine	0.01 ± 0 a	0.02 ± 0 b	0.02 ± 0 b
Threonine	0.1 ± 0.0 a	0.1 ± 0.01 a	0.2 ± 0.01 b
Tyrosine	0.34 ± 0.0 a	1 ± 0.06 b	1.3 ± 0.1 b
Glutamine	71.1 ± 1.8 a	91.7 ± 5.6 b	106 ± 6.6 c
Glutamic acid	54 ± 3.1 a	67 ± 4.2 b	90 ± 8.9 c
Organic Acids			
Oxalic	2.11 ± 0.2 a	2.84 ± 0.2 a	3.04 ± 0.1 a
Malic	2.08 ± 0.2 a	3.08 ± 0.19 b	4.72 ± 1.5 c
Isobutyric	3.1 ± 0.1 a	3.2 ± 0.2 a	4.1 ± 1.37 a
Fumaric	0.73 ± 0.01 a	0.93 ± 0.01 a	0.95 ± 0.3 a
Succinic	1.24 ± 0.5 a	3.24 ± 0.33 b	4.15 ± 1.1 c
Citric	2.1 ± 0.2 a	3.5 ± 0.3 b	5.4 ± 1.8 c

Different small letters (a, b, c) within a row indicate significant differences between means at $p \leq 0.05$. Units: sugar (mg/g FW), amino acid (mg/100 g FW), organic acid (mg/g FW).

3.3. Laser Treatment Increased the Levels of Essential Oils in Cymbopogon proximus Sprouts

As laser light application increased the photosynthetic rate in *Cymbopogon proximus* sprouts, the biosynthesis of sugars, organic acids, and essential oils should be increased in a rhythmic manner. To verify this, an array of 18 essential oils was screened in the current

study (Table 4). Essential oils are oily liquids produced through secondary metabolism in plants and can exert beneficial biological effects, which is the reason behind considering them as candidates for new drug screening [46]. As illustrated in Table 4, in the laser-treated sprouts of *C. proximus*, there were significant increases in the levels of α-eudesmol (66%), β-eudesmol (72%), γ-eudesmol (68%), elemol (52%), terpinene (66%), p-cymene (65%), 1,8-cineole (65%), ocimene (66%), piperitone (40%), elemene (65%), terpinene (62%), and 3-carene (66%) in comparison to control sprouts. Additionally, control sprouts showed significantly ($p < 0.05$) richer values of 10 out of 18 essential oils than seeds (Table 4). On the other hand, the laser-treated sprouts showed slight increases in the levels of linalool (65%), linalyl acetate (64%), and bourbobene (63%) in comparison to the control group. In a recent study, the essential oils elemol, piperitone, α-eudesmol, and β-eudesmol, separated from *Cymbopogon proximus* sprouts, were able together to protect rats against isoproterenol-induced cardiac hypertrophy and fibrosis [19]. In agreement with this, piperitone, elemol, α eudesmol, limonene, and β- eudesmol are reported to be the main components of *Cymbopogon proximus* [15]. Interestingly, the essential oil of *Cymbopogon proximus* sprouts has been previously investigated for its cardio-protective effects [19]. Additionally, essential oils from *Cymbopogon Flexuosus* Steud. were utilized in the control of some vector-borne diseases [47].

Table 4. Effect of He-Ne laser light treatment on essential oil contents of *Cymbopogon proximus* sprouts and seeds. Data are represented by means ± standard errors.

Essential Oils (mg/gFW)	Seed-Control	Sprout-Control	Sprout-Laser
α-Pinene	0.5 ± 0.2 a	0.8 ± 0.17 b	1.3 ± 0.3 b
α-Eudesmol	0.2 ± 0.0 a	0.3 ± 0.05 b	0.5 ± 0.01 c
Elemol	7.4 ± 0.2 a	9.4 ± 0.71 b	14.2 ± 0.4 c
α-Terpinene	1 ± 0.1 a	1 ± 0.15 a	1.6 ± 0.2 a
p-Cymene	3.4 ± 0.6 a	5.6 ± 0.64 b	9.2 ± 0.1 c
1,8-Cineole	2.5 ± 0.09 a	2.2 ± 0.1 a	3.7 ± 0.3 b
Piperitone	37.6 ± 2.1 a	45.6 ± 1.4 b	64 ± 2.9 c
(E)-β-Ocimene	0.7 ± 0.03 a	1.28 ± 0.2 b	1.9 ± 0.3 c
γ-Terpinene	0.3 ± 0.04 a	0.3 ± 0.04 a	0.47 ± 0.07 b
α-Terpinolene	0.01 ± 0 a	0.01 ± 0 a	0.01 ± 0 a
Linalool	1.1 ± 0.2 a	1 ± 0.15 a	1.6 ± 0.25 a
Linalyl acetate	0.07 ± 0.0 a	0.14 ± 0.03 b	0.23 ± 0.05 c
β-Bourbobene	0.24 ± 0.01 a	0.3 ± 0.02 a	0.49 ± 0.04 b
β-Elemene	0.32 ± 0.0 a	0.62 ± 0.06 b	1.0 ± 0.1 b
γ-Eudesmol	0.10 ± 0.0 a	0.18 ± 0.03 a	0.31 ± 0.04 b
β-Eudesmol	0.11 ± 0.0 a	0.28 ± 0.05 b	0.47 ± 0.08 c
(Z)-β-Ocimene	0.22 ± 0.01 a	0.64 ± 0.06 b	1.06 ± 0.11 c
δ-3-Carene	0.4 ± 0.02 a	0.5 ± 0.01 a	0.83 ± 0.01 b

Different small letters (a, b, c) within a row indicate significant differences between means at $p \leq 0.05$.

3.4. Improved Levels of Antioxidant Metabolites in Laser-Treated Cymbopogon proximus Sprouts

It is known that the nutritive value of plants is greatly dependent on their contents of secondary metabolites such as phenolic compounds [48]. Phenolic compounds, as well as flavonoids, are well-known as antioxidants [49] and hence their levels represent a direct reflection of the nutritive value of plants. Previous studies have reported potential phytotherapeutic effects of phenolic products from *Cymbopogon citratus* [50]. In addition,

for plants, these compounds act as defense mechanisms against environmental stress and attack by other organisms [49]. Here, the concentrations of seven phenolic acids and seven flavonoids, as well as the total phenolic content (TPC) and total flavonoid content (TFC), were quantified in the sprouts of *Cymbopogon proximus*. Interestingly, control sprouts showed significantly ($p < 0.05$) higher levels of eight out 14 measured phenolics and flavonoids compared to seeds. Furthermore, they were significantly richer in total phenolic and flavonoid compounds than seeds (Table 5). In addition, the laser-treated group of *Cymbopogon proximus* sprouts showed significant ($p < 0.05$) elevations in the levels of gallic acid (43%), caffeic acid (77%), protocatechuic acid (27%), luteolin (33%), apigenin (32%), and TPC (26%) in comparison with control sprouts. Additionally, the laser-treated group of *Cymbopogon proximus* sprouts showed slight increases in the levels of p-coumaric acid (4%), chicoric acid (16%), rosmarinic acid (23%), kaempferol (16%), chlorogenic acid (25%), and TFC (31%) in comparison to control sprouts. However, there were equivalent levels of quercetin, naringenin, and rutin in both laser-treated and control groups of sprouts (Table 5). Several of these reported phenolic compounds have been previously detected in *Cymbopogon citratus* [51]. A positive influence of laser light treatment on the growth and metabolism in seedlings of white lupine and faba beans has been reported [52]. Our previous study revealed laser light application as a powerful tool for improving the antioxidant levels of buckwheat sprouts [21].

Table 5. Effect of He-Ne laser light treatment on phenolic and flavonoid contents of *Cymbopogon proximus* sprouts and seeds. Data are represented by means ± standard errors.

Phenolics and Flavonoids (mg/gFW)	Seed-Control	Sprout-Control	Sprout-Laser
Gallic acid	0.21 ± 0.03 a	0.29 ± 0.02 a	0.5 ± 0.05 b
Caffeic acid	0.22 ± 0.0 a	0.42 ± 0.03 b	0.74 ± 0.1 c
p-Coumaric acid	1.10 ± 0.1 a	2.27 ± 0.1 b	2.3 ± 0.16 b
Chicoric acid	1.1 ± 0.04 a	1.1 ± 0.04 a	1.2 ± 0.04 a
Rosmarinic acid	1.0 ± 0.07a	1.0 ± 0.07 a	1.7 ± 0.05 b
Protocatechuic acid	1.1 ± 0.01 a	3.1 ± 0.06 b	4.9 ± 0.3 b
Quercetin	0.053 ± 0.01 a	0.055 ± 0.0 a	0.057 ± 0.0 a
Isoquercetrin	0.026 ± 0.0 a	0.045 ± 0.0 b	0.046 ± 0.0 b
Kaempferol	0.042 ± 0.0 a	0.06 ± 0.0 b	0.07 ± 0.0 b
Luteolin	0.06 ± 0.01 a	0.06 ± 0.0 a	0.08 ± 0.0 b
Apigenin	0.03 ± 0.02 a	0.06 ± 0.02 b	0.09 ± 0.0 c
Naringenin	0.004 ± 0.0 a	0.01 ± 0.0 b	0.023 ± 0.01 b
Rutin	0.001 ± 0.0 a	0.007 ± 0.0 b	0.021 ± 0.0 c
Chlorogenic acid	0.0 ± 0.0 a	0.0 ± 0.0 a	0.017 ± 0.0 b
Total phenols	6.43 ± 0.4 a	9.43 ± 0.1 b	11.87 ± 0.3 c
Total Flavonoids	1.45 ± 0.0 a	2.05 ± 0.07 b	2.91 ± 0.1 c

Different small letters (a, b, c) within a row indicate significant differences between means at $p \leq 0.05$.

3.5. Phenolic Compound Accumulation by Laser Treatment Improved the Overall Antioxidant Capacity of Cymbopogon proximus Sprouts

As a consequence of increasing the antioxidant metabolites in *Cymbopogon proximus* sprouts, higher antioxidant activities were observed, as indicated by different antioxidant assays (FRAP, ORAC, DPPH) (Figure 1). Antioxidant activity is considered a key indicator of the nutritive value in edible plant parts [53]. In this regard, the antioxidant capacities of essential oils of *Cymbopogon proximus* have been previously reported [15]. There was also a shred of direct evidence for the link between the total phenolic and flavonoid content and the total antioxidant activities in many plant species [54]. As laser treatment

resulted in increasing the levels of flavonoids, as well as phenolic compounds, in sprouts of *Cymbopogon proximus*, as clearly observed in Table 5, the laser-treated group of *Cymbopogon proximus* sprouts expressed significantly elevated levels of FRAP (Figure 1A), ORAC (Figure 1B), and DPPH (Figure 1C) in comparison to control sprouts. Furthermore, the control sprouts presented significantly higher values of FRAP, ORAC, and DPPH than control seeds (Figure 1). Moreover, a highly significant antioxidant activity (DPPH, ABTS, scavenging of the superoxide anion, and lipid peroxidation) has also been previously exhibited by *Cymbopogon citratus* [55]. In line with our study, the application of laser light increased the total antioxidant capacities (DPPH, FRAP, and ABTS) in buckwheat sprouts [21]. The observed enhanced antioxidant capacity after laser light application augments the previously reported enhancing effects of laser light on the total phenolic content of some plants such as soybeans [27] and sunflowers [23].

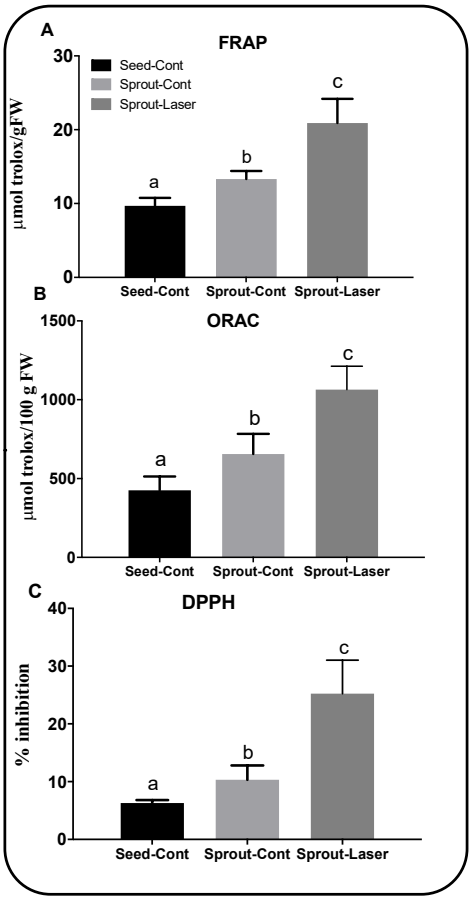

Figure 1. Effect of He-Ne laser light treatment on the total antioxidant capacity of *Cymbopogon proximus* sprouts and seeds. (**A**) FRAP (ferric reducing antioxidant power); (**B**) ORAC (oxygen radical absorbance); (**C**) DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate). Data are represented by means ± standard errors. Different small letters (a, b, c) above bars indicate significant differences between means at $p < 0.05$.

3.6. Enhanced Cholesterol-Lowering Activity of Laser Light-Treated Cymbopogon proximus Sprouts

As high cholesterol is the sixth risk factor for death in the world, cholesterol-lowering agents are considered to be of special relevance and many plant species have been investigated for their hypocholesterolemic potential [56]. The incorporation of dietary cholesterol into micelles is critical for absorption across the enterocytes into circulation. Consequently, the inhibition of cholesterol micellar solubility (CMS) is considered a parameter for anti-cholesterol activity measurement. As observed in Figure 2A, the percentage of CMS inhibition in the laser-treated group of *Cymbopogon proximus* sprouts is higher than that in the control group of sprouts. Additionally, the half maximal inhibitory concentration (IC50)

of the tested sprouts against pancreatic amylase activity (Figure 2B) and pancreatic lipase activity (Figure 2C) were clearly reduced in the laser-treated group of *Cymbopogon proximus* sprouts in comparison to control sprouts. Moreover, these hypocholesterolemic activities were significantly ($p < 0.05$) greater in control sprouts than in control seeds (Figure 2). Although the hypocholesterolemic effect of *Cymbopogon spp.* has been confirmed before [57], no studies, to the best of our knowledge, have reported this effect in *Cymbopogon proximus* or studied the enhancing effect of laser application on this effect. In this regard, *Cymbopogon citratus* extracts were able to reduce total cholesterol, triglycerides, and low-density lipoprotein (LDL) levels [58]. Thus, *Cymbopogon citratus* might contribute to maintaining a balance of cholesterol levels. This hypocholesterolemic activity is correlated with the availability of secondary metabolites such as phenolic compounds, which could play a role in getting rid of cholesterol in the feces [59]. Taken together, our current data illustrate the paramount importance of laser light application in the field of agricultural research, and further research is still needed to investigate the detailed mechanisms of these effects.

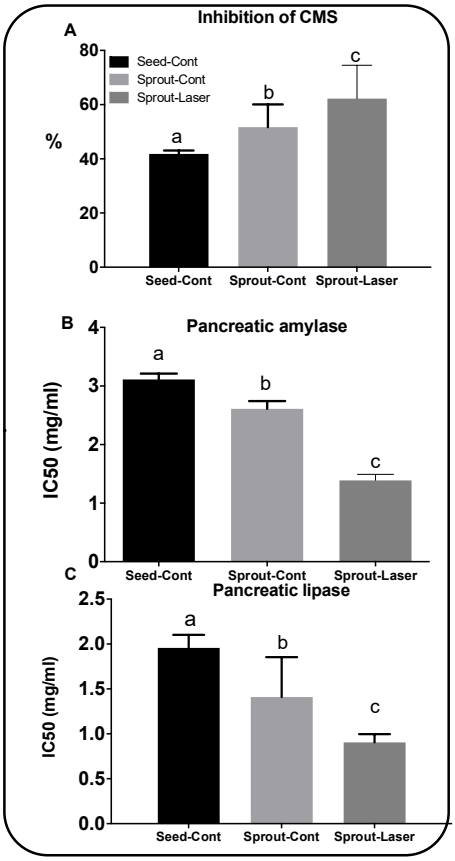

Figure 2. Effect of He-Ne laser light treatment on the anti-cholesterol activity of *Cymbopogon proximus* sprouts and seeds. (**A**) inhibition of CMS (cholesterol micellar solubility); (**B**) Pancreatic amylase; (**C**) Pancreatic lipase. Data are represented by means ± standard errors. Different small letters (a, b, c) above bars indicate significant differences between means at $p \leq 0.05$.

3.7. Improved Anti-Diabetic Activity of Laser-Treated Cymbopogon proximus Sprouts

Furthermore, we assessed the effect of both sprouting and laser treatment separately and in combination on the anti-diabetic activity of laser-treated *Cymbopogon proximus* (Figure 3). Remarkably, control sprouts had a significantly lower glycemic index (Figure 3A) ($p < 0.05$) and higher anti-diabetic activities—namely, α-glucosidase inhibition activity (Figure 3B) and α-amylase inhibition activity (Figure 3C)—than control seeds. In addition, laser-treated sprouts had significantly higher glycemic index values and α-glucosidase inhibition activity, as well as slightly higher α-amylase inhibition activity (Figure 3). The glycemic index concept was

developed as a tool to select healthy foods for diabetic patients. According to its effect on blood glucose levels after consumption, it is classified as low (≤55), medium (56–69), and high (≥70) [58]. Glycemic index mainly relies on the absorption of carbohydrates, i.e., lowering the absorption of carbohydrates causes low blood glucose levels and therefore a low glycemic index. The composition and microstructure of food, such as the sugar profile and antioxidant secondary metabolites, are contributing factors in a food's glycemic index [59,60]. Thus, the observed low glycemic index of sprouts, particularly after laser light treatment, could be due to the accumulation of soluble sugars (sucrose, fructose), essential oils and amino acids, and antioxidant polyphenols [59,60]. Increased inhibition percentages of α-amylase and α-glucosidase by sprouting and seed treatment with laser light can be correlated with high levels of tocopherol and phenolics [61,62]. For instance, the high availability of quercetin, ρ-coumaric, and gallic acid possibly exert the effect on α-amylase and α-glucosidase inhibition [61]. Appositive correlation with total antioxidant capacity (DPPH) was observed [59].

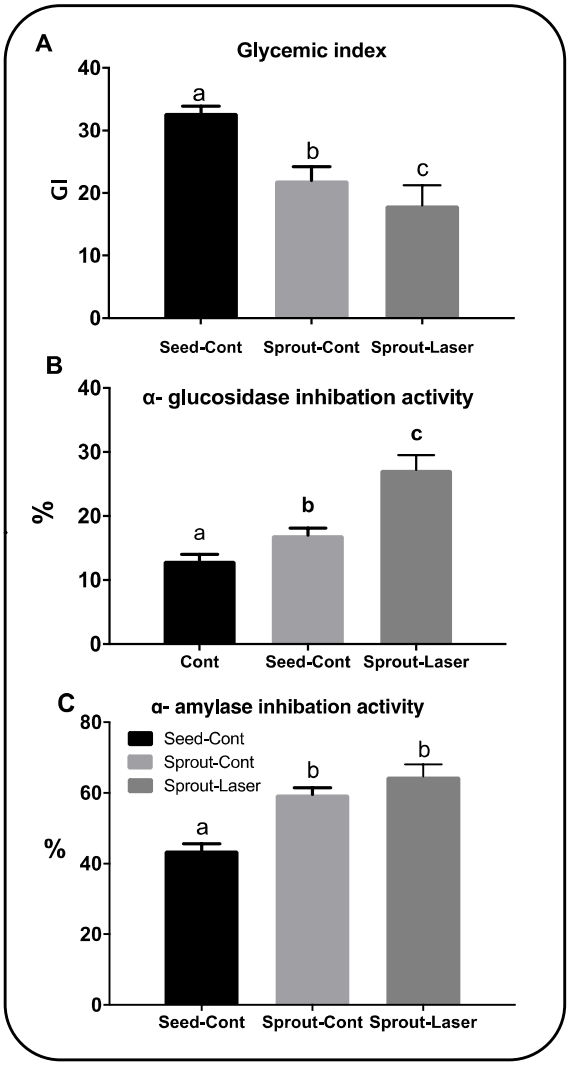

Figure 3. Effect of He-Ne laser light treatment on the anti-diabetic activity of *Cymbopogon proximus* sprouts and seeds. (**A**) glycemic index; (**B**) α-glucosidase inhibition activity; (**C**) α-amylase inhibition activity. Data are represented by means ± standard errors. Different small letters (a, b, c) above bars indicate significant differences between means at $p < 0.05$.

3.8. Cluster Analysis

The hierarchical clustering data of 66 measured parameters, shown in Figure 4, confirm the synergistic effect of laser light treatment with sprouting on most of the measured

parameters. Given that the lowest values of most measured metabolites and biological activities were found in untreated seeds, whereas sprouts cultivated from untreated seeds showed moderate values, sprouts grown from laser-treated seeds had the highest values of most metabolites and the highest biological activity results. Only for chlorogenic acid were the lowest values detected in laser light-treated sprouts. Furthermore, no effect of laser treatment nor sprouting was detectable in the case of alpha-terpinolene. Higher values of leucine were also found in seeds and treated sprouts than in control sprouts. The rest of the parameters can be divided into three large clusters, highlighting the synergism of sprouting with laser treatment.

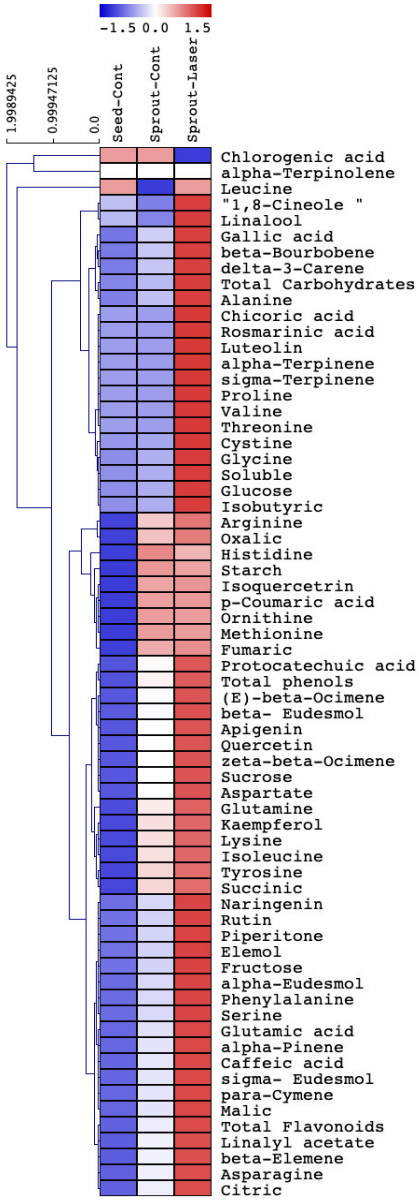

Figure 4. Heatmap showing the effect of He-Ne laser light treatment on growth, photosynthesis, respiration, metabolites, and biological activity of *Cymbopogon proximus* sprouts and seeds.

4. Conclusions

Laser light improved the photosynthetic activity, respiration, and hence the fresh weight of *Cymbopogon proximus* sprouts. Sprouting increased the bioactive primary and secondary metabolites as compared to seeds. Enhanced photosynthesis by laser light further improved the synthesis of amino acids, organic acids, and essential oils, as well as

phenolics and flavonoids. Accordingly, laser treatment significantly improved antioxidant, hypocholesterolemic, and antidiabetic activities. Similar to most physical factors, laser light modifies only the physiological and biochemical processes in seeds, so it is safe for humans and the environment [63]. Moreover, in contrast to high doses of laser light, which induce plant mutation, the applied low dose of laser light in the current study only induced biostimulation effects. Overall, the synergism of laser light treatment and sprouting of plants could be deemed as a promising tool for improving the nutraceutical and biological values of edible plants. This is especially true for plants that have been documented to possess highly enriched contents of bioactive compounds and biologically relevant metabolites that can be used in promising functional food and therapeutic applications.

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