




Article

The Effect of Botanical Extracts Obtained through Ultrasound-Assisted Extraction on White Head Cabbage (*Brassica Oleracea* L. Var. *Capitata* L.) Seedlings Grown under Controlled Conditions

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Abstract: This research presents the possibility of using innovative botanical extracts as biostimulants of plant growth to improve plant nutritional value, growth, and development. It is important to increase agricultural production but this process should be carried out in a sustainable way, without causing risks for both the environment and consumers. For this reason, we have focused on the use of 14 natural raw materials and ultrasound assisted extraction for the production of biostimulants. Results proved that higher plants can be used to obtain valuable products for the application in modern horticulture and agriculture. For instance, extract based on *Urtica dioica* L. showed the highest biostimulatory properties: in the group sprayed with 0.1% extract cabbage seedlings were longer by 31%, while with 1.0% extract of *Polygonum aviculare* L. roots were longer by 72% than in the control group treated with water. Extracts based on *Equisetum arvense* L. (0.5%) and *Urtica dioica* L. (leaf) (0.1%) increased the fresh weight of sprouts by 113% and 112%, respectively. The highest root weight was observed in groups treated with *Equisetum arvense* L. (0.5%), *Polygonum aviculare* L. (0.5%), and *Urtica dioica* L. (leaf) (2.5%)—heavier by 207%, 206%, and 205%, respectively. Most of biostimulants increased the content of pigments involved in photosynthesis (e.g. 156% more chlorophyll for 0.1% *Hypericum perforatum* L. extract), decreased the content of polyphenols (e.g. 47% less for 2.5% *Trifolium pretense* extract), and showed a varied impact on antioxidant activity. There is an increasing interest in botanical extracts due to their high content of biologically active compounds and wide variety of application possibilities.

Keywords: biostimulants; extracts of higher plants; ultrasound-assisted extraction; white head cabbage seedlings; foliar spray

1. Introduction

The major problem facing the modern agriculture science is the maintenance of food production with the aim of satisfying needs of a growing global population without compromising natural resources for future generations [1]. The United Nations Food and Agriculture Organisation (FAO) predicts that by 2050 the population will increase to 10 billion [2]. According to FAO estimates, the number of undernourished world population has expanded from around 804 million (2016) to almost 821 million (2017). Due to some progress, these levels still remain very high. Nearly 151 million children

under five were affected by stunting in 2017. A lack of food security redounded to undernutrition, overweight, and obesity in many countries. The greater risk of overweight and obesity can be related to the stress of food insecurity, higher price of nutritious food, and physiological adaptations to food restriction. It is estimated that over 672 million people are obese and this number increases every year [3].

The condition of the proper nutrition, ensuring normal functioning of the body, is to provide sufficient amounts of clean water and adequate energy which, depending on the age, gender and occupation of the individuals, is about 2400 kcal, supplemented with optimal levels of nutritional and health-promoting substances. Currently, the intensification of agriculture has reached a critical point as a result of which negative effects occur such as non-reversible global climate change and losses in many ecosystem services [4]. The primary agricultural pollutants that pose a threat to the environment are pesticides, nitrates (resulting from nitrogen-rich fertilisers) and phosphorus [5]. The greatest opportunity for increasing food production is the intensification of yields and quality that can be achieved through the rational use of mineral and organic fertilisers, plant protection products, and water. This process should proceed in a sustainable way—without causing risks for both the environment and consumers [4]. Therefore, in the last decades, crop production has concerned the ameliorate of cheap, sustainable, and eco-friendly systems to provide high yields of nutritious food [5].

Biostimulants of plant growth have lately attained increasing attention worldwide [5]. By improving the resilience of plants against stresses, biostimulants contribute to increased quantity and quality of yield, lack of pesticide residues and richness in healthy substances [4]. Such technological solutions would benefit the environment, by limiting the use of pesticides and nitrogen fertilizers, which is of particular importance in Poland due to the implementation of the Nitrates Directive [6]. The sector of plant biostimulants has been emerging and is still being defined. According to The European Biostimulant Industry Council (EBIC) "Plant biostimulants contain substance(s) and/or micro-organisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality" [7]. Furthermore, they can boost the activity of soil enzymes, rhizosphere microbes, the production of hormones and/or growth regulators, and the photosynthesis [5]. Specific activities such as enhanced roots and shoots growth, tolerance to biotic and abiotic stresses, water intake, reduction of transplant shock etc., have been also highlighted. For this reason, a critical role for biostimulants in the agriculture is expected. The market of biostimulants is evaluated to have been worth US\$1.4 billion in 2014 and is projected to reach \$2.5 billion by 2019. The anticipated factors of this increase involve (1) increasing significance for organic products; (2) growth of biostimulants use in developing countries; and (3) greater acceptance of biostimulants among customers globally [8]. Nowadays, the regulatory situation for this type of products is very complex owing to the shortage of any defined framework in Europe or United States. In the EU, they are placed on the market by one of two routes: (1) the national regulations on fertilisers, or (2) the European pesticides lawlinking the national and supranational regulations regarding the launch of plant protection products to the market [4]. The scientific literature classifies biostimulants as: humic and fulvic acids [9], protein hydrolysates and other N-containing compounds [10], seaweed and botanical extracts [11], chitosan and other biopolymers [12], inorganic compounds [13], beneficial fungi [14], and beneficial bacteria [15].

In the literature, there are many reports of the application of various seaweed extracts in plant cultivation. The fresh seaweeds had been used as fertilisers and a source of organic matter since ancient times, but the biostimulating properties were not recognised [16]. Algae-based products are universal and can be applied on soils, in hydroponic solutions (they reduce the use of fertilisers and concentration of nutrient solutions) or as foliar or seed treatments [17]. Several plant growth promotion constituents are unique to the algae species, which clarifies the increasing attention of growers, scientists, and industry for this group of photosynthetic organisms [18]. They contain for example polysaccharides responsible for water retention, gel formation, and soil aeration and polyanionic compounds participating in cations fixation and exchange. Seaweeds also positively

affect soil microorganisms due to the promotion of plant growth-promoting bacteria and pathogen antagonists in suppressive soils. The biostimulating activity on crop plants (e.g., the impact on seed germination, plant establishment, growth and development) is related to hormonal activity [4]. There are many articles concerning seaweed/algal biostimulants as effective preparations increasing the yield and biochemical value of plants [19–24]. Compared with algae, less is reported about the activity of extracts derived from higher plants. Substances obtained from plants can be used in pharmaceutical, cosmetic, and plant products, as well as food ingredients. The use of higher plants (e.g., oak, soy, carrot root, blueberry fruits) for the production of biostimulants arouses growing interest of scientists [25–30]. The use of botanical extracts shows a prospective future in the functional plant nutrition associated with increased food quality parameters. Biostimulants have a rich composition and may contain, e.g., plant hormones, hormone-like substances, proteins, betaines, amino acids, peptides, sugars, lipids, vitamins, elements, phenolic compounds, furostanol glycosides, sterols. The content of a particular chemical compound does not necessarily mean that it is functional [31].

Selected raw materials for this research are common plants, especially in Europe, containing a wide range of compounds with potential biostimulatory effects (e.g., inulin, phytosterols, tannins, minerals). For the production of innovative biostimulants, we selected the following raw materials: aloe (*Aloë vera* L. Burm. f.) (leaf), chokeberry (*Aronia melanocarpa* (Michx) Elliott) (fruit), red beet (*Beta vulgaris* L.) (root), horsetail (*Equisetum arvense* L.) (herb), common sea-buckthorn (*Hippophae rhamnoides* L.) (fruit), hypericum (*Hypericum perforatum* L.) (herb), red lentil (*Lens culinaris* Medik.) (seeds), common bracken (*Pteridium aquilinum* L.) (leaf), knotgrass (*Polygonum aviculare* L.) (herb), pea (*Pisum sativum* L.) (seeds), broadleaf plantain (*Plantago major* L.) (herb), red clover (*Trifolium pratense* L.) (flower), nettle (*Urtica dioica* L.) (leaf and root). The health-promoting benefits of these raw materials result from the presence of numerous bioactive compounds (Table 1).

Table 1. Biologically active compounds in the examined plant biomass.

Species	Active Compounds	References
Aloe <i>Aloe vera</i> L. Burm. f.	Vitamins (A, C, E, B1, B2, B6, B9, B12), choline, enzymes (alialase, alkaline phosphate, amylase, bradykinase, carboxypeptidase, catalase, cellulose, lipase, peroxidase), minerals (Ca, Cr, Cu, Se, Mg, Mn, K, Na, Zn, Fe, P), sugars: monosaccharides (glucose, fructose), polysaccharides (glucomannans/polymannose), anthraquinones (aloin, emodin), fatty acids (cholesterol, campesterol, β -sisosterol, lupeol), hormones (auxins, gibberellins), amino acids (A, R, D, E, G, H, I, L, K, M, F, P, T, Y, V), proteins (lectins), salicylic acid, lignin, saponins, carbohydrates (pure mannan, acetylated mannan, cellulose, pectic substance, xylan)	[32–34]
Aronia <i>Aronia melanocarpa</i> (Michx) Elliott	Carbohydrate (glucose, fructose, sucrose, sorbitol), dietary fibre (pectins), fat, proteins, organic acids (l-malic acid, citric acid, isocitric acid, tartaric acid, quinic acid, succinic acid, fumaric acid), vitamins (C, B1, B2, B3, B5, B6, B9, E, K), minerals (Na, K, Ca, Mg, Fe, Zn, I), phytochemicals (carotenoids: β -carotene, β -cryptoxanthin; phenols; amygdalin), nitrate, nitrite	[35–37]
Red beet <i>Beta vulgaris</i> L. subs. <i>vulgaris</i>	Nitrate, phenolics (flavonoids, phenolic acids, phenolic amides), vitamins (A, B), carotenoids, betalains (betacyanins: betanin, isobetanin; betaxanthins: vulgaxanthin I, vulgaxanthin II, indicaxanthin), minerals (Mg, Na, K, P, Ca), sugars, proteins, fibre	[38–40]
Horsetail <i>Equisetum arvense</i> L.	Alkaloids, phytosterols, tannin, triterpenoids, phenolics (flavonoids, styrylpyrones, phenolic acids), aromatic compounds (benzothiazole, homovanillic acid, isovanillin), terpenes (linalool, β -caryophyllene), isoprenoid derivatives (α -ionone, (E, Z)—pseudoionone), silicic acid	[41–43]
Common sea-buckthorn <i>Hippophae rhamnoides</i> L.	β -carotene, organic acids, essential oils, polyphenols, flavonoids, phytosterols, tocopherols, vitamins (K, C, E, B complex), polyunsaturated fatty acids, coumarins, triterpenes, protein (globulins, albumins), amino acids, carbohydrates, minerals (N, Ca, K, Na, Mg, Cu, Fe, Zn, Mn)	[44–46]
Hypericum <i>Hypericum perforatum</i> L.	Naphthodianthrones, phloroglucinols, flavonoids, biflavones, phenylpropanes, proanthocyanidins, tannins, xanthenes, essential oils, amino acids, procyanidins, hypericin, pseudohypericin, hyperforin, melatonin, minerals (B, Cu, Fe, Mg, Mn, Mo, Zn)	[47–49]
Red lentil <i>Lens culinaris</i> Medik.	Protein (lectins, defensins, protease inhibitors), bioactive peptide, complex carbohydrate fractions, particularly the resistant starches, oligosaccharides, dietary fibre, antioxidants, non-nutritive bioactive phytochemicals, minerals (Ca, Fe, Mg, P, K, Na, Zn, Cu, Mn, Se), vitamins (C, A, E, K, B1, B2, B3, B5, B6, B9), carotene, choline, lipids (fatty acids, cholesterol), polyphenolics, phytic acid, saponins, phytosterols	[50–52]
Common bracken <i>Pteridium aquilinum</i> L.	Crude fibre, holocellulose, α -cellulose, lignin, crude protein, crude fat, macroelements (N, K, P, S, Ca, Mg), microelements (Fe, Mn, Zn, Cu, Cr), thiaminase, cyanogen glycosides, pterosins, flavonoids (kaempferol, apigenin, quercetin), phenoloids (cinnamic acid, benzoic acid, phenoloid polymers: tannins), volatile compounds (benzaldehyde, limonene, linalool)	[53–55]

Table 1. Cont.

Species	Active Compounds	References
Knotgrass <i>Polygonum aviculare</i> L.	Flavonoids (kaempferol, baicalin, quercetin, myricetin, isoquercetin, luteolin, avicularin, rutin, astragalin), quinones (chrysophanol, emodin, rhein, aloe-emodin), phenylpropanoids, terpenoids (triterpenoids, diterpene), phenolic acids (gallic acid, protocatechuic acid, chlorogenic acid), stilbene glycosides, alkaloids, rosemary acid, caffeic acid, coumaric acid, hirsutine, tadeonal, isotadeonal, apianen lactones, phytohormones	[56–58]
Pea <i>Pisum sativum</i> L.	Proteins (albumin, vicilin, legumin), amino acids, carotenoids, fibre, enzyme inhibitor, lectin, choline, phytic acid, phenolics, carbohydrates (starch, oligosaccharides, amylose), vitamins (A, B, C), lipids, saponins, tannins, minerals (Ca, Fe, Mg, P, K, Na, Zn, Se)	[59–61]
Broadleaf plantain <i>Plantago major</i> L.	Phenylethanoid glycosides, triterpenoids, polysaccharides, phenolic acids, alkaloids, phenolic compounds (caffeic acid derivatives), coumarins, fats and oils, mucilage, sterols, volatile substances, flavonoids, vitamins (C, A)	[62–64]
Red clover <i>Trifolium pratense</i> L.	Isoflavones (biochanin A), pterocarpans, coumestrols, lignans, medicagol, coumarin, soyasaponins, clovamides, flavonoids, afrormosin, daidzein, genistein, methyl orobol, irilin, irilone, formononetin, tyramine, fisetin, calycosin, quercetin, naringenin, pratensein, kaempferol, pseudobaptigenin, irilone, prunetin	[65–67]
Nettle <i>Urtica dioica</i> L.	Agglutinin, acetophenone, alkaloids, acetylcholine, chlorogenic acid, butyric acid, chlorophyll, caffeic acid, carbonic acid, choline, histamine, coumaric acid, formic acid, pantothenic acid, kaempferol, coproporphyrin, lectin, lecithin, lignan, linoleic and linolenic acids, palmitic acid, quercetin, quinic acid, serotonin, stigmasterol, terpenes, violaxanthin, succinic acid, fixed oil, fatty substance, albumins, protein, vitamins (A, C, B1, K), provitamin A, carotenoids, xanthophyll, oxalate, histamine, acetylcholine, sistosterin, ferric oxide, minerals (N, K, Ca, Si), flavonoids, tannins, volatile compounds, polysaccharides, sterols, amino acids, phytohormones (cytokinins)	[68–70]

2. Materials and Methods

2.1. Chemicals

All the reagents (acetone, calcium carbonate, sodium carbonate, ethanol, methanol, potassium persulphate, acetic acid, sodium acetate, Folin-Ciocalteu's phenol reagent, Trolox, gallic acid, DPPH, ABTS, FRAP, TPTZ) were of analytical grade and purchased from IDALIA (Radom, Poland) and Archem (Lany, Poland).

2.2. Plant Materials

In our previous article [71] we have conducted research on 12 biomasses, whereas in this article we performed a second series of tests for 14 another medicinal plants popular and easily available in Poland, which have never been tested as a raw material to receive biostimulants of plant growth. The time of the raw materials collection were adjusted to the plant growth stage that exhibits the highest level of active constituents. They were collected/purchased once in 2017 in the amount needed to carry out all the research. We examined the biostimulatory effect of the following biomasses: aloe (*Aloë vera* L. Burm. f.) (leaf) (marked as: Al L), chokeberry (*Aronia melanocarpa* (Michx) Elliott) (fruit) (Ar Fr), red beet (*Beta vulgaris* L.) (root) (Bv R), horsetail (*Equisetum arvense* L.) (herb) (Eq H), common sea-buckthorn (*Hippophae rhamnoides* L.) (fruit) (Hr Fr), hypericum (*Hypericum perforatum* L.) (herb) (Hp H), red lentil (*Lens culinaris* Medik.) (seeds) (Lc S), common bracken (*Pteridium aquilinum* L.) (leaf) (Paq L), knotgrass (*Polygonum aviculare* L.) (herb) (Pav L), pea (*Pisum sativum* L.) (seeds) (Pi S), broadleaf plantain (*Plantago major* L.) (herb) (Pm H), red clover (*Trifolium pratense* L.) (flower) (Tp F), nettle (*Urtica dioica* L.) (leaf and root) (Ur L, Ur R). The raw materials were purified, dried (50°C), grinded (500 µm), averaged, and appropriately stored in bags.

2.3. Extracts Production

The ultrasound assisted extraction (UAE) was used for the production of environmentally friendly and rich in sensitive bioactive compounds biostimulants. The deionised water was used as a solvent. The methodology of extracts production is presented by Godlewska et al. [71]. The dried and milled raw materials were stirred with deionised water (1:20). After 30 minutes ($23 \pm 2^\circ\text{C}$), ultrasounds (40 W, 30 min, homogenizer UP 50H). Subsequently, mixtures were centrifuged (4500 rpm, 10 min, Heraeus Megafuge 40, rotor TX-750, Thermo Scientific, Waltham, MA, USA). The resulting solutions (100% extracts) were stored in a refrigerator in dark glass bottles.

2.4. Utilitarian Properties of Extracts

To investigate the impact of produced extracts on the white head cabbage growth, the screening tests under controlled conditions (germination tests) were conducted. These studies indicate also the potential phytotoxicity of the tested extracts. The general scheme of experiments is presented in Figure 1.

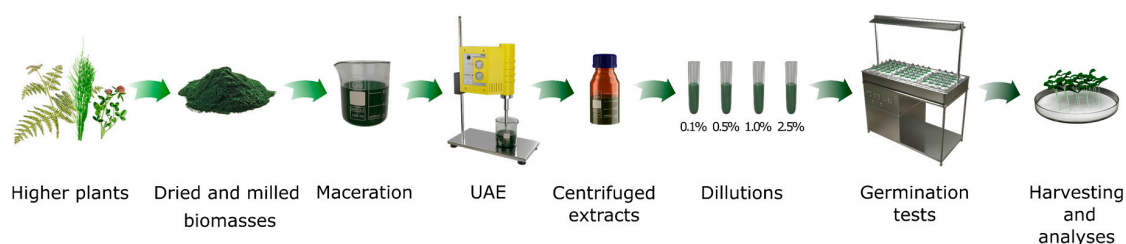


Figure 1. The schematic methodology of the experiment.

2.4.1. Germination Tests

In the germination tests, 25 seeds of white head cabbage (cultivar ‘Socrates F₁’, Syngenta) were placed on the filter paper in Petri dishes which were put on the Jacobsen apparatus (Laborset, Lodz, Poland) [71]. For the experiment a completely randomized design was used. After six, eight, and ten days dishes were sprayed with 1 mL of extracts (examined concentrations: 0.1, 0.5, 1.0, and 2.5%) or deionised water (C) or commercial biostimulant (CB). The tests lasted 14 days, and after that time plants were collected, measured, weighed, and subjected to further analyses. The hypocotyl with cotyledons is named as seedling shoot later in the text.

2.4.2. The Concentration of Pigments and Greenness Index of the Leaf

The total chlorophyll ($a + b$) and carotenoids concentrations were analysed in the fresh cotyledon (0.2 g) of cabbage seedlings grounded up with few drops of 80% acetone, pinch of sand and calcium carbonate, and then filtered, quantitatively transferred, and filled up to 50 mL. The absorbances (663, 645, and 470 nm) were measured in three replicates using UV-Vis spectrophotometer (HACH DR1900). The exact methodology and calculation formulas are described in our research [71]. The greenness index of five cotyledons from each Petri dish was measured in three replicates using chlorophyll meter SPAD 502 Plus (Konica Minolta, Europe).

2.4.3. The Concentration of Polyphenols in Cabbage Seedlings

The concentrations of total phenolic compounds (TPC) in cabbage seedlings were made in accordance to the Folin-Ciocalteu method presented by Jałoszyński et al. [72] with modifications [71]. The fresh, grounded plants (2 g) were sonicated with 20 mL of 80% aqueous methanol for 15 minutes and centrifuged (10 min, 4500 rpm). The obtained supernatants (0.1 mL) were mixed with Folin-Ciocalteu’s phenol reagent (0.2 mL), distilled water (2.0 mL), and incubated at room temperature for 3 minutes. Next, 20% sodium carbonate (1.0 mL) was added, and left for 1 hour in the dark. The absorbance (765 nm) was determined using a portable spectrophotometer (HACH DR1900). The results were expressed as gallic acid equivalents (GAE) ($\text{mg} \cdot 100 \text{ g}^{-1} \text{ f.m.}$). All measurements were made in triplicate.

2.4.4. The Antioxidant Activity (DPPH, ABTS, FRAP) of Cabbage Seedlings

To determine the antioxidant activities, obtained supernatants were tenfold diluted with 80% methanol. The DPPH radical scavenging activity was determined according to Yen and Chen [73] with slight modifications [71]. The supernatants (0.5 mL) were mixed with ethanol (1.5 mL), and fresh DPPH solution (0.5 mL), shaken, and left at room temperature in the dark. After 10 minutes, the absorbance was measured at 517 nm. The ABTS assay was made as stated by Re et al. [74] and Almeida et al. [75] with modification [71]. To the plant supernatants (30 μL), 3 mL of blue-green ABTS solution were added and allowed to react with in the dark condition. After 6 minutes, the absorbance at 734 nm was measured. The FRAP antioxidant capacity was carried out in accordance with the procedure of Benzie et al. [76]. Freshly prepared ferric reducing antioxidant power reagent (3 mL) was added to supernatants (1 mL) and allowed to react for 10 minutes. Next, the absorbance at 593 nm was measured. The results for antioxidant activities were expressed in μM Trolox per 1 g f.m. and all measurements were made in triplicate.

2.5. Statistical Analysis

The collected data were subjected to statistical analysis using Statistica 13.1 (StatSoft Polska Sp. z o.o., Kraków, Poland). Shapiro–Wilk test was used for the normality of distribution assessment. For the normal distribution, Brown–Forsythe test was used. Statistically significant differences were estimated with the (HSD) Tukey test. Results were considered as significantly different when $p < 0.05$. For distribution other than normal, Kruskal–Wallis test was applied. The foliar spray of produced botanical extracts affected the growth and development of cabbage seedlings in a diversified way.

Statistically significant differences between examined extracts and the control group (water) were marked with “a”, while between examined extracts and commercial product with “b”.

3. Results

Obtained results showed a statistically significant influence of the tested botanical extracts on the growth, composition and antioxidant activity of cabbage seedlings. The effect of produced biostimulants was compared with the control groups (C and CB) examined in our previous work [71].

3.1. The Effect of Extracts on the Length of Shoots and Roots

The effect of plant extracts on the length of shoots and roots of white head cabbage seedlings is presented in Figures 2 and 3, respectively. Almost all tested extracts positively influenced the mean root length, including red beet (Bv R), horsetail (Eq H), common sea-buckthorn (Hr Fr), hypericum (Hp H), knotgrass (Pav H), pea (Pi S), broadleaf plantain (Pm H, beside concentration 0.1%), red clover (Tp F), nettle root and leaf (Ur R, Ur L). The values of root length were higher than in the control group. A weaker effect was observed for extracts produced from aloe (Al L), chokeberry (Ar Fr), red lentil (Lc S) and common bracken (Paq L). The concentration of plant extracts had no significant effect (beside horsetail (Eq H), pea (Pi S) and red clover (Tp F)) on the mean root length.

There is no direct relationship between concentration of a given extract and mean shoot length. For extracts produced from chokeberry (Ar Fr), horsetail (Eq H), common sea-buckthorn (Hr Fr), red clover (Tp F) the best concentration was 2.5 %. Extract—1.0% from aloe (Al L) provided the highest shoot length of cabbage. Further, 0.5% concentration of extracts produced from red beet (Bv R), red lentil (Lc S), common bracken (Paq L), knotgrass (Pav H), and pea (Pi S) stimulated the shoot growth in the highest extent. The lowest concentrations of extracts from hypericum (Hp H), broadleaf plantain (Pm H), nettle (Ur L and Ur R) resulted in the highest mean shoot length of cabbage seedlings. Generally, 0.5% concentration of plant extracts occurred most often among other tested.

The best results were for extract obtained from knotgrass (Pav H), applied in a concentration of 1.0%, and the mean root length was 72% higher than in the control group (water) and 76% than in the group with commercial biostimulant.

Taking into account the examined concentration of extracts, 2.5% was the best for extracts from chokeberry (Ar Fr), red beet (Bv R), broadleaf plantain (Pm H); 1.0% for extracts from horsetail (Eq H), knotgrass (Pav H), pea (Pi S), red clover (Tp F), nettle (Ur R); 0.5% for extracts from aloe (Al L), hypericum (Hp H), common bracken (Paq L), broadleaf plantain (Pm H) and 0.1% for extracts from common sea-buckthorn (Hr Fr), red lentil (Lc S), nettle (Ur L). Among tested extracts and their concentrations, 1.0% occurred the most often and can be recommended for further research.

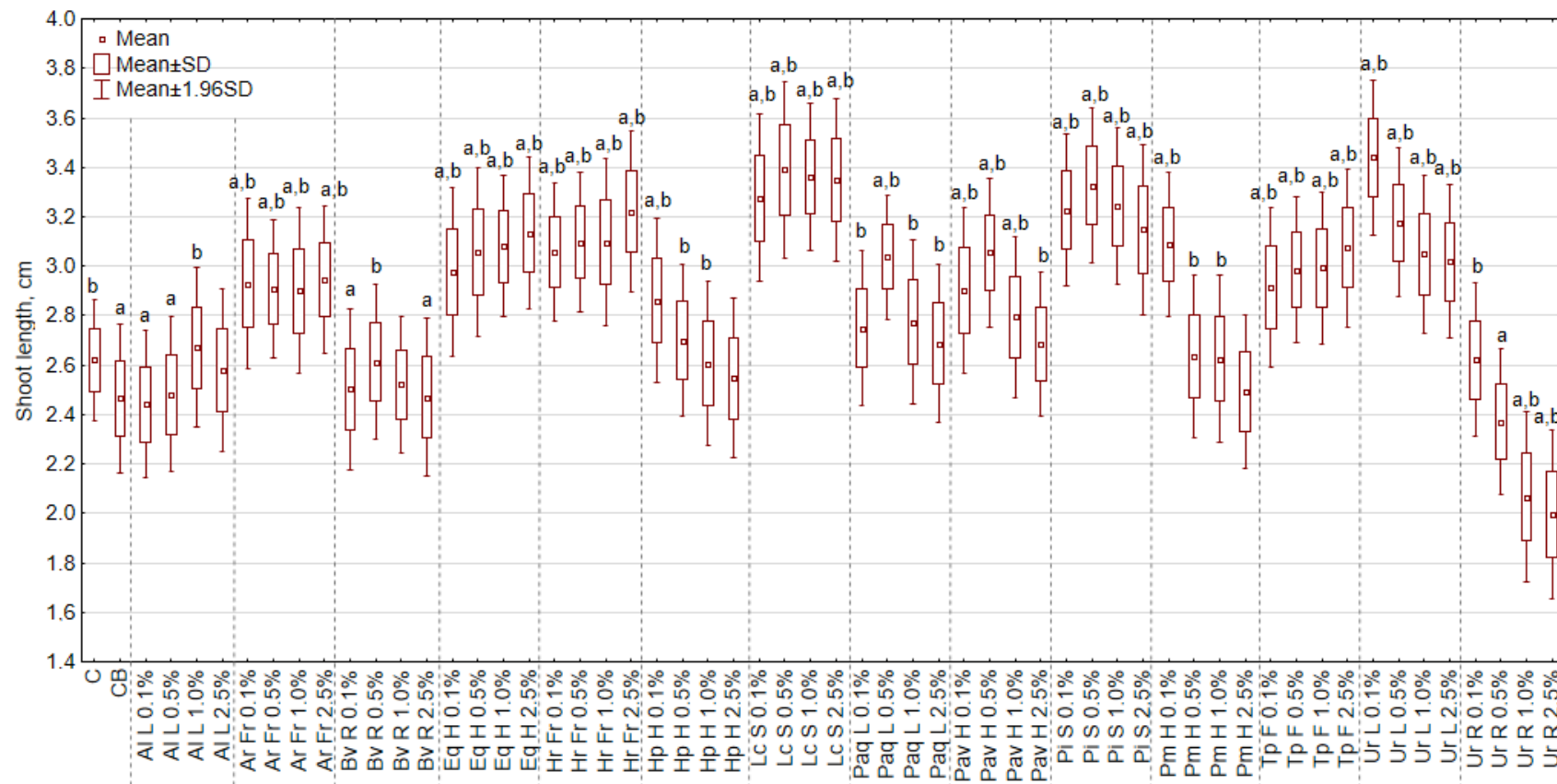


Figure 2. The shoot length of white head cabbage seedlings after application of plant extracts. (a) Statistically significant differences ($p < 0.05$) between the control group (C) and extracts. (b) Statistically significant differences ($p < 0.05$) between commercial biostimulant (CB) and extracts. Abbreviations: Al L, *Aloë vera* L. Burm. f. (leaf); Ar Fr, *Aronia melanocarpa* (Michx) Elliott (fruit); Bv R, *Beta vulgaris* L. (root); Eq H, *Equisetum arvense* L. (herb); Hr Fr, *Hippophae rhamnoides* L. (fruit); Hp H, *Hypericum perforatum* L. (herb); Lc S, *Lens culinaris* Medik. (seeds); Paq L, *Pteridium aquilinum* L. (leaf); Pav L, *Polygonum aviculare* L. (herb); Pi S, *Pisum sativum* L. (seeds); Pm H, *Plantago major* L. (herb); Tp F, *Trifolium pratense* L. (flower); Ur L, *Urtica dioica* L. (leaf); Ur R, *Urtica dioica* L. (root).

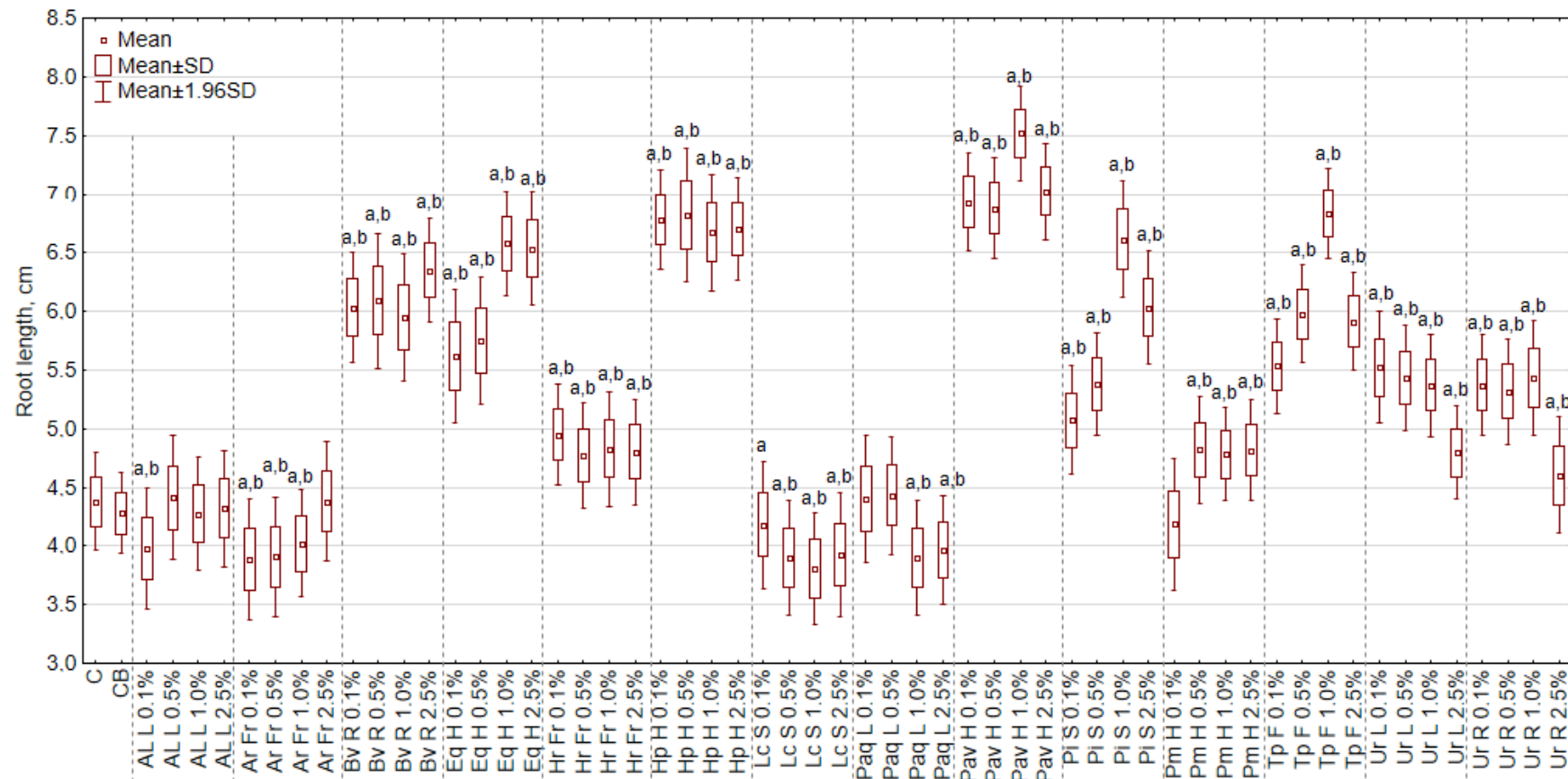


Figure 3. The root length of white head cabbage seedlings after application of plant extracts. (a) Statistically significant differences ($p < 0.05$) between the control group (C) and extracts. (b) Statistically significant differences ($p < 0.05$) between commercial biostimulant (CB) and extracts. Abbreviations as in Figure 2.

3.2. The Effect of Extracts on the Fresh Weight of Shoots

The effect of plant extracts on the shoot fresh weight of white head cabbage seedlings is presented in Figure 4. Generally, the results in all tested groups were better than in the control groups. The best results, taking into account the mean fresh weight of shoots, were obtained for nettle (Ur L, all examined concentrations) and for horsetail (Eq H, only 0.5% concentration). For common nettle (Ur L, 0.1%) and horsetail (Eq H, 0.5%), the mean fresh weight of shoots was almost two times higher than in the control group (both water and commercial biostimulant). Significantly higher values of the mean fresh weight of shoots were obtained also for cabbage seedlings treated with extracts from red lentil (Lc S) and pea (Pi S). These data correspond to the results presented in Figure 2 (shoot length). The highest shoot length, 3.4 cm, was obtained for nettle (Ur L, 0.1%) and the highest mean fresh weight of shoots, 1.8 g, also for nettle (Ur L, 0.1%). The dominant extracts affecting both mean shoot length and mean fresh weight of shoots were the extracts produced from red lentil and pea.

Considering the concentration of plant extracts, 0.1% was the best for extracts produced from chokeberry (Ar Fr), pea (Pi S), common bracken (Paq L), red lentil (Lc S), red clover (Tp F) and nettle (Ur L). Concentration 0.5% dominated only for horsetail (Eq H), whereas 1.0% for extracts from red beet (Bv R), hypericum (Hp H), knotgrass (Pav H), broadleaf plantain (Pm H) and common nettle (Ur R). The highest concentration 2.5% provided the highest mean fresh weight of shoots for extracts from aloe (Al L) and common sea-buckthorn (Hr Fr). This confirms one of the properties of plant growth biostimulants, that they are especially active at low concentrations. Based on these data, the dose 0.1% can be selected for further testing.

3.3. The Effect of Extracts on the Fresh Weight of Roots

The effect of plant extracts on the root fresh weight of white head cabbage seedlings is showed in Figure 5. The highest fresh weight of roots was observed for horsetail (Eq H, 0.5 and 1.0%), knotgrass (Pav H, 0.5% and 1.0%) and nettle (Ur L, 2.5%). It was approximately three times higher than in the control group with water and two times higher than in the group with commercial biostimulant. In the case of knotgrass (Pav H, 1.0%), the highest root length was also measured (Figure 3).

The weakest results (but still higher than for water and comparable to commercial biostimulant) were obtained for extracts of aloe (Al L) and common bracken (Paq L). Results for these two groups coincide with the results obtained for the root length (Figure 3).

There is no clear relationship between the root fresh weight and the concentration of plant extracts. 0.1% increased the fresh weight of cabbage seedlings after application of extracts from red beet (Bv R), red lentil (Lc S), common bracken (Paq L), red clover (Tp F), 0.5% for extracts from horsetail (Eq H) and knotgrass (Pav H), 1.0% for extracts from pea (Pi S) and broadleaf plantain (Pm H), and finally 2.5% for extracts from aloe (Al L), chokeberry (Ar Fr), common sea-buckthorn (Hr Fr), hypericum (Hp H), nettle leaf (Ur L) and nettle root (Ur R). Among tested extracts and their concentrations, 2.5% occurred the most often.

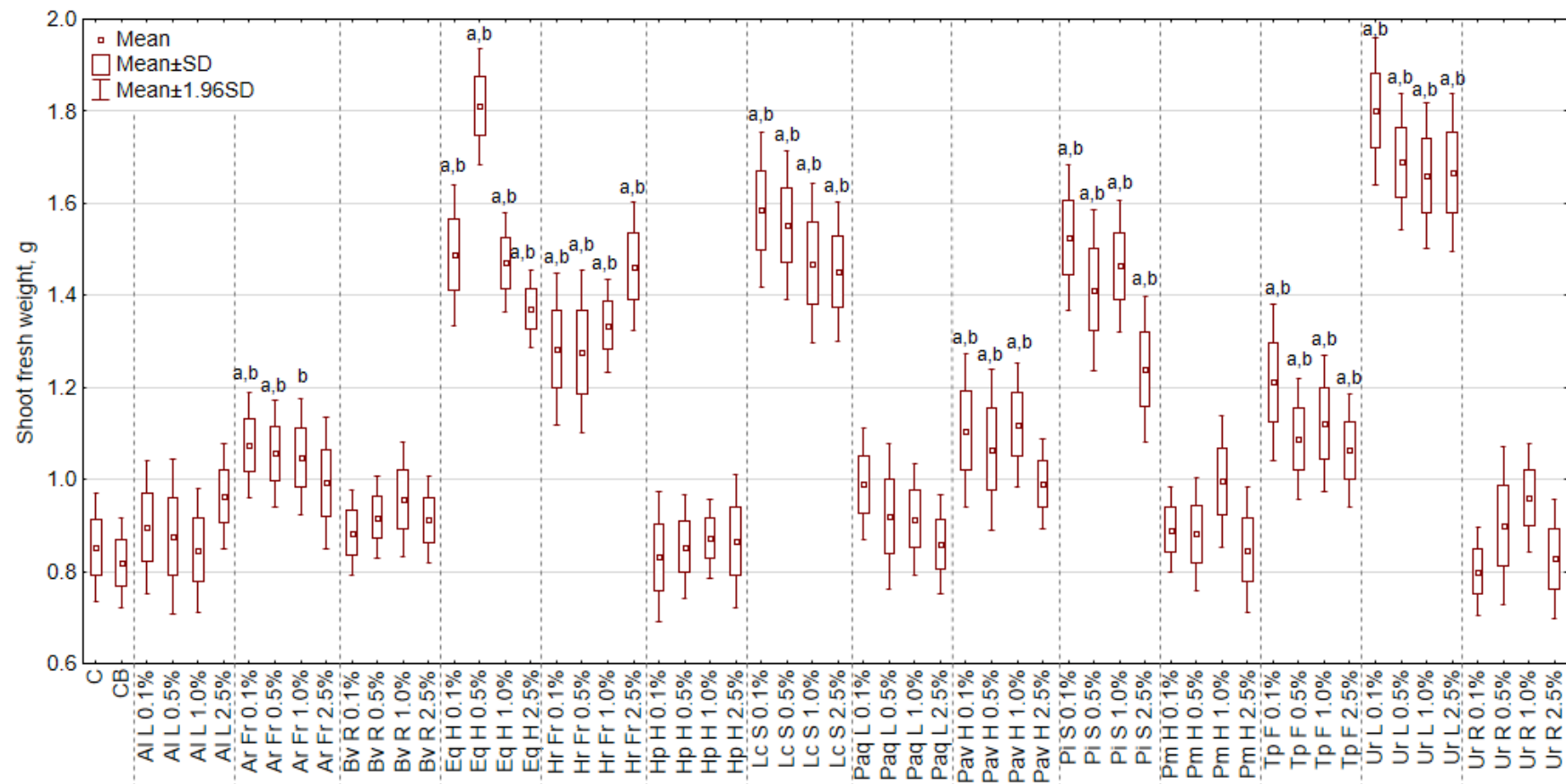


Figure 4. The shoot fresh weight of white head cabbage seedlings after application of plant extracts. (a) Statistically significant differences ($p < 0.05$) between the control group (C) and extracts. (b) Statistically significant differences ($p < 0.05$) between commercial biostimulant (CB) and extracts. Abbreviations as in Figure 2.

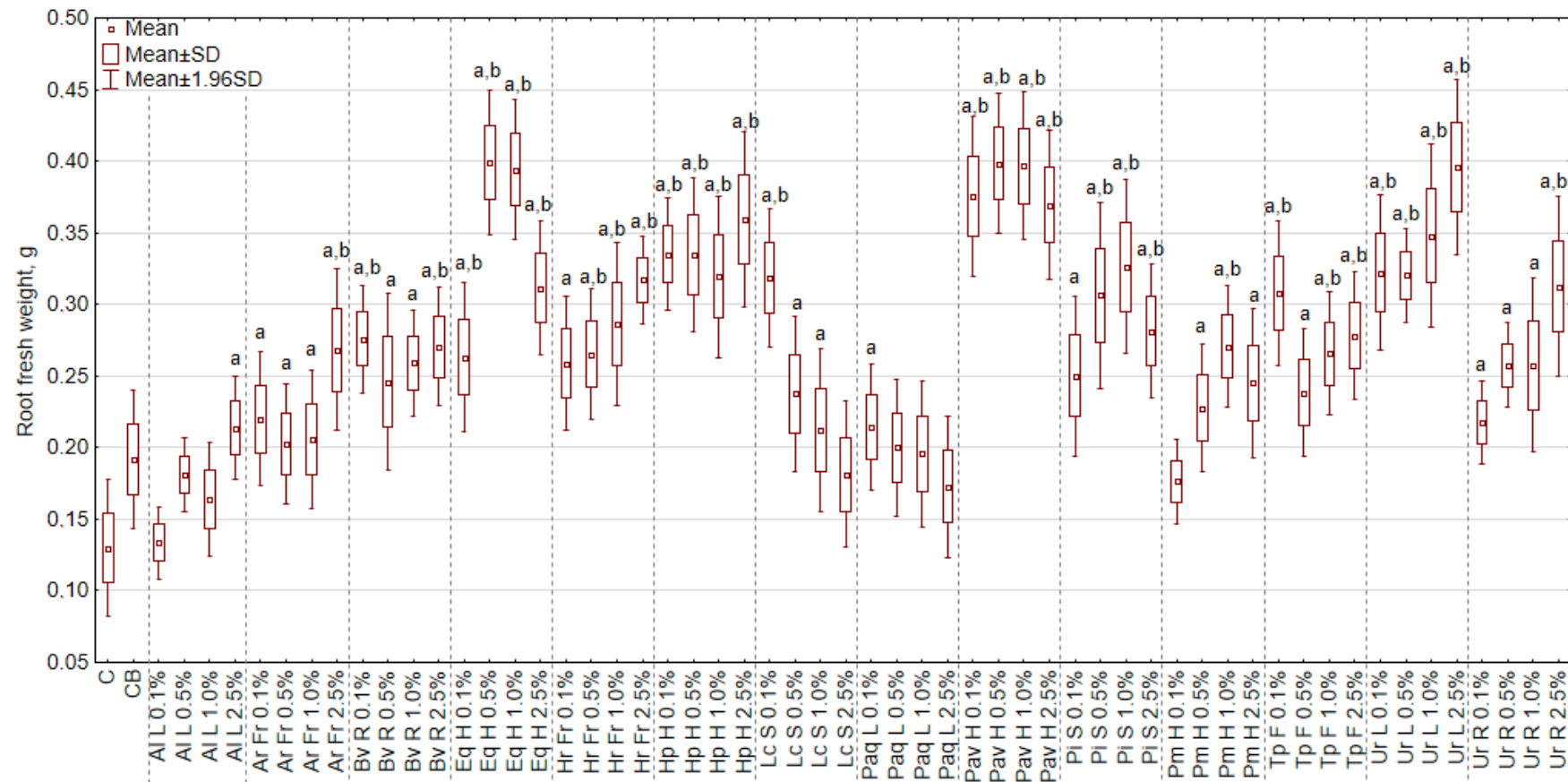


Figure 5. The root fresh weight of white head cabbage seedlings after application of plant extracts. (a) Statistically significant differences ($p < 0.05$) between the control group (C) and extracts. (b) Statistically significant differences ($p < 0.05$) between commercial biostimulant (CB) and extracts. Abbreviations as in Figure 2.

3.4. The Effect of Extracts on the Dry Weight of Shoots

All tested extracts in all concentrations (with exception of only nettle – root 0.1%) provided higher dry weight of cabbage shoots than both control groups (Figure 6). Statistically significant differences were observed between dry weight in the control group and the following extracts: horsetail (Eq H), common sea buckthorn (Hr Fr), red lentil (Lc S), pea (Pi S), red clover (Tp F) and common nettle (Ur L).

The best results, taking into account the dry weight of cabbage seedlings shoots, were obtained in the group treated with extract from nettle (Ur L, 0.1%), then horsetail (Eq H, 0.5%) and pea (Pi S, 0.1%). For nettle, it was 64% higher than in C and 67% higher than in CB, for horsetail by 55% higher than in C and 58% higher than in CB and for pea 50% higher than in C and 52% higher than in CB. These results coincide with the results of shoot fresh weight presented in Figure 4.

Taking into account concentrations of the applied extracts, 0.1% was the best for chokeberry (Ar Fr), red lentil (Lc S), common bracken (Paq L), knotgrass (Pav H), pea (Pi S), red clover (Tp F) and nettle (Ur L). The linear correlation was observed: the lower concentration of plant extract, the higher dry weight of cabbage shoots. Concentration 0.5% was effective only for extract from horsetail (Eq H) and 1.0% for extracts from red beet (Bv R), broadleaf plantain (Pm H) and nettle (Ur R). The highest concentration, 2.5%, was the best for extract from aloe (Al L), common sea buckthorn (Hr Fr), and hypericum (Hp H). For these three extracts, with the increase of concentration, the dry weight of cabbage shoots increases.

When the fresh and dry weight of cabbage shoots is considered as a crucial parameter of plant growth, extracts from nettle (Ur L), horsetail (Eq H) and pea (Pi S) at lower concentrations are recommended.

3.5. The Effect of Extracts on the Dry Weight of Roots

All examined plant extracts demonstrated a positive effect on this parameter when compared to the control group treated with water (Figure 7). In the case of commercial biostimulant, weaker effect was observed only for extracts of aloe (Al L, 0.1%, 0.5% and 1.0%), red lentil (Lc S, 2.5%), common bracken (Paq L, 2.5%) and broadleaf plantain (Pm H, 0.1%).

The best results for dry weight of roots were obtained for extracts produced from knotgrass (Pav H, 0.5%), nettle (Ur L, 2.5%) and horsetail (Eq H, 0.5%). Dry weight of cabbage roots in the group treated with knotgrass (Pav H, 0.5%) was higher by 53% than in the control group and by 33% than in the group with commercial biostimulant, for the group treated with common nettle (Ur L, 2.5%) by 52% and 32%, respectively and for the group treated with horsetail (Eq H, 0.5%) by 51 and 32%, respectively. These data correspond to the results presented in Figure 5, where the effect of plant extracts on root fresh weight is showed.

Taking into account the concentration of the examined plant extracts, 0.1% was the best for red beet (Bv R), red lentil (Lc S), common bracken (Paq L), red clover (Tp F)—for these extracts, dry weight of roots decreases with the increase of extract concentration. In the case of 0.5% extracts, significant effect was observed for the following biomasses - horsetail (Eq H), knotgrass (Pav H) and broadleaf plantain (Pm H). 1.0% concentration was the best for extracts produced from pea (Pi S) and broadleaf plantain (Pm H), whereas the highest concentration of extracts—2.5% for aloe (Al L), chokeberry (Ar Fr), common sea-buckthorn (Hr Fr), hypericum (Hp H), nettle (Ur L and Ur R). For this concentration with the increase of extract concentration, dry weight of roots increases. Similarly, as for fresh weight of roots (Figure 5), the concentration 2.5% occurred the most often and can be recommended for further research.

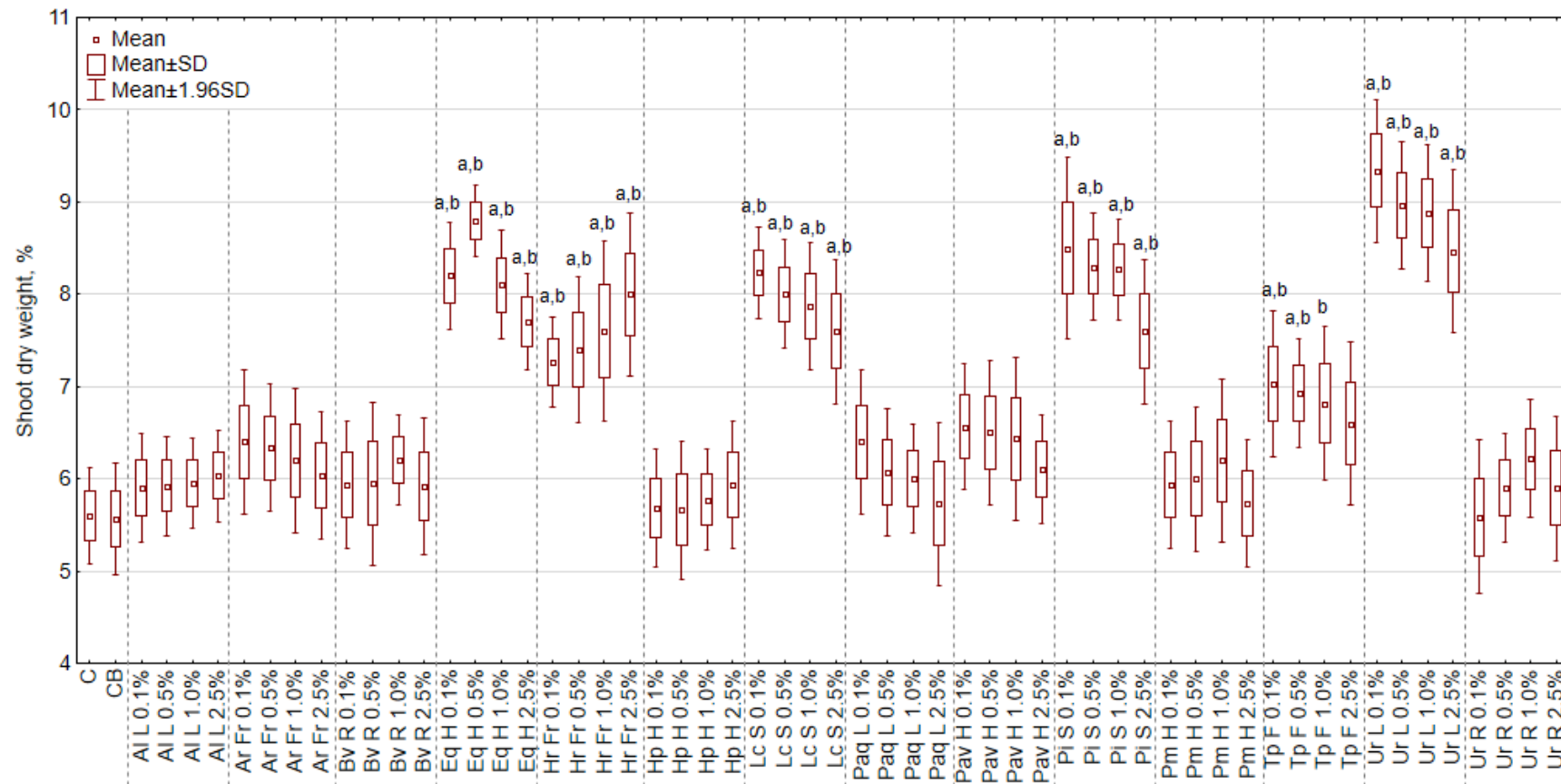


Figure 6. The shoot dry weight of white head cabbage seedlings after application of plant extracts. (a) Statistically significant differences ($p < 0.05$) between the control group (C) and extracts. (b) Statistically significant differences ($p < 0.05$) between commercial biostimulant (CB) and extracts. Abbreviations as in Figure 2.

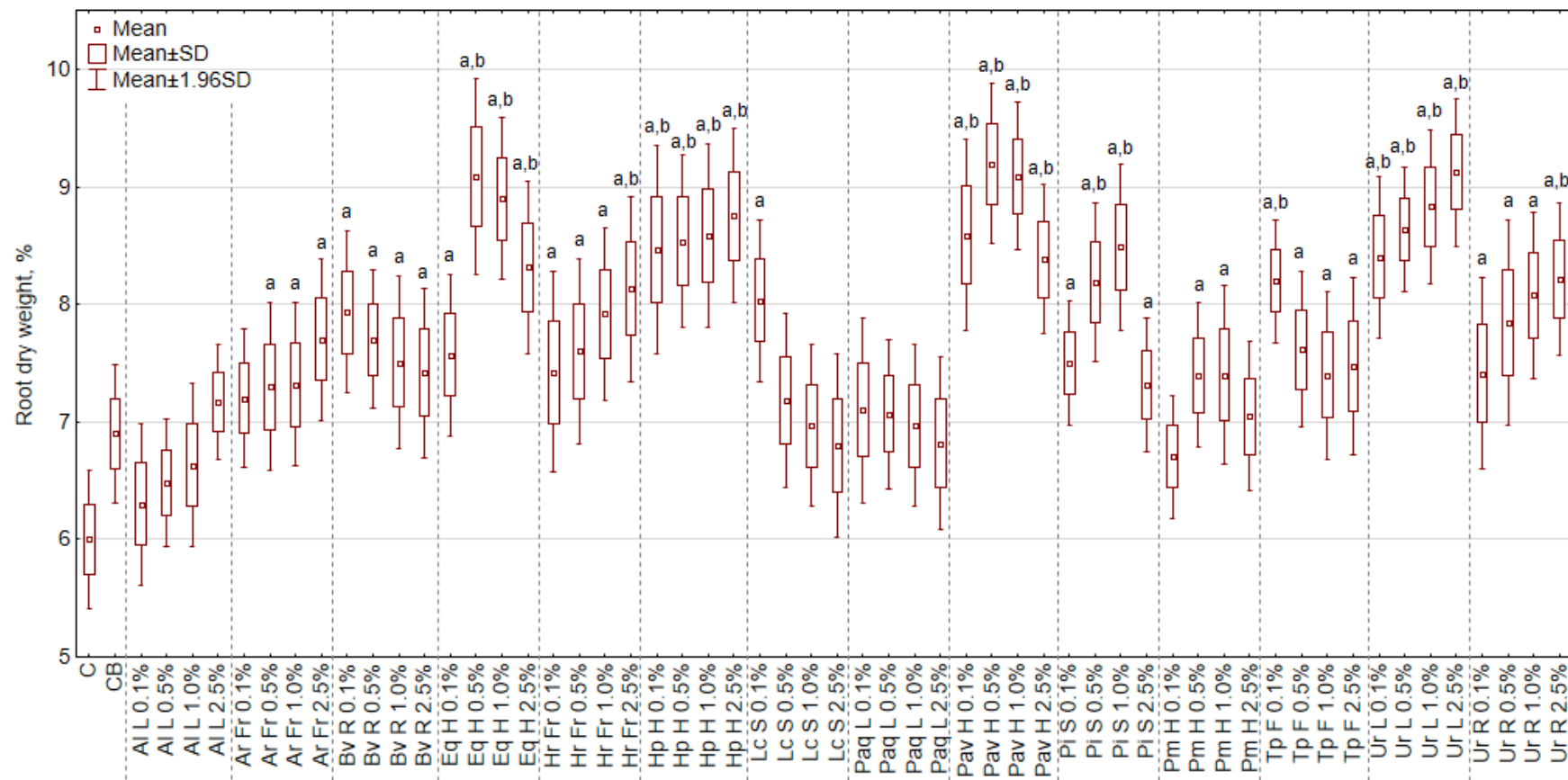


Figure 7. The root dry weight of white head cabbage seedlings after application of plant extracts. (a) Statistically significant differences ($p < 0.05$) between the control group (C) and extracts. (b) Statistically significant differences ($p < 0.05$) between commercial biostimulant (CB) and extracts. Abbreviations as in Figure 2.

3.6. The Effect of Extracts on the Content of Chlorophyll and Carotenoids, and the Greenness Index of Leaf (SPAD)

In general, chlorophyll content in the cabbage seedlings was higher in the tested groups than in the control group with water, beside aloe (Al L, 0.1%), red beet (Bv R, 1.0 and 2.5%), red lentil (Lc S, 0.5 and 1.0%), common bracken (Paq L, 0.1%), knotgrass (Pav H, all concentrations), broadleaf plantain (Pm H, 0.1%) and nettle (Ur R, 1.0% and 2.5%) (Figure 8). But in the case of commercial biostimulant, higher contents of chlorophyll were observed in plants treated with extracts only from chokeberry (Ar Fr, all concentrations) and hypericum (Hp H, 0.1% and 0.5%). The best results were obtained for hypericum (Hp H, 0.1%)—the chlorophyll content was higher about 2.5 times than in the control group with water and higher by 22% than for commercial biostimulant. For chokeberry (Ar Fr, 2.5%), these values were as follows—about 2.5 times higher and 20% higher, respectively.

Chlorophyll content in cabbage seedlings was also measured using non-destructive method—with SPAD equipment (Figure 9). Largely, these results correspond with data presented in Figure 8. The highest SPAD index was observed for the extract produced from hypericum (Hp H, 0.1%) and it was higher by 34% than in the control group with water and only by 4% when compared with commercial biostimulant. In the case of chokeberry (Ar Fr, 2.5%), these values were as follows—by 30% and 1.1%, respectively.

Mosly, all plant extracts showed a greater effect on the content of chlorophyll in the cabbage seedlings compared to the control group treated only with water. Again, the weakest results, i.e., the lowest chlorophyll content (even compared to the control group with water), were obtained for extract produced from knotgrass (Pav H, all concentrations).

We also examined the effect of plant extracts on the content of carotenoids of in cabbage seedlings (Figure 10). General trends are similar to those observed for chlorophyll measured spectrophotometrically and with the SPAD meter. Carotenoids content was the highest in the group of cabbage, treated with 0.1% extract from hypericum (Hp H). It was two times higher than in the control group treated with water and by 11% when compared with commercial biostimulant. In the case of 2.5% extract from chokeberry (Ar Fr), the carotenoids content in seedlings was the same as for commercial biostimulant and almost two times higher than in the control group with water.

Likewise, as in the case of chlorophyll content, the smallest effect on the content of carotenoids in the cabbage biomass (even smaller than for the control group with water) had the extract from knotgrass (Pav H, all concentrations). Additionally unsatisfactory effect was observed in the case of extracts produced from common bracken (Paq L, 0.1% and 2.5%) and red lentil (Lc S, 0.5% and 1.0%).

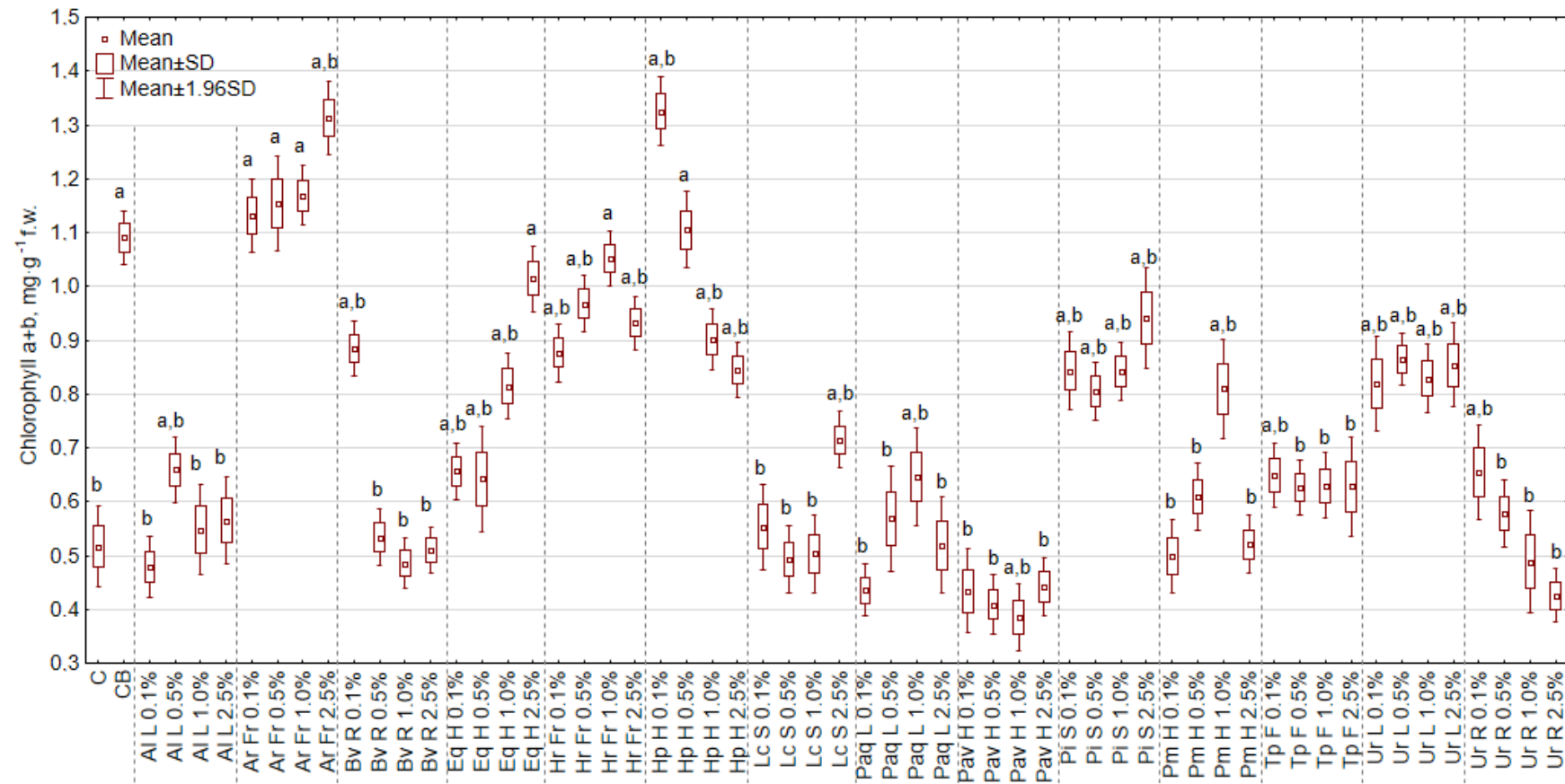


Figure 8. The of chlorophyll *a* + *b* of white head cabbage seedlings after application of plant extracts. (a) Statistically significant differences (*p* < 0.05) between the control group (C) and extracts. (b) Statistically significant differences (*p* < 0.05) between commercial biostimulant (CB) and extracts. Abbreviations as in Figure 2.

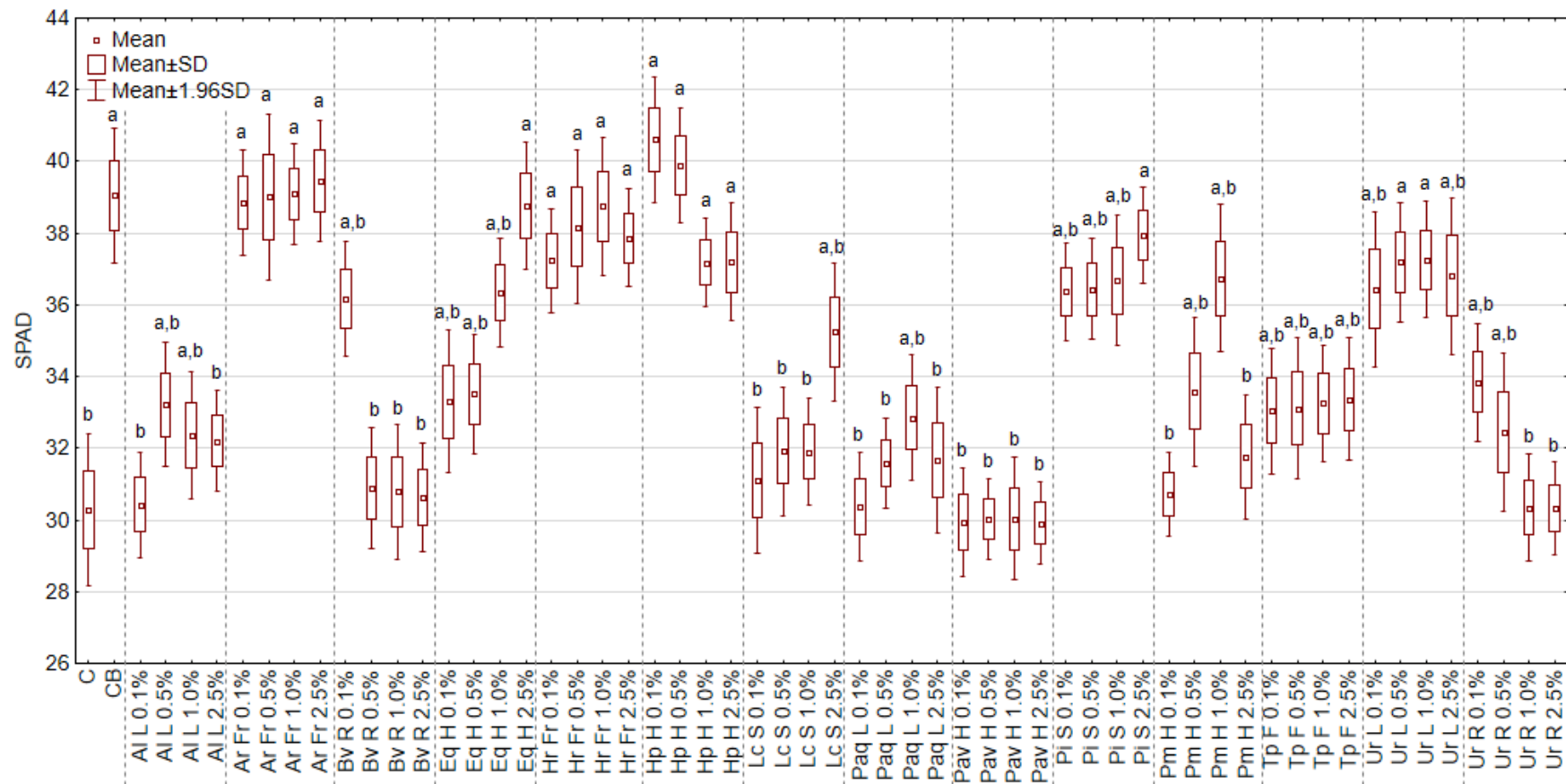


Figure 9. The SPAD index of white head cabbage seedlings after application of plant extracts. (a) Statistically significant differences ($p < 0.05$) between the control group (C) and extracts. (b) Statistically significant differences ($p < 0.05$) between commercial biostimulant (CB) and extracts. Abbreviations as in Figure 2.

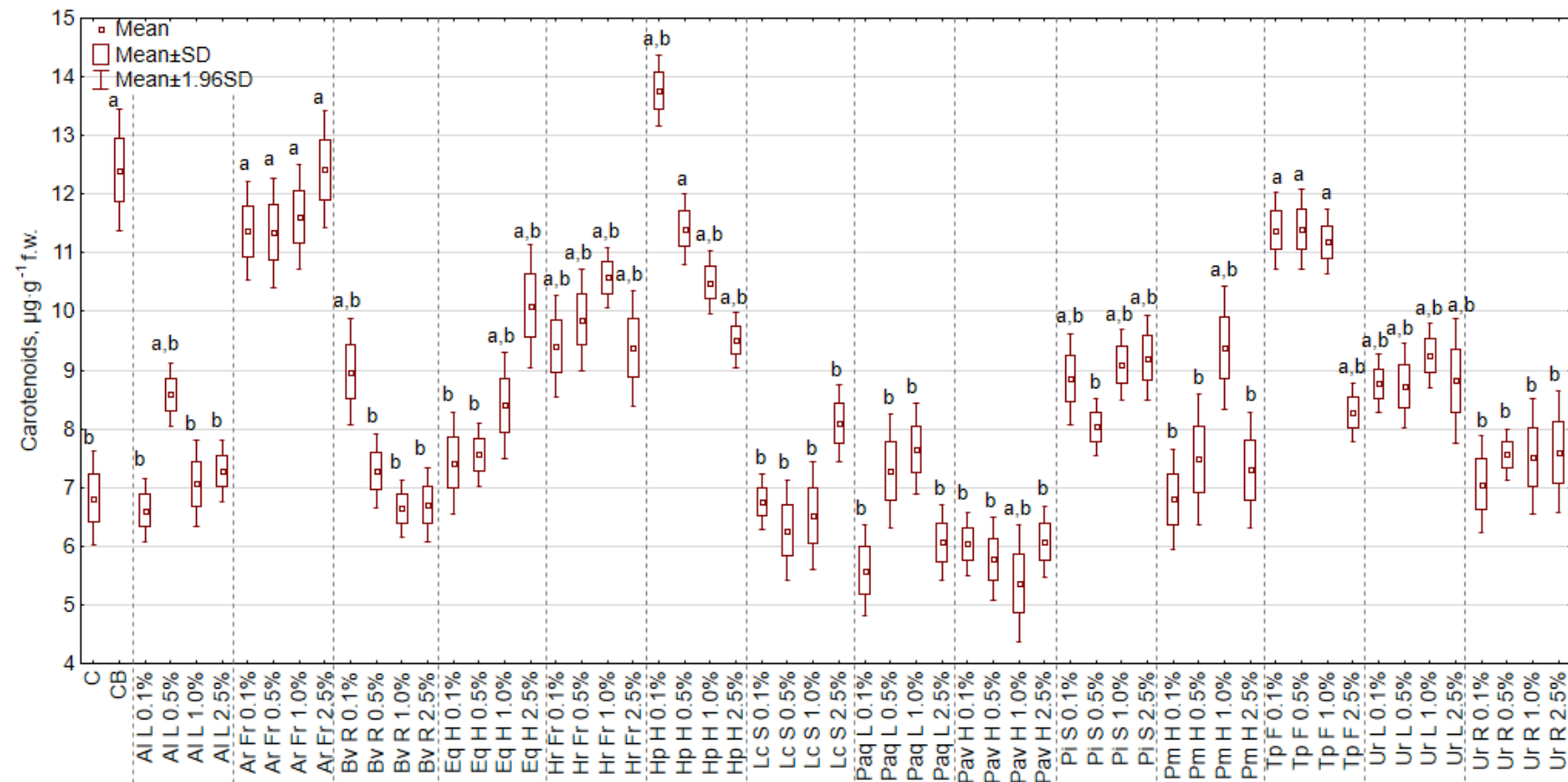


Figure 10. The content of carotenoids of white head cabbage seedlings after application of plant extracts. (a) Statistically significant differences ($p < 0.05$) between the control group (C) and extracts. (b) Statistically significant differences ($p < 0.05$) between commercial biostimulant (CB) and extracts. Abbreviations as in Figure 2.

3.7. The Effect of Extracts on the Content of Polyphenols

Most of the examined plant extracts didn't influence positively the polyphenols content (Figure 11). In most tested groups, it was lower than in the control groups—C and CB. Higher values of polyphenols, than in the group with commercial biostimulant, were observed only for hypericum (Hp H, 0.1%), broadleaf plantain (Pm H, 0.5%), nettle (Ur L, 0.5%) and nettle (Ur R, 0.5% and 1.0%). The best experimental group was extract from hypericum (Hp H, 0.1%), where the mean polyphenol content was higher by 25% than in the control group with water and by 9% than in the group with commercial biostimulant. Extracts from aloe (Al L, 0.1%, 0.5%, and 1.0%) increased the content of polyphenols in cabbage seedlings in comparison with the control group with water.

3.8. The Effect of Extracts on the Antioxidant Activity (DPPH, ABTS, FRAP)

The antioxidant activity of cabbage seedlings measured with DPPH technique was smaller in the experimental groups than in the control group with commercial biostimulant (Figure 12). In the case of the control group treated with water, higher antioxidant activity was observed only for extracts produced from common bracken (Paq L, 0.5%), knotgrass (Pav H, 0.5% and 1.0%), broadleaf plantain (Pm H, 0.5%) and nettle (Ur R, 1.0%). Among them, knotgrass extract exhibited the highest stimulatory effect on the antioxidant activity, 8% higher than in the control group (C) for 0.5% extract and by 5% for 1.0% extract.

In the case of the ABTS assay (Figure 13), diversity was observed between control and experimental groups. All examined extracts stimulated the antioxidant activity of cabbage seedling when compared with the control group with water. The antioxidant activity in the CB group was almost three times higher than in C group. Much higher results than in CB group, were obtained for the following extracts: chokeberry (Ar Fr, 0.1%, 0.5%, and 1.0%), horsetail (Eq H, 1.0%), common sea-buckthorn (Hr Fr, all concentrations), hypericum (Hp H, all concentrations), common bracken (Paq L, 0.1%, 0.5%, and 1.0%), pea (Pi S, all concentrations), nettle (Ur L, all concentrations). Among all examined plant extracts, the highest antioxidant activity of cabbage seedlings was determined for common sea-buckthorn (Hr Fr, 0.1%) and pea (Pi S, 0.1%). In both cases it was almost 7 times higher than in the control group (C) and more than two times higher than for CB. From Figure 13 it can be noticed, that most of antioxidant activity of cabbage seedlings decreased with the increase of plant extract concentration (beside chokeberry, Ar Fr), hypericum (Hp H) and red clover (Tp F). In order to obtain a higher antioxidant activity of cabbage seedlings, low concentrations of plant extracts are recommended.

Most of the plant extracts showed lower antioxidant activity, measured by FRAP assay (Figure 14), than examined control groups: C and CB, which coincides with the results presented in Figure 12 (DPPH). Higher values of antioxidant activity of cabbage seedlings than for commercial biostimulant were observed for the following plant extracts: chokeberry (Ar Fr, 1.0%), hypericum (Hp H, 0.1%), common bracken (Paq L, 0.1% and 0.5%), broadleaf plantain (Pm H, all concentrations), red clover (Tp F, 1.0%) and nettle (Ur L, 0.5%). The best results were obtained for broadleaf plantain (Pm H) applied at concentration 2.5%: which was by 50% higher than in the control group with water and by 31% than in the control group with commercial biostimulant. In addition, higher antioxidant activity of cabbage seedlings than in the control group with water group was determined for the extracts—aloe (Al L, 0.1%), chokeberry (Ar Fr, 0.5%), hypericum (Hp H, 0.5 and 1.0 %), common bracken (Paq L, 2.5%) and red clover (Tp F, 0.1%, 0.5%, and 2.5%) (almost the same groups like for the commercial biostimulant).

There is no apparent relationship between the three used assays (DPPH, ABTS, FRAP) and the antioxidant properties of cabbage seedlings. However, on the basis of the findings, it can be concluded that the extract from broadleaf plantain showed the highest antioxidant activity and can be recommended for further studies.

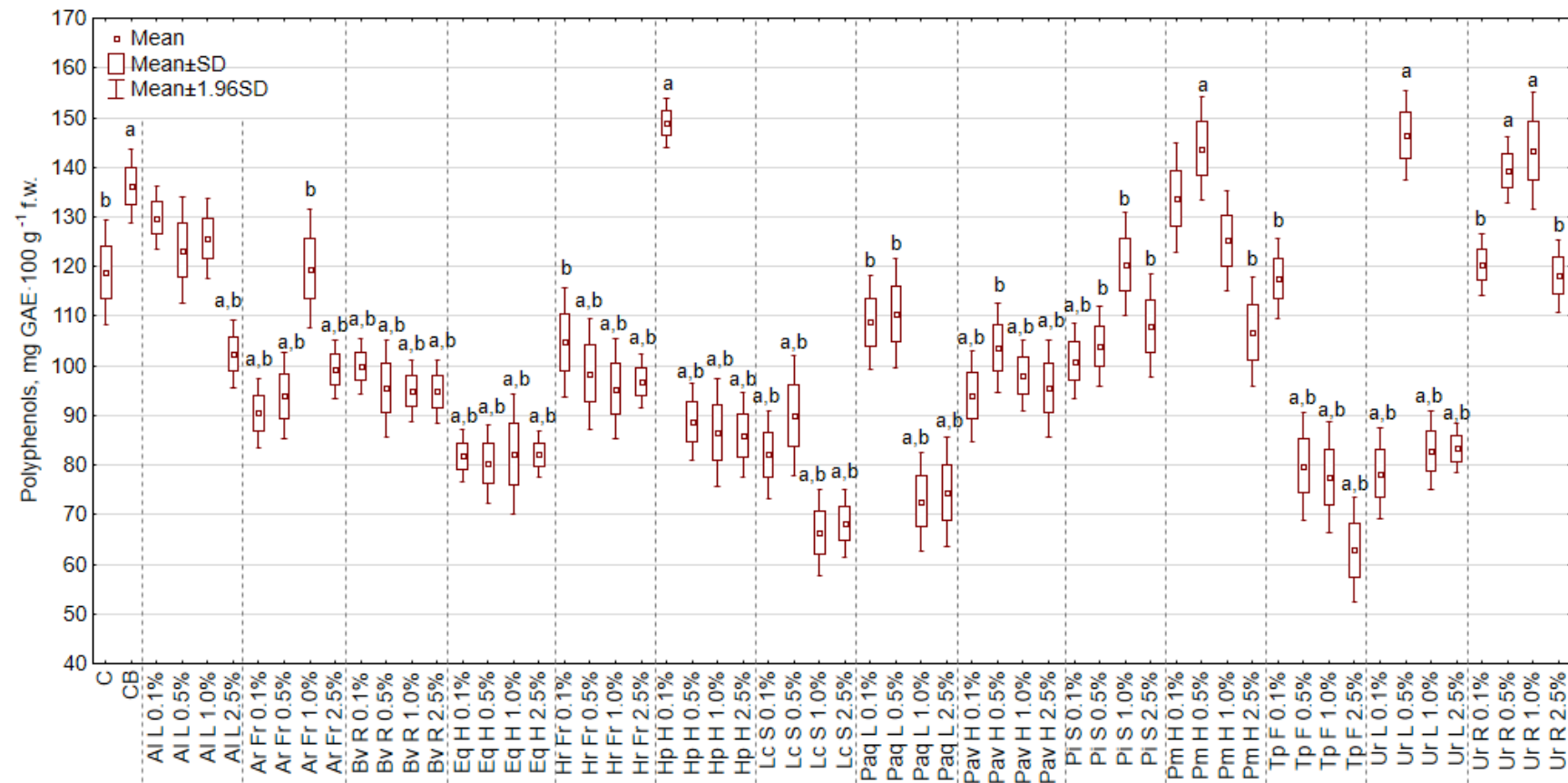


Figure 11. The content of polyphenols of white head cabbage seedlings after application of plant extracts. (a) Statistically significant differences ($p < 0.05$) between the control group (C) and extracts. (b) Statistically significant differences ($p < 0.05$) between commercial biostimulant (CB) and extracts. Abbreviations as in Figure 2.

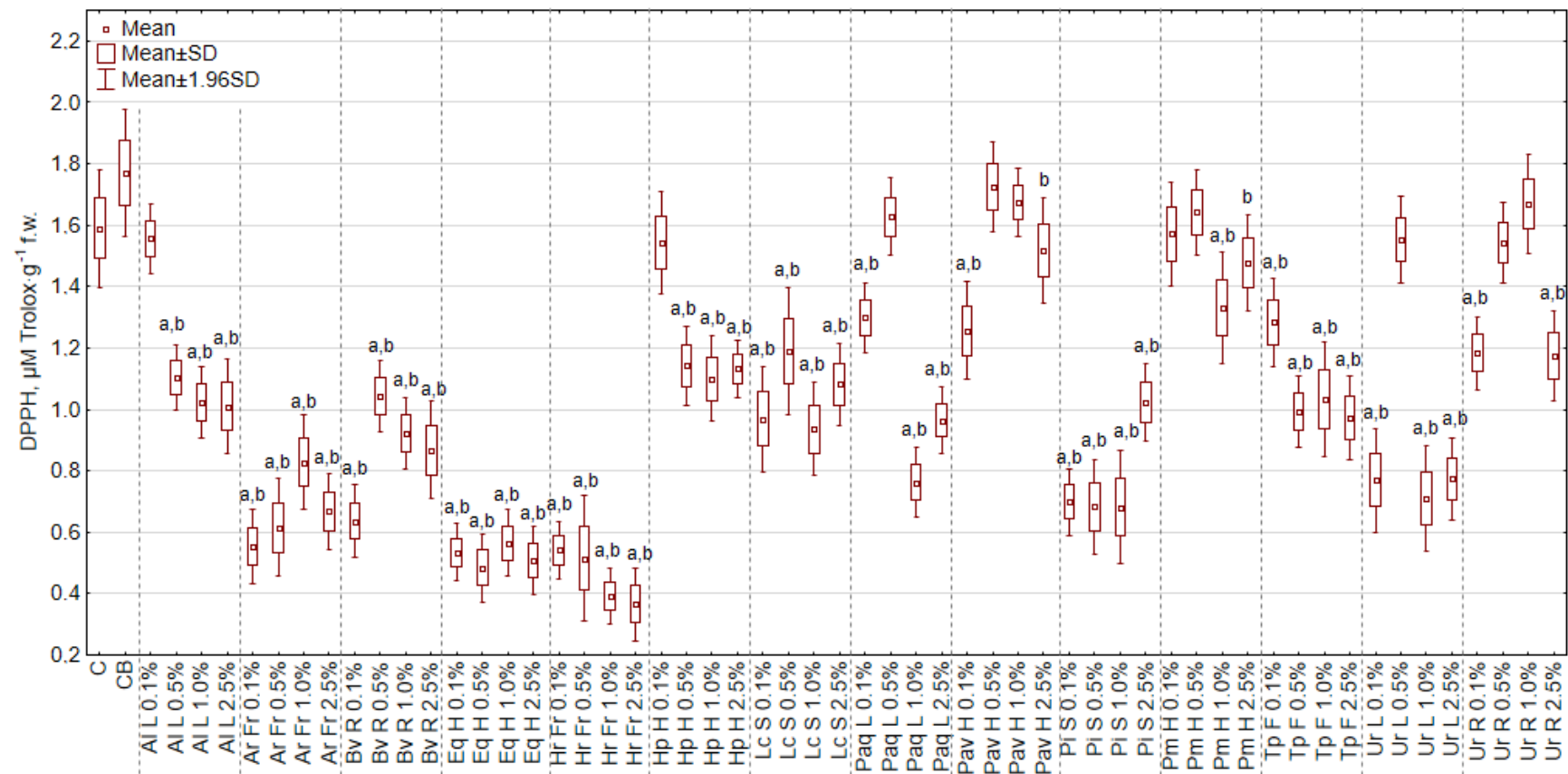


Figure 12. The antioxidant activity (DPPH) of white head cabbage seedlings after application of plant extracts. (a) Statistically significant differences ($p < 0.05$) between the control group (C) and extracts. (b) Statistically significant differences ($p < 0.05$) between commercial biostimulant (CB) and extracts. Abbreviations as in Figure 2.

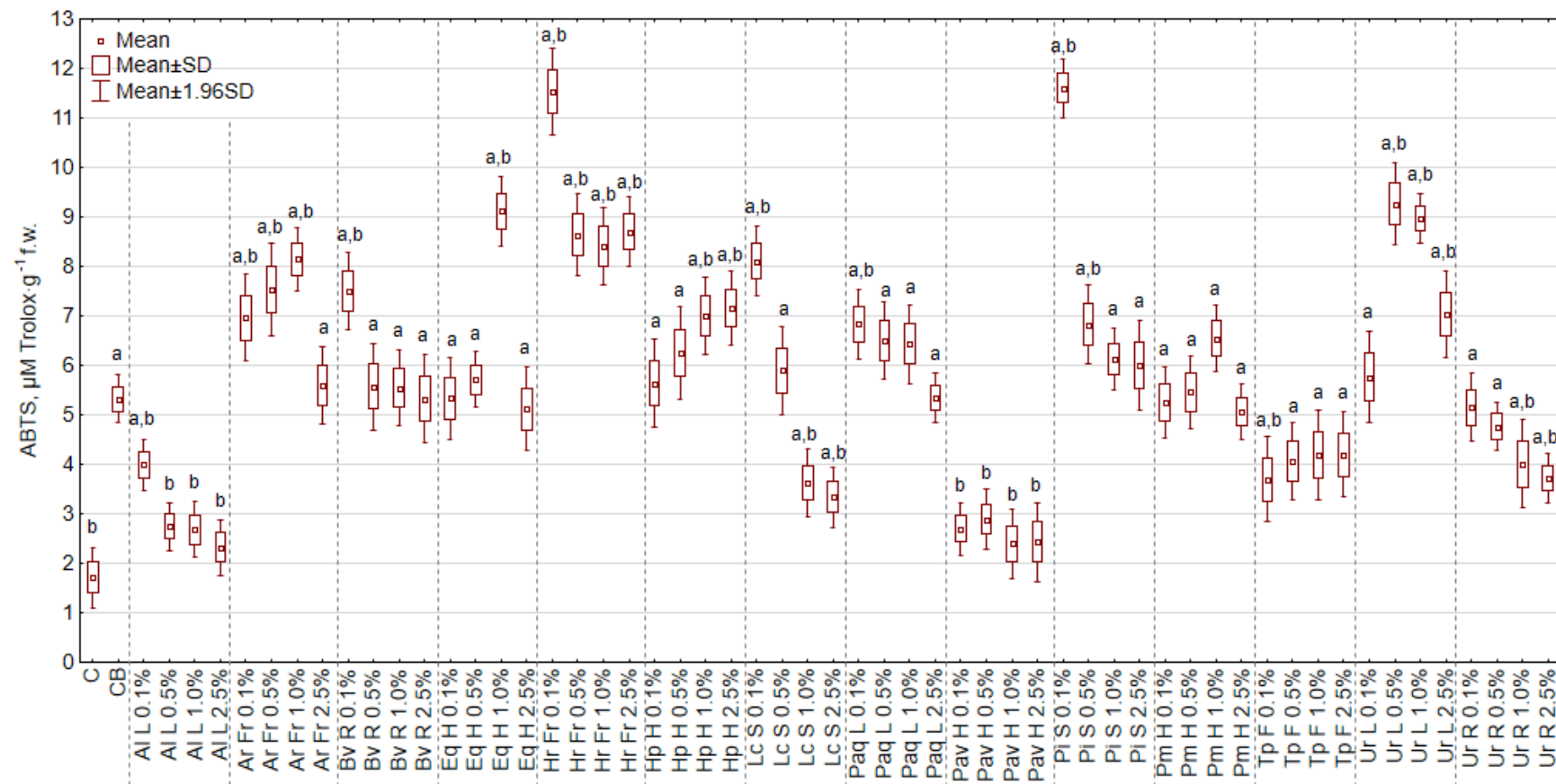


Figure 13. The antioxidant activity (ABTS) of white head cabbage seedlings after application of plant extracts. (a) Statistically significant differences ($p < 0.05$) between the control group (C) and extracts. (b) Statistically significant differences ($p < 0.05$) between commercial biostimulant (CB) and extracts. Abbreviations as in Figure 2.

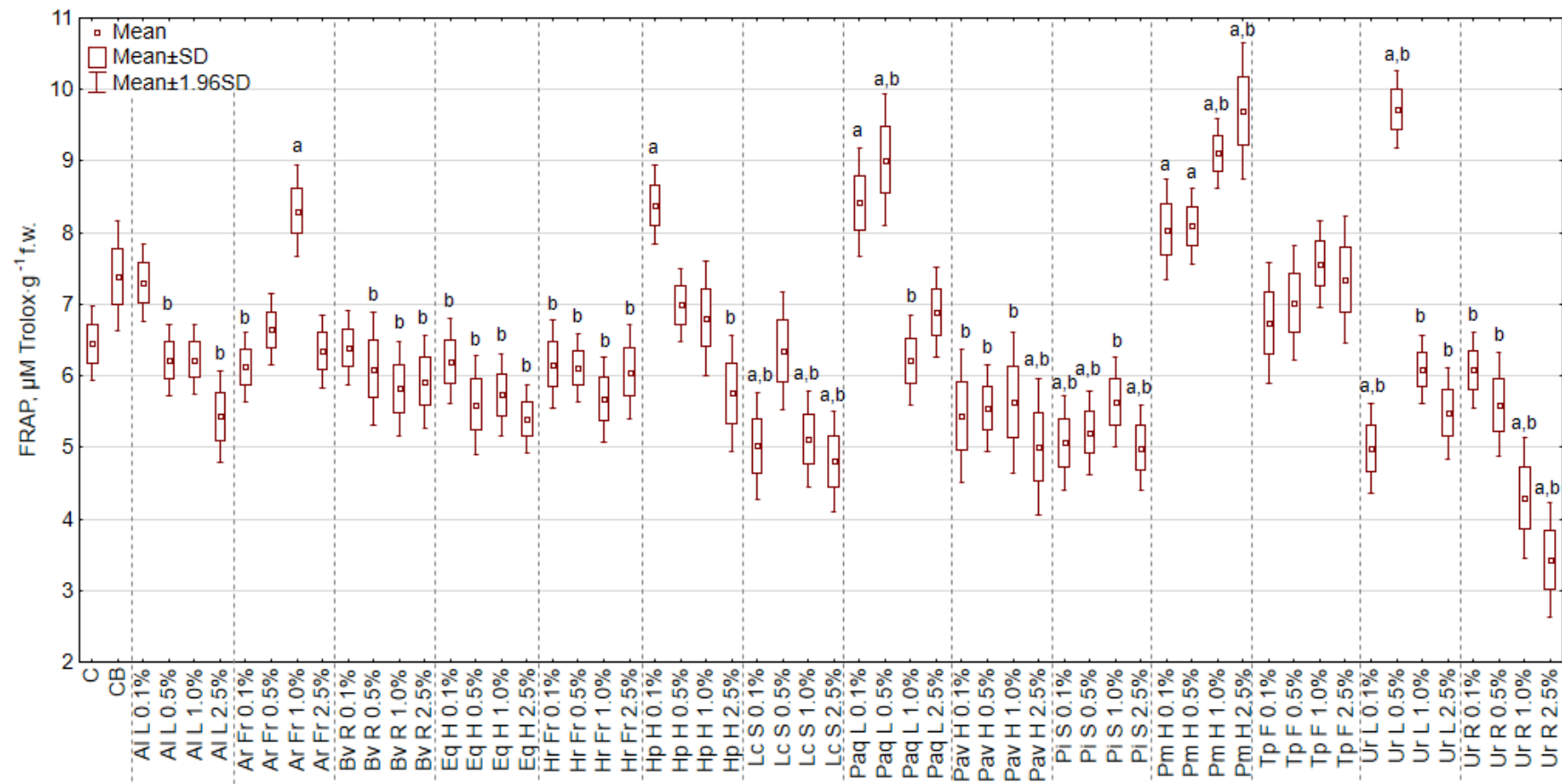


Figure 14. The antioxidant activity (FRAP) of white head cabbage seedlings after application of plant extracts. (a) Statistically significant differences ($p < 0.05$) between the control group (C) and extracts. (b) Statistically significant differences ($p < 0.05$) between commercial biostimulant (CB) and extracts. Abbreviations as in Figure 2.

4. Discussion

In this paper, we examined the effect of botanical extracts on the growth (shoot and root development) and some properties (content of chlorophyll, carotenoids, polyphenols and antioxidant properties) of white head cabbage seedlings. Different extracts of 14 medicinal plants, as well as their concentrations influenced examined parameters in different ways, i.e., there was a positive effect on plant growth (shoot and root) and the content of pigments and a weak effect on polyphenol content and antioxidant activity of cabbage seedlings.

During the germination experiments no toxic effects of plant extracts on cabbage growth were observed. Germination tests in the controlled environmental conditions are necessary in order to define the best application methods of tested biostimulants, their concentration, timing, and rates. This approach provides also information on the phytotoxicity of the tested preparations [8].

The positive effect of examined plant extracts on cabbage growth can result from the rich variety of biologically active compounds in the raw material used for extraction (as it was shown in Table 1), that can stimulate plant growth. However, in the future it will be necessary to conduct detailed studies of the chemical composition of extracts obtained by ultrasound assisted extraction, that showed in the present study the best biostimulatory properties of cabbage growth, development and composition. In the literature, there are many evidence that plant-based biostimulants increase plant parameters such as fresh and dry weight, leaf area, plant height, pigments content. For example, Ertani et al. [30] showed that extracts produced from blueberry fruits, red grape and hawthorn leaves influenced positively maize (*Zea mays* L.) growth and composition (increased leaf and root biomass, chlorophyll, sugar and protein content). Amirkhani et al. [29] examined the effect of a soy protein-based biostimulant, used for seed coating, on shoot, root and broccoli seedling length. Soy biostimulant provided greater root and shoot growth, as well as chlorophyll content in broccoli seedlings when compared with the non-coated control. This biostimulatory effect can result from the content of proteins which can enhance plant growth and uptake of nitrogen in plants. As can be seen in Table 1, most of the examined plant materials used for extraction, contain proteins, peptides, amino acids which possess the ability to stimulate plant growth and increase resistance to the abiotic stress [10,27]. In our study, the highest shoot length and the highest fresh weight of shoots were observed for extracts from red lentil (Lc S, 0.5%) and nettle (Ur L, 0.1%). Both biomasses can serve as a valuable source of active proteins. It was also noted that when the fresh and dry weight of cabbage shoots is considered as a crucial parameter of plant growth, extracts from nettle (Ur L), horsetail (Eq H) and pea (Pi S) at lower concentrations (0.1% and 0.5%) are recommended. This is one of the advantages of biostimulants of plant growth that are active at low concentrations (diluted as 1:1000 or even more) [77]. Also, Abbas and Akladios [27] showed that a lower concentration ($0.25 \text{ g}\cdot\text{mL}^{-1}$) of carrot root extract was more effective in the stimulation of shoot and root length, number of leaves, fresh and dry weight of cowpea seedlings, chlorophyll, and carotenoids content in seedlings than a higher concentration ($0.5 \text{ g}\cdot\text{mL}^{-1}$).

In the present study we also observed the positive effect of most of the plant extracts on the cabbage root length and weight. The longest cabbage roots were measured in the group treated with knotgrass extract (Pav H, 1.0%), whereas the highest fresh weight of roots was observed for horsetail (Eq H, 0.5% and 1.0%), knotgrass (Pav H, 0.5% and 1.0%) and nettle (Ur L, 2.5%). These results correspond with dry weight of roots obtained for extracts produced from knotgrass (Pav H, 0.5%), nettle (Ur L, 2.5%) and horsetail (Eq H, 0.5%). The increase of cabbage roots length treated with plant extracts can be attributed to the presence of phytohormones, for example in knotgrass: abscisic acid (ABA), indole-3-acetic acid (IAA), cis-zeatin (c-Z), trans-zeatin (t-Z), zeatin riboside (ZR), zeatin-O-glucoside (ZOG), isopentenyladenosine (iPa), isopentenyladenine (iP), which also increase the resistance to the abiotic stress (e.g., high salinity) [78], or in nettle, cytokinins which are root-born phytohormones, transported into the shoot [79]. Additionally, horsetail can be important from the agricultural point of view because is able to synthesize numerous volatiles which exhibit pesticide and/or arthropod repellent properties [42].

Our study confirms also that natural botanical extracts increase the content of pigments (chlorophylls and carotenoids) in the cultivated plants. In our research, the highest chlorophyll content in cabbage seedlings was obtained for hypericum (Hp H, 0.1%) and chokeberry (Ar Fr, 2.5%), whereas carotenoids content in the group, treated with 0.1% extract from hypericum (Hp H). Crucial for the formation of chlorophyll are micro- and macroelements such as K, Mg, Fe and Cu which are present in plant extracts [27]. As it was shown in the work of Sekeroglu et al. [80], hypericum can serve as a source of B, Cu, Fe, Mg, Mn, Mo, and Zn, whereas chokeberry of the following minerals: Na, K, Ca, Mg, Fe, Zn, and I [35,37]. Furthermore, carotenoids which are essential in photosynthesis act also as free radical scavengers and protect chlorophyll from photo-oxidative destruction [27].

The weakest effect of examined plant extracts was noticed for the polyphenols content in the cabbage seedlings and their antioxidant activity. Our results were much smaller than for the commercial biostimulant and comparable or lower than in the control group with water. The highest content of polyphenols in seedlings was measured for the extract from hypericum (Hp H, 0.1%), whereas the antioxidant activity for the extract from broadleaf plantain (Pm H). As it was shown in Table 1, the biomass of hypericum and broadleaf plantain is a rich source of phenolic compounds. In our study, germination tests were performed under optimal conditions, without biotic or abiotic stress. Usually, the increase of the biosynthesis of plant phenols is a response to unfavourable cultivation conditions and these compounds increase the plant resistance to stress and the regulation of plant physiological stages [27].

Results obtained in this study are in agreement with the previous research, where vegetal extracts obtained from higher plants (basil, calendula, chamomile, comfrey, dandelion, giant goldenrod, mugwort, purple coneflower and valerian) mostly increased the cabbage shoots, length of roots, weight of cabbage shoots and roots, the content of photosynthetic pigments (chlorophyll and carotenoids) and decreased the polyphenols content [71].

This study shows that there is a possibility to use renewable bioresources for the production of extracts that can be used in sustainable agricultural systems. These natural biostimulants of plant growth could be successfully applied, especially in organic crop production.

5. Conclusions

Nowadays, one of the biggest challenges for scientist and food producers is to meet the nutritional needs of growing world population but without the negative impact on the environment. Obtained results may be considered as a one of promising solutions to this problem. The foliar application of botanical extracts improved the growth and development of model vegetable seedlings. Treated plants had significantly longer shoots and roots. They were heavier and produced more photosynthetic pigments in comparison to control groups. However, in most cases, the plants contained lower concentration of polyphenols or showed lower antioxidant activity. Those extracts that increased the content of polyphenols could be used for enhancing the pigmentation, reproduction and resistance of crop plants. In addition to environmental aspects, the utilisation of natural biostimulants meets economic requirements—they exhibit biostimulating properties in very low concentrations. In the relation to the findings of this research, nettle leaf extract at 0.5% could be considered as a suitable foliar spray in organic agriculture to enhance plant yield. Obviously, it is of great importance that any product used in the cultivation of a plant crop is safe, with a low impact on the environment, is convenient to use, effective at much lower application rates, cheap, and less toxic to non-target species. Obtaining a stable formulation (extracts enriched with e.g., surfactants, antifreeze agent, and preservatives) that will deliver more bioavailable compounds, elongate the time of wetting the leaf surface, increase the adhesion of the leaf drop, and allow faster transport and absorption of nutrients in plants under standard and abiotic stress conditions is a priority. Future research should be devoted to the explanation of the mechanism of action of natural extracts, and should consider the larger scale of experiments, such as pot test or field trials reflecting more realistic conditions for food producers. Due to the benefits of using biostimulants, this type of product will gain more popularity in the near future.

Author Contributions: K.G. designed and conducted the research, analyzed obtained data and wrote the paper; A.B. proposed the research question, methodology, supervision, writing—review and editing paper; I.M. analyzed obtained data and wrote the paper, P.P. prepared the graphics, analyzed obtained data and wrote the paper. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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