**Abstract:** The colored grain of wheat (*Triticum aestivum* L.) contains a large number of polyphenolic compounds that are biologically active ingredients. The purpose of this work was a comparative metabolomic study of extracts from anthocyaninless (control), blue, and deep purple (referred to here as black) grains of seven genetically related wheat lines developed for the grain anthocyanin pigmentation trait. To identify target analytes in ethanol extracts, high-performance liquid chromatography was used in combination with Bruker Daltonics ion trap mass spectrometry. The results showed the presence of 125 biologically active compounds of a phenolic (85) and nonphenolic (40) nature in the grains of *T. aestivum* (seven lines). Among them, a number of phenolic compounds affiliated with anthocyanins, coumarins, dihydrochalcones, flavan-3-ols, flavanone, flavones, flavonols, hydroxybenzoic acids, hydroxycinnamic acids, isoflavone, lignans, other phenolic acids, stilbenes, and nonphenolic compounds affiliated with alkaloids, carboxylic acids, carotenoids, diterpenoids, essential amino acids, triterpenoids, sterols, nonessential amino acids, phytohormones, purines, and thromboxane receptor antagonists were found in *T. aestivum* grains for the first time. A comparative analysis of the diversity of the compounds revealed that the lines do not differ from each other in the proportion of phenolic (53.3% to 70.3%) or nonphenolic (46.7% to 29.7%) of the total number of identified compounds. However, diversity of the compounds was significantly lower in the proportion of phenolic (53.3% to 70.3% of the total number of identified compounds) and nonphenolic compounds (46.7% to 29.7%), but diversity of the compounds was significantly lower in grains of the control line. Even though the lines are genetically closely related and possess similar chemical fingerprints and allow to distinguish each line from the six others. Finally, the influence of the genotype on the chemical profiles of the wheat grains is discussed.

**Keywords:** *Triticum aestivum*; colored wheat; anthocyanin; HPLC-MS/MS; tandem mass spectrometry; phenolic compound; biologically active compound

1. Introduction

Among nutritional sources of antioxidant compounds necessary for human health, cereal products, which contain flavonoid pigments (plant compounds of a phenolic na-
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1. Introduction

Among nutritional sources of antioxidant compounds necessary for human health, flavonoid compounds in certain grain components of cereal plants give rise to a distinct color (Figure 1). As a result of the biosynthesis of anthocyanins, cereal seeds can have a color of various shades from bluish gray and reddish to dark purple and almost black. Other classes of flavonoid compounds give the grain a reddish-brown color (proanthocyanidins) or a dark-brown color (phlobaphenes). Anthocyanins have the highest antioxidant potential among the above-mentioned compounds [2]. These substances can accumulate in vegetative and reproductive parts of the plant, where their main physiological role is to protect the plant from excessive UV radiation. Additionally, the concentration of anthocyanins usually increases during exposure to adverse environmental factors [3].

Anthocyanins have been shown to play an important part in the prevention of neurodegenerative diseases [4], atherosclerosis, diabetes, and obesity and to have vasoprotective and anti-inflammatory properties [5,6]. As a consequence, the food industry is interested in researching colored cereals.

In the most common cereal species, soft wheat (Triticum aestivum L.), the grain can have either an unremarkable color or a reddish-brown, bluish-gray, or purple hue. The differences in color are due to the accumulation of certain flavonoid pigments in various layers of wheat grain envelopes [7–9]. The biosynthesis of proanthocyanidins in the seed coat causes a reddish-brown hue (trait: “red grain”) and is controlled by R genes localized on chromosomes of homoeologous group 3 [10]. The bluish-gray hue appears to be due to the biosynthesis of anthocyanins in the aleurone layer (trait: “blue aleurone”) and is regulated by Ba genes introduced into the common wheat genome from wild relatives such as wheatgrass Thinopyrum ponticum, Triticum boeoticum, and Thinopyrum bessarabicum owing to translocations in the chromosomes of homoeologous group 4 or via a substitution of one of the chromosomes of homoeologous group 4 [11–13]. The purple color is a consequence of

Figure 1. Diversity of colors among the analyzed wheat lines (having anthocyanin-rich grains). (A) The grains of the wheat lines used in this study: control line Saratovskaya 29 (S29) (1) in the upper left-hand corner and next in clockwise order S29 BLACK (4Th-4B) (2), S29 BLACK (4Th-4D) (3), S29 BLUE (4Th-4D) (4), BW BLACK (4Th-4D) (5), S29 BLUE (4Th-4B) (6), and E22 BLACK (4Th-4D) (7). (B) Grains of S29 BLUE (4Th-4B).
of the biosynthesis of anthocyanins in cells of the pericarp (trait: “purple pericarp”). This trait is regulated by two complementary genes, \( Pp-1 \) and \( Pp3 \), mapped to chromosomes of homoeologous group 7 and chromosome 2A, respectively [8,14,15]. The inclusion of the genes that control the biosynthesis of anthocyanins in the grain into the breeding process may ultimately increase nutritional value of whole-grain products [1,16]. This notion has been demonstrated in end-use bakery products prepared from anthocyanin-rich wheat grains such as whole-grain bread [17,18], biscuits [16,19], pasta [20], pancakes, porridge, crackers, and candy bars [21]. The amount of anthocyanins in whole-grain blue-wheat bread and purple-wheat bread made according to a traditional Czech recipe has been determined, and how thermal parameters, such as temperature and baking time, affect individual anthocyanins and the total amount of anthocyanins in bread has been shown [18].

The predominant anthocyanin in purple and blue wheat varieties is cyanidin-3-glycoside, and each wheat variety is reported to have a specific anthocyanin profile [22]. Thirteen anthocyanins have been identified in purple wheat, among which cyanidin-3-glucoside is the most abundant, followed by cyanidin-3-galactoside and malvidin-3-glucoside. In purple wheat, anthocyanins are also present in the form of pelargonidin-3-glucoside and anthocyanins glycosylated with arabinose [23]. Besides anthocyanins, other bioactive phytochemicals such as phenolic acids, carotenoids, tocopherols, and phytosterols can be found in the wheat grain [24], and determination of their profiles in colored wheat grains will allow to take full practical advantage of colored wheat varieties. Therefore, the aim of this study was to identify anthocyanins and the total polyphenolic profile along with accompanying sterols and stilbenes in seven wheat lines having grains of different colors by high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS) on an ion trap instrument. The lines have been previously developed for the grain anthocyanin pigmentation trait in a common genetic background of anthocyaninless cultivar Saratovskaya 29 (S29) (four lines) used here as a control and in the genetic background of the breeding lines promising in terms of cultivation in Western Siberia (two lines). Two blue-grained lines—S29 BLUE (4Th-4B) and S29 BLUE (4Th-4D)—are substitution lines where chromosomes 4B and 4D are substituted with \( T. \) ponticum chromosome 4Th carrying the \( Ba \) gene determining the blue pigmentation of grains [25,26]. Two deep-purple-grained, i.e., almost black-grained lines—S29 BLACK (4Th-4B) and S29 BLACK (4Th-4D)—in addition to chromosomes 4B and 4D substituted with 4Th, feature introgressions in chromosomes 7D and 2A, where the dominant alleles of genes \( Pp-D1 \) and \( Pp3 \), respectively, are located [25]. Two other black-grained lines—BW BLACK (4Th-4D) and E22 BLACK (4Th-4D)—were developed in the genetic background of breeding line BW49880 (BW) and \( cv. \) \( E22 \). Element 22 (E22), respectively, by means of S29 sister lines as donors of genes \( Pp \) and \( Ba \) in the current study. The use of such genetically related lines in a metabolomic study should enable us to draw conclusions about the effects of chromosome substitutions and introgression fragments on the chemical profile of the grains. To our knowledge, this is the first extensive study on anthocyanins and related polyphenols in unpigmented, blue-grained, and purple-grained wheat lines.

2. Results
2.1. Chemical Identification of the Wheat Grain Metabolites

A total of 300 peaks were detected in the chromatogram (Figure 2). After a comparison of the \( m/z \) values, retention times, and the fragmentation patterns with the MS/MS spectral data retrieved from the cited articles and after a database search (MS2T, MassBank, HMDB), a comprehensive table was compiled of the molecular masses of the analytes of interest isolated from ethanolic extracts of \( T. \) aestivum grains for ease of annotation (Appendix A). The 125 identified biologically active compounds are presented in Table 1. Among them, 85 compounds belong to various polyphenolic families: anthocyanins, flavones, flavonols, flavan-3-ols, flavanones, hydroxycinnamic acids, hydroxybenzoic acids, stilbenes, and coumarins, and the other 40 compounds are nonphe-
nolic substances. In addition to previously reported metabolites, a number of metabolites were found for the first time in *T. aestivum* grains. Among them, there were anthocyanins (cyanidin 3-(2′′-galloylglucoside) and petunidin), coumarins (fraxetin and fraxetin-7-O-sulfate), dihydrochalcones (phlorizin), flavan-3-ols (epicatechin and gallochelin), flavanone (naringenin), flavones (acetin C-glucoside methylmalonylated, apigenin, apigenin 6-C-deoxyhexoside-8-C-pentoside, dihydroxy tetramethoxyflavanol, cirsiilol, genistein C-glucosylglucoside, hydroxy dimethoxyflavone hexoside, myricetin, orientin 7-O-deoxyhexoside, pentahydroxy dimethoxyflavone, pentahydroxy dimethoxyflavone hexoside, pentahydroxy trimethoxy flavone, tetrahydroxy-dimethoxyflavone-hexoside, trihydroxy methoxyflavone triacetate, and vitexin 6′′-O-glucoside), flavonols (ampelopsin, isorhamnetin, kaempferide, kaempferol, rhamnetin I, rhamnetin II, taxifolin-3-O-glucoside, and taxifolin-O-pentoside), hydroxybenzoic acids (cis-salvianolic acid J, gallic acid hexoside, hydroxy methoxy dimethylbenzoic acid, salvianolic acid D, salvianolic acid F, and salvianolic acid G), hydroxycinnamic acids (1-cafeoyl-β-D-glucose, 1-O-sinapoyl-β-D-glucose, caffeic acid derivative, caftaric acid, and ferulic acid methyl ester), isoflavones (wighteone-O-glucoside), lignans (dimethyl-secoisolariciresinol, podophyllotoxin), other phenolic acids (1-O-cafeoyl-5-O-feruloylquinic acid, 4-O-Caffeoyl-5-O-p-coumaroylquinic acid, and feruloyl sulfate), stilbenes (pinosylvin, polydatin, and resveratrol), alkaloids (berberine and sespendole), carboxylic acids (9,10-dihydroxy-8-octadec-12-enoic acid, 11-hydroperoxy-octadecatrienoic acid, dihydroxy docosanoic acid, docosenoic acid, myristoleic acid, pentacosenoic acid, salvianic acid C, and undecanediol acid), carotenoids (cryptoxanthin and (3S, 3′S, all-E)-zeaxanthin), diterpenoids (isocryptotanshinone II and tanshinone III), essential amino acids (L-histidine, L-tryptophan, and L-valine), triterpenoids (β-amyrin, squalene, uvaol, and ursolic acid), steroids (avenasterol, brassicasterol, β-sitosterol, β-sitosterin, campestenone, ergosterol, fucosterol, oxo-hydroxy sitosterol, vebolon), steroids (cyclopassifiloic acid glucoside), nonessential amino acids (tyrosine), phytohormone (GA8-hexose gibberellin), propionic acid (ketoprofen), purine (adenosine), and thromboxane receptor antagonist (vapiprost).

**Figure 2.** Chemical profiles of the BW BLACK (4Th-4D) sample presented as a total ion chromatogram from the EtOH extract.

The flavone family featured the greatest number of members (32 substances) among the analyzed wheat grains; the flavonol family (10), anthocyanins (10), cinnamic acids (seven), lignin (five), and hydroxybenzoic acids (six) were found much less frequently. Among other identified compounds, i.e., nonphenolic substances, sterols (six compounds), higher-molecular-weight carboxylic acids (seven), and di- and triterpenoids (eight) were detected most often.
Table 1. A detailed table of the biologically active substances found in the analyzed colored-grain lines of the wheat *T. aestivum*. Different color marks the presence of certain compounds in particular lines.

| ID | Classes and Families of Compounds | Name | S29 Control | S29 BLUE 4Th-4B | S29 BLUE 4Th-4D | S29 BLACK 4Th-4B | S29 BLACK 4Th-4D | E22 BLACK 4Th-4D | BW BLACK 4Th-4D |
|----|----------------------------------|------|-------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1  | Phenolics                        | Anthocyanin | Cyanidin 3-(2’’-galloylglucoside) | yes | yes | yes | yes |
| 2  | Phenolics                        | Cyanidin-3-O-3’’/8’’-O-Dimalonylglucoside | yes | yes | yes | yes |
| 3  | Phenolics                        | Malvidin 3-O-rutinoside | yes | yes | yes | yes |
| 4  | Phenolics                        | Peonidin 3-O-rutinoside | yes | yes | yes | yes |
| 5  | Phenolics                        | Peonidin 3-rutinoside-5-glucoside | yes | yes | yes | yes |
| 6  | Phenolics                        | Peonidin 3-rutinoside-5-glucoside | yes | yes | yes | yes |
| 7  | Phenolics                        | Cinnamic acid derivative | Ferulic acid methyl ester | yes | yes | yes | yes |
| 8  | Hydroxycinnamic acid             | 1-Caffeoyl-β-D-glucose | yes | yes | yes | yes |
| 9  | Hydroxycinnamic acid             | 1-O-Sinapoyl-β-D-glucose | yes | yes | yes | yes |
| 10 | Hydroxycinnamic acid             | Caffeic acid derivative | yes | yes | yes | yes |
| 11 | Hydroxycinnamic acid             | Chlorogenic acid | yes | yes | yes | yes |
| 12 | Hydroxycinnamic acid             | Cyclamic acid | yes | yes | yes | yes |
| 13 | Hydroxycinnamic acid             | Fraxetin-7-O-sulfate | yes | yes | yes | yes |
| 14 | Hydroxycinnamic acid             | Dihydrochalcone | Phlorizin | yes | yes | yes | yes |
| 15 | Flavan-3-ol                      | Catechin [D-Catechol] | yes | yes | yes | yes |
| 16 | Flavan-3-ol                      | Epicatechin | yes | yes | yes | yes |
| 17 | Flavan-3-ol                      | Galloacatechin [+(-)Galloacatechin] | yes | yes | yes | yes |
| 18 | Flavanone                        | Naringenin [Naringetol; Naringenine] | yes | yes | yes | yes |
| 19 | Flavone                          | 6-C-hexosyl-chrysoeriol O-rhamnoside-O-hexoside | yes | yes | yes | yes |
| 20 | Flavone                          | Acacetin C-glucoside methyl malonylated | yes | yes | yes | yes |
| 21 | Flavone                          | Apigenin | yes | yes | yes | yes |
| 22 | Flavone                          | Apigenin 2’’-O-sinapoyl, C-hexosyl, C-pentosyl | yes | yes | yes | yes |
| 23 | Flavone                          | Apigenin 6,8-di-C-pentoside | yes | yes | yes | yes |
| 24 | Flavone                          | Apigenin 6-C-deoxyhexoside-8-C-pentoside | yes | yes | yes | yes |
| 25 | Flavone                          | Apigenin 6-C-β-galactosyl-8-C-β-glycosyl-O-glycuromopyranoside | yes | yes | yes | yes |
| 26 | Flavone                          | Apigenin 8-C-hexoside-6-C-pentoside | yes | yes | yes | yes |
| 27 | Flavone                          | Apigenin 8-C-hexoside-8-C-pentoside | yes | yes | yes | yes |
| 28 | Flavone                          | Chrysoeriol [Chryseriol] | yes | yes | yes | yes |
| 29 | Flavone                          | Chrysoeriol C-hexoside-C-pentoside | yes | yes | yes | yes |
| 30 | Flavone                          | Cirsiliol | yes | yes | yes | yes |
| 31 | Flavone                          | Diosmetin | yes | yes | yes | yes |
| 32 | Flavone                          | Diodyxymethoxyflavone | yes | yes | yes | yes |
Table 1. Cont.

| ID | Classes and Families of Compounds | Name | S29 Control | S29 BLUE 4Th-4B | S29 BLACK 4Th-4D | S29 BLACK 4Th-4D | E22 BLACK 4Th-4D | BW BLACK 4Th-4D |
|----|----------------------------------|------|-------------|-----------------|------------------|------------------|-----------------|-----------------|
| 39 |                                  | Genistein 7-glucosyl glucoside | yes           |                 |                  |                  |                 |                 |
| 40 |                                  | Hydroxy dimethoxyflavone hexoside | yes          |                 |                  |                  |                 |                 |
| 41 |                                  | Luteolin                       | yes          |                 |                  |                  |                 |                 |
| 42 |                                  | Luteolin 8-C-Glucoside          | yes          |                 |                  |                  |                 |                 |
| 43 |                                  | Luteolin 8-C-hexoside-6-C-pentoside | yes     |                 |                  |                  |                 |                 |
| 44 |                                  | Luteolin 8-C-pentoside-6-C-hexoside | yes | yes           | yes             | yes             | yes             | yes             |
| 45 |                                  | Myricetin                   | yes          |                 |                  |                  |                 |                 |
| 46 |                                  | Orientin 7-O-deoxyhexoside     | yes          |                 |                  |                  |                 |                 |
| 47 |                                  | Pentahydroxy dimethoxyflavone  | yes          |                 |                  |                  |                 |                 |
| 48 |                                  | Pentahydroxy dimethoxyflavone hexoside | yes | yes           | yes             |                  |                 |                 |
| 49 |                                  | Pentahydroxy trimethoxy flavone | yes          | yes           | yes             | yes             | yes             | yes             |
| 50 |                                  | Tricin                        | yes          | yes           | yes             | yes             | yes             | yes             |
| 51 |                                  | Tetrahydroxy dimethoxyflavone-hexoside | yes | yes           | yes             | yes             | yes             | yes             |
| 52 |                                  | Trifluorobenzoyl flavone triacetate | yes         |                 |                  |                  |                 |                 |
| 53 |                                  | Vicenin-2 [Apigenin-8,8-Di-C-Glucoside] | yes | yes           | yes             |                  |                 |                 |
| 54 |                                  | Vitexin 2”-O-glucoside         | yes          | yes           | yes             |                  |                 |                 |
| 55 |                                  | Vitexin 8”-O-glucoside         | yes          | yes           | yes             |                  |                 |                 |
| 56 |                                  | Wighteone-O-glucoside          | yes          |                 |                  |                  |                 |                 |
| 57 |                                  | Flavonol                      | yes          |                 |                  |                  |                 |                 |
| 58 |                                  | Ampelopsin                     | yes          | yes           | yes             |                  |                 |                 |
| 59 |                                  | Isohamnetin                    | yes          | yes           | yes             |                  |                 |                 |
| 60 |                                  | Kaempferide                    | yes          | yes           | yes             |                  |                 |                 |
| 61 |                                  | Quercetin                      | yes          | yes           | yes             |                  |                 |                 |
| 62 |                                  | Rhamnetin I                    | yes          |                 |                  |                  |                 |                 |
| 63 |                                  | Rhamnetin II                   | yes          | yes           | yes             |                  |                 |                 |
| 64 |                                  | Selgin                        | yes          | yes           |                  | yes             | yes             | yes             |
| 65 |                                  | Taxifolin-3-O-glucoside        | yes          | yes           | yes             |                  | yes             | yes             |
| 66 |                                  | Taxifolin-O-pentoside          | yes          | yes           | yes             |                  | yes             | yes             |
| 67 |                                  | Gallotannin \[β-Glucogallin [1-O-Galloyl-β-D-Glucose]\] yes | yes | yes | yes | yes | yes | yes |
| 68 |                                  | 4-Hydroxybenzoic acid          | yes          |                 |                  |                  |                 |                 |
| 69 |                                  | Cis-salvianolic acid J         | yes          |                 |                  |                  |                 |                 |
| 70 |                                  | Hydroxy methoxy dimethylbenzoic acid | yes | yes | yes |                  | yes             |                 |
| 71 |                                  | Salvianolic acid D             | yes          | yes           | yes             |                  | yes             | yes             |
| 72 |                                  | Salvianolic acid F             | yes          | yes           |                  | yes             | yes             | yes             |
| 73 |                                  | Salvianolic acid G             | yes          |                  | yes             |                  | yes             | yes             |
| 74 |                                  | Dimethyl-secoisolariresinol    | yes          |                 |                  |                  |                 |                 |
| 75 |                                  | Hinokinin                     | yes          | yes           | yes             |                  | yes             |                 |
| 76 |                                  | Pinoresinol                   | yes          |                 |                  |                  |                 |                 |
| ID | Classes and Families of Compounds | Name | S29 Control | S29 BLUE 4Th-4B | S29 BLUE 4Th-4D | S29 BLACK 4Th-4B | S29 BLACK 4Th-4D | E22 BLACK 4Th-4D | BW BLACK 4Th-4D |
|----|----------------------------------|------|-------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 77 | Podophyllotoxin                   | Podofilox; Condylox; Condylone; Podophyllinic acid lactone | yes | yes | yes | yes | yes | yes | yes |
| 78 | Syringaresinol                     | yes | yes | yes | yes | yes | yes | yes | yes |
| 79 | Phenolic acid                     | yes | yes | yes | yes | yes | yes | yes | yes |
| 80 | 4-O-Caffeoyl-5-O-feruloylquinic acid | yes | yes | yes | yes | yes | yes | yes | yes |
| 81 | Feruloyl sulfate                  | yes | yes | yes | yes | yes | yes | yes | yes |
| 82 | Phenolic glucoside                | Gallic acid hexoside | yes | yes | yes | yes | yes | yes | yes |
| 83 | Stilbene                          | yes | yes | yes | yes | yes | yes | yes | yes |
| 84 | Polydatin (Picelid; trans-Piceid) | yes | yes | yes | yes | yes | yes | yes | yes |
| 85 | Resveratrol                       | yes | yes | yes | yes | yes | yes | yes | yes |
| 86 | Alpha, omega-dicarboxylic acid    | Undecanedioic acid | yes | yes | yes | yes | yes | yes | yes |
| 87 | Carboxylic acid                   | yes | yes | yes | yes | yes | yes | yes | yes |
| 88 | Higher-molecular-weight carboxylic acid | yes | yes | yes | yes | yes | yes | yes | yes |
| 89 | 5,10-Dihydroxy-8-oxooctadec-12-enoic acid | yes | yes | yes | yes | yes | yes | yes | yes |
| 90 | Dihydroxy docosanoic acid         | yes | yes | yes | yes | yes | yes | yes | yes |
| 91 | Docosenoic acid [2-Docosenoic acid] | yes | yes | yes | yes | yes | yes | yes | yes |
| 92 | Hydroxy methoxy dimethylbenzoic acid | yes | yes | yes | yes | yes | yes | yes | yes |
| 93 | Pentacosenoic acid                | yes | yes | yes | yes | yes | yes | yes | yes |
| 94 | Salvianic acid C                  | yes | yes | yes | yes | yes | yes | yes | yes |
| 95 | Anabolic steroid                  | Vebonol | yes | yes | yes | yes | yes | yes | yes |
| 96 | Cycloartanol [Steroids]           | Cyclopassifloric acid glucoside | yes | yes | yes | yes | yes | yes | yes |
| 97 | Carotenoid (3S,3′S,all-E)-zeaxanthin [Zeaxanthin; (3S,3′S)-Zeaxanthin] | yes | yes | yes | yes | yes | yes | yes | yes |
| 98 | Cryptoxanthin [β-cryptoxanthin]   | yes | yes | yes | yes | yes | yes | yes | yes |
| 99 | Diterpenoid Isocryptotanshinone II | yes | yes | yes | yes | yes | yes | yes | yes |
| 100 | Tanshinone IIB                    | yes | yes | yes | yes | yes | yes | yes | yes |
| 101 | Pentacyclic diterpenoid            | yes | yes | yes | yes | yes | yes | yes | yes |
| 102 | Gibberellic acid                  | yes | yes | yes | yes | yes | yes | yes | yes |
| 103 | Triterpenic acid                  | yes | yes | yes | yes | yes | yes | yes | yes |
| 104 | Ursolic acid                      | yes | yes | yes | yes | yes | yes | yes | yes |
| 105 | Triterpenoid                     | Squalene | yes | yes | yes | yes | yes | yes | yes |
| 106 | Uvaol                             | yes | yes | yes | yes | yes | yes | yes | yes |
| 107 | Essential amino acid              | yes | yes | yes | yes | yes | yes | yes | yes |
| 108 | L-Tryptophan [Tryptophan; (S)-Tryptophan] | yes | yes | yes | yes | yes | yes | yes | yes |
| 109 | L-Valine                          | yes | yes | yes | yes | yes | yes | yes | yes |
| 110 | Nonessential amino acid           | Tyrosine | yes | yes | yes | yes | yes | yes | yes |
Table 1. Cont.

| ID | Classes and Families of Compounds | Name                                                                 | S29 Control | S29 BLUE 4Th-4B | S29 BLACK 4Th-4D | S29 BLACK 4Th-4D | E22 BLACK 4Th-4D | BW BLACK 4Th-4D |
|----|----------------------------------|----------------------------------------------------------------------|-------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 111| Indole sesquiterpene alkaloid    | Suspendesol                                                          |             | yes             | yes             | yes             | yes             | yes             |
| 112| Isoquinoline alkaloid            | Berberine               [Berberin; Umbelletine; Berbericine]          | no          | yes             | yes             | yes             | yes             | yes             |
| 113| Phytohormone                     | GAβ-hexose gibberelin                                              | yes         | yes             | yes             | yes             | yes             | yes             |
| 114| Sesquiterpenoid plant hormone    | Abscisic acid [Dormin; Abscisin II; (S)-(+)-Abscisic acid]         | yes         | yes             | yes             | yes             | yes             | yes             |
| 115| Propionic acid                   | Ketoprofen [Orudis; 2-(3-Benzoylphenyl)propionic acid]              | yes         | yes             | yes             | yes             | yes             | yes             |
| 116| Purine                           | Adenosine                                                           | yes         | yes             | yes             | yes             | yes             | yes             |
| 117| Phytosterol                      | Ergosterol [Provitamin D2; Ergosterin]                             | yes         | yes             | yes             | yes             | yes             | yes             |
| 118| Sterol                           | Avenasterol                                                          | yes         | yes             | yes             | yes             | yes             | yes             |
| 119| β-Sitosterone                    | Stigmast-4-En-3-One; Sitostenone                                   | yes         | yes             | yes             | yes             | yes             | yes             |
| 120| β-Sitosterol [β-Sitosterol]      |                                                                      | yes         | yes             | yes             | yes             | yes             | yes             |
| 121| Campestosterone                  |                                                                      | yes         | yes             | yes             | yes             | yes             | yes             |
| 122| Fucosterol                       |                                                                      | yes         | yes             | yes             | yes             | yes             | yes             |
| 123| Oxo-hydroxy sitosterol           |                                                                      | yes         | yes             | yes             | yes             | yes             | yes             |
| 124| Thromboxane receptor antagonist  | Vapiprost                                                            | yes         | yes             | yes             | yes             | yes             | yes             |
| 125| Unsaturated fatty acid           | Hexadecatrienoic acid [Hexadeca-2,4,6-trienoic acid]                |             | yes             | yes             | yes             | yes             | yes             |

2.2. Similarities and Differences in Metabolites among the Lines

According to Table 1 and Figure 3, the largest number of biologically active compounds (55) was found in lines S29 BLUE (4Th-4B) and S29 BLACK (4Th-4D), and the smallest (18) in the control line (differences of S29 from all the other lines are significant according to a two-sided test for proportions [Spearman’s rank correlation analysis], \( p = 0.00001–0.0177 \)). Similar data were obtained for the polyphenol family: the largest (33 and 36) and smallest (12) numbers of such compounds were detected in the same lines as mentioned above. Phenolic compounds were found more often than nonphenolic compounds (\( p = 0.00001–0.0016 \)) in all studied lines except for S29 BLUE (4Th-4D). In this line, the two classes of compounds showed almost equal numbers of members (\( p = 0.3428 \)). Overall, in terms of the numbers of substances of a phenolic nature (53.3–70.3% of the total number of identified compounds) and a nonphenolic nature (46.7–29.7%) the studied lines were similar.

The results of cluster analysis of all the compounds (Figure 4) showed that two clusters can be distinguished in the dendrogram. The first cluster is formed by lines S29 BLUE (4Th-4B) and S29 BLUE (4Th-4D) and the adjacent S29 BLUE (4Th-4B) line. The second cluster consists of the control line S29 and of E22 BLACK (4Th-4D). Lines BW BLACK (4Th-4D) and S29 BLACK (4Th-4D) did not end up in any clusters. Analysis of Spearman’s rank correlations confirmed the results of the cluster analysis. It was found that pairs of isogenic lines “S29 BLUE (4Th-4B)/S29 BLUE (4Th-4D)” and “S29 BLUE (4Th-4B)/S29 BLACK (4Th-4D)” (located in one cluster) are close to each other (\( R_S = 0.346–0.409, p < 0.05 \)). Similar results were obtained on the second cluster in the “S29/E22 BLACK (4Th-4D)” pair (\( R_S = 0.333, p < 0.05 \)). In addition, statistically significant correlation coefficients (\( R_S = 0.243–0.287, p < 0.05 \)) were obtained in the comparison of the pair of lines with a substituted 4D chromosome “S29 BLUE (4Th-4D)/E22 BLACK (4Th-4D)” and a pair of lines with the black seed color “S29 BLACK (4Th-4B)/E22 BLACK (4Th-4D)”.

Figure 3. The number of the phenolic (red) and non-phenolic (green) compounds that were detected in the differently colored grains of the seven wheat lines. Error bars denote standard deviation; the number of individual compounds and its proportion among all the annotated compounds (125) are shown above the bars. * A significant difference from the control line.

Figure 4. This tree was constructed by the unweighted pair group method with arithmetic mean (UPGMA) (based on Euclidean distances) from the data on 125 phenolic and nonphenolic substances of the seven *T. aestivum* lines.
Plotting of dendrograms separately for phenolic and nonphenolic families of substances indicated that nonphenolic compounds differentiate lines by grain color (Figure S1). Even clearer separation by grain color was noted when the lignin family of compounds was utilized for the tree construction. Similar data were obtained on anthocyanins, flavones, and terpenoids. Unambiguous separation by substituted chromosomes was not achieved by means of any one family of substances. In some cases (e.g., for sterols and flavonols), one cluster was distinguished on the basis of the seed color, and the other cluster on the basis of chromosome substitution (Figure S1).

Examination of the chemical composition of wheat grains by the families of compounds within the phenolic and nonphenolic classes revealed that the lower number of biologically active substances detected in the control line can be explained by the absence of seven families of phenolic substances: coumarins, flavan-3-ols, flavanones, flavonols, phenolic acids, dihydrochalcone, and stilbenes. Flavonols were found in all the lines except for the control (S29). Furthermore, in S29, the number of substances belonging to the most numerous (in this study) “flavones” was 1.4–3.2-fold lower as compared to the other lines. The lower number of nonphenolic substances detected in the control line can be explained by the absence of the following families: alkaloids, anabolic steroids, carboxylic acids, carotenoids, cycloartanols, di- and triterpenoids, propionic acids, purines, sesquiterpenoid plant hormones, thromboxane receptor antagonists, and unsaturated fatty acids. Accordingly, the colored-grain lines showed a 3–6-fold greater number of substances in the carboxylic acid family, 2–3-fold in the sterol family, 2–4-fold in the anthocyanin family, and 1.5–2.7-fold in the flavone family as compared to the unpigmented-grain control line (S29). It should also be noted that among the phenolic compounds, selgin (from the flavonol family) and abscisic acid [dormin; abscisin II; (S)−(−)-abscisic acid] from the class of nonphenolic compounds (sesquiterpenoid plant hormone family) were found only in lines with a substitution of chromosome 4B. A number of compounds (peonidin-3-O-glucoside, caffeic acid derivative, apigenin, isorhamnetin, kaempferol, rhamnetin II, taxifolin-O-pentoside, salvianolic acid G, undecanedioic acid, cyclopassifloic acid glucoside, sespendole, berberine, and β-sitostenedone) were found only in some lines with a substituted 4D chromosome (Table S1, 13 substances in total). In addition, some detected substances proved to be characteristic of only wheat with blue grains (malvidin 3-O-rutinoside-5-O-glucoside, petunidin 3-O-rutinoside-5-O-glucoside, apigenin 2′′-O-sinapoyl, C-hexosyl, C-pentosyl, vicenin-2, isocryptotanshinone II, and vapiprost) or black grains (isorhamnetin and taxifolin-O-pentoside). The latter case includes only the lines with a substitution of chromosome 4D.

Among the 125 compounds identified in this study in wheat grains, 58 substances turned out to be unique, that is, each was detectable in only one of the seven analyzed lines. The lowest number of unique compounds (three and four) was found in lines S29 BLACK (4Th-4B) and S29, respectively, and the highest number (17 and 15) in S29 BLACK (4Th-4D) and BW BLACK (4Th-4D). The rest of the lines were somewhere in between. These data are in good agreement with the contribution of the unique compounds to the total pool of detected substances (Figure 5). It is worth mentioning that the difference between the proportions estimated by ratios—(1) the number of unique substances in a line to the sum of unique substances for all wheat lines under study and (2) the number of unique substances in a line to the total number of biologically active substances in this line—was 3.2-fold in the control line S29: the largest difference among the seven lines (Figure 5). In all the studied lines, the contribution of phenolic compounds to the pool of unique substances was predominant (60.0–88.2%).
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Effects of various factors on the compounds’ diversity in the seven lines were analyzed by one-way ANOVA on ranks (Table 2). It was found that factors “Chromosome Substitution”, “Grain Color”, and “Genotype of Line” affect the diversity of the chemical compounds, but “Genotype of Parental Line/Cultivar” does not. A multiple pairwise comparison of proportions of compounds (in the total number of compounds) in the groups of the lines having substituted chromosomes 4B and 4D did not uncover any differences between the lines \((p = 0.688, \text{Duncan test})\). No such differences were revealed in the groups of lines with blue and black colors of grains \((p = 0.229, \text{Duncan test})\). Nonetheless, an effect of an interaction of two factors “Chromosome Substitution \(\times\) Grain Color” on the diversity of compounds was detected (Figure 6A). In the group of the lines with substituted chromosome 4D, there were no differences between the blue- and black-grained lines \((p = 0.807, \text{Duncan test})\), whereas in the group of the lines with substituted chromosome 4B, such differences between the blue- and black-grained lines were found \((p = 0.0023, \text{Duncan test})\), with significantly lower diversity of the compounds in the latter group. In the group of blue-grained lines, lower diversity of the compounds was observed in the lines with substituted chromosome 4D, while in the black-grained lines, the effect of chromosome substitutions was opposite: higher diversity (Figure 6B).
Table 2. Effects of various factors on the diversity of phenolic and nonphenolic compounds in the seven wheat lines according to the Kruskal-Wallis H test (i.e., one-way ANOVA on ranks; df: degrees of freedom).

| Factor               | Group                      | Group Size | df | Sum of Ranks | Mean Rank | H Criterion | p Value  | Significant Result |
|----------------------|----------------------------|------------|----|--------------|------------|-------------|----------|-------------------|
| Chromosome Substitution | 4Th-4B                    | 2          | 2  | 111,625.0    | 446.5      | 23.58       | 0.00001  | yes               |
|                      | 4Th-4D                    | 4          | 2  | 227,187.5    | 454.4      |             |          |                   |
|                      | Control                   | 1          |    | 44,437.5     | 355.5      |             |          |                   |
| Genotype of Parental Line/Cultivar | BW                     | 1          |    | 57,562.5     | 460.5      | 2.26        | 0.322    | no                |
|                      | E22                       | 1          | 2  | 52,750.0     | 422.0      |             |          |                   |
|                      | S29                       | 5          |    | 272,937.5    | 436.7      |             |          |                   |
| Grain Color          | Black grains              | 4          | 2  | 443,875.0    | 443.8      | 25.52       | 0.00001  | yes               |
|                      | Blue grains               | 2          | 2  | 116,875.0    | 467.5      |             |          |                   |
|                      | Control                   | 1          |    | 44,437.5     | 355.5      |             |          |                   |
| Genotype of Line     | S29 BLUE (4Th-4B)         | 1          |    | 60,625.0     | 485.0      | 38.29       | 0.00001  | yes               |
|                      | S29 BLUE (4Th-4D)         | 1          |    | 56,250.0     | 450.0      |             |          |                   |
|                      | S29 BLACK (4Th-4B)        | 1          |    | 51,000.0     | 408.0      |             |          |                   |
|                      | S29 BLACK (4Th-4D)        | 1          |    | 60,625.0     | 485.0      |             |          |                   |
|                      | E22 BLACK (4Th-4D)        | 1          |    | 57,562.5     | 460.5      |             |          |                   |
|                      | BW BLACK (4Th-4D)         | 1          |    | 52,750.0     | 422.0      |             |          |                   |
|                      | Control                   | 1          |    | 44,437.5     | 355.5      |             |          |                   |

Figure 6. Effects of the factors “Chromosome Substitution” and “Grain Color” on diversity of chemicals in wheat grain assessed in groups of lines combined based on grain color (A) and substituted chromosomes (B), respectively, according to two-way ANOVA (Fisher’s F test). Vertical bars denote 0.95 confidence intervals.

3. Discussion

Successful extraction of polyphenolic compounds depends on two sequential actions: dissolution of each polyphenolic compound at the cellular level in the matrix of plant material and its diffusion into the external medium (the solvent). This is why it is difficult to develop an extraction procedure suitable for all phenolic compounds. For the extraction of phenolic compounds, various organic solvents are commonly used, such as methanol, ethanol, acetone, ethyl acetate, or combinations thereof, often with different proportions of water. Additionally, an important factor directly affecting the solubility and extraction of...
these compounds is pH of the extraction medium, which determines the solubility of the soluble compounds and affects the possible solubilization of the hydrolyzable fraction.

Liquid chromatography is a versatile and well-established separation technique often employed for a variety of analytical tasks and allowing the separation of fairly complex mixtures of low- and high-molecular-weight compounds. This method is also suitable for different polarities and acid-base properties of various matrices.

In this study, 125 biologically active compounds of a phenolic and nonphenolic nature were identified in differently pigmented wheat grains by HPLC coupled with Bruker Daltonics ion trap MS/MS (Table 1). Our annotation results are consistent with the extensive mass-spectrometric literature data on the wheat T. aestivum [27–33] and other plant matrices, e.g., Passiflora incarnata [34], Bituminaria [35], Phyllostachys nigra [36], Carpobrotus edulis [37], and Vaccinium macrocarpon [38]. For example, the collision-induced dissociation spectrum (in negative ion mode) of a flavone called apigenin 2′′-O-sinapoyl, C-hexosyl, C-pentosyl from extracts of T. aestivum grains [line S29 BLUE (4Th-4D)] is given in Figure 7. The [M − H]− molecular ion gave rise to three molecular ions at m/z 545.02, 724.18, and 425.07 (see Figure 7). The molecular ion with m/z 545.02 yielded one daughter ion at m/z 425.07. The molecular ion with m/z 425.07 broke up into three daughter ions with m/z 365.00, 335.04, and 185.04. It was identified in the literature about extracts from T. aestivum [29].

Among the identified compounds, 87 were identified in wheat grains for the first time; they are affiliated with such phenolic compounds families as anthocyanins, coumarins, dihydrochalcones, flavan-3-ols, flavanone, flavones, flavonols, hydroxybenzoic acids, hydroxycinnamic acids, isoflavone, lignans, other phenolic acids, stilbenes, and nonphenolic compounds families as alkaloids, carboxylic acids, carotenoids, diterpenoids, essential amino acids, triterpenoids, sterols, nonessential amino acids, phytohormones, purines, and thromboxane receptor.

The diversity of phytochemicals may underlie diverse biological activities of the raw material. For instance, under the common name anthocyanins, there are up to 600 individual chemicals [39]. Biological activity of some individual anthocyanins has been tested, and distinct effects on physiological processes in animals and humans (or a lack of any) have been described. Antioxidant activity of anthocyanins is reported to be dependent on structural features of the molecules such as the number of hydroxyl and methyl groups and patterns of glycosylation [40]. Among anthocyanins, the highest antioxidant activity is featured by derivatives of delphinidin and cyanidin, followed by derivatives of malvidin, peonidin, pelargonidin, and petunidin [41]. In addition, a glycoside and rutinoside of cyanidin accelerate the regeneration of rhodopsin, while the derivatives of delphinidin have no effect [42]. Anthocyanins have been demonstrated to be better inhibitors of cell proliferation than anthocyanins [43], with delphinidin and cyanidin having the best growth-inhibitory property and pelargonidin and malvidin devoid of such effects [44,45].
From these observations, we may conclude that the more compounds are present in plant material, the wider is the expected spectrum of biological activities. Investigation of such diversity is a promising field for the development of functional food programs and for pharmacological research.

Here, we compared the diversity of compounds among colored-grain wheat lines and observed that the anthocyaninless line S29 is characterized by the lowest diversity of all the identified compounds, phenolic compounds in particular (Figure 3). The lower diversity of biologically active compounds in S29 is explained by the absence of seven families of phenolics (coumarins, flavan-3-ols, flavanones, flavonols, phenolic acids, dihydrochalcones, and stilbenes) and 12 families of nonphenolic compounds (alkaloids, anabolic steroids, carboxylic acids, carotenoids, cycloartanols, di- and triterpenoids, propionic acids, purines, sesquiterpenoid plant hormones, thromboxane receptor antagonists, and unsaturated fatty acids). These data imply that the genes of wheatgrass chromosome 4Th and chromosome fragments introgressed into 2A and 7D (including the genes regulating anthocyanin biosynthesis) are responsible for the presence of the above compounds in the grain and thus affect the diversity of biologically active substances in the wheat grain.

Although the black-grained lines contain $Pp$ genes in addition to $Ba$ and one may expect an increased number of biologically active compounds in these lines, there were no significant differences in the number of identified compounds between blue- and black-grained lines having chromosome 4D substituted by 4Th; moreover, a statistically significant decrease in the diversity of compounds was observed in the black-grained lines in comparison with the blue-grained lines having a chromosome 4B substitution. According to the results of our one-way ANOVA on ranks, the diversity of the chemicals is affected by such genetic factors as “Chromosome Substitution,” “Grain Color,” and “Genotype of Line,” but not “Genotype of Parental Line/Cultivar.” (Table 2) In support of these data, some differences in the chemical profile were noted among the lines with distinct substitutions of chromosomes and among lines with different colors of grains (Table S1). For example, two compounds belonging to the classes of phenolic and nonphenolic substances—selgin and a sesquiterpenoid plant hormone, respectively—were identified only in the lines with substituted chromosome 4B [S29 BLUE (4Th-4B) and S29 BLACK (4Th-4B)]. This observation suggests that this chromosome carries regulatory factors suppressing the synthesis of these compounds. Removing them by substitution of the chromosomes carrying these repressors activates the synthesis of the compounds in the substitution lines. Some common features can be found among the chemical profiles of the lines with similar chromosomes composition. Even though the sister lines of S29 are genetically related (and there is a line based on E22 that has S29 in its pedigree [46]; Figure S2), some line-specific (unique) compounds were identified (Table 1, Figure 5). They constitute unique chemical fingerprints of each line, allowing to distinguish each line from the six others. The unique compounds of each line are hardly explained by the genetic relationships among the lines but can be considered the main reason for the separation of the analyzed lines into two subclusters observed in the dendrogram and the separation of lines S29 BLACK (4Th-4D) and BW BLACK (4Th-4D), which are characterized by the highest percentage of unique compounds (Figures 4 and 5).

4. Materials and Methods

4.1. Materials

The chemical profiles were analyzed in seven wheat lines with different grain colors and characterized genetic pedigrees (Table 3, Figure S2). The control group of (anthocyaninless) grains consisted of cv. Saratovskaya 29 (S29). Blue grains were represented by two wheat-wheatgrass substitution lines S29 BLUE (4Th-4B) and S29 BLUE (4Th-4D) developed in the S29 background but carrying $Ba$ gene–containing wheatgrass chromosome 4Th, which replaced wheat chromosomes 4B and 4D, respectively [25,26]. Black grains were represented by four lines, two of them—S29 BLACK (4Th-4B) and S29 BLACK (4Th-4D)—have been developed previously in the S29 background by crossing the above-mentioned lines
with purple-grained near-isogenic wheat line S29 PURPLE Pp-D1Pp3 carrying introgressions in chromosomes 7D and 2A, onto which the dominant alleles of genes Pp-D1 and Pp3, respectively, have been mapped [47,48]. Two other black-grained lines—E22 BLACK (4TH-4D) and BW BLACK (4TH-4D)—were developed in the current study by marker-assisted transfer of genes Pp-D1+Pp3 and Ba from donor lines [S29 PURPLE Pp-D1Pp3 and S29 BLUE (4TH-4D), respectively] into cv. Element 22 (E22) (P.A. Stolypin Omsk State Agrarian University, Omsk, Russia) and breeding line BW49880 (CIMMYT, INT, México-Veracruz, Mexico) (Figure S2).

Table 3. Genetic characteristics of the wheat lines used in this study.

| Genotype            | Recurrent Parent | Grain Color  | Ba   | Pp-D1 + Pp3 | Substituted Chromosome | References |
|---------------------|------------------|--------------|------|-------------|------------------------|------------|
| S29 BLUE(4TH-4D)    | S29              | blue         | +    | -           | 4D                     | [25]       |
| S29 BLUE(4TH-4B)    | S29              | blue         | +    | -           | 4B                     | [26]       |
| S29 BLACK(4TH-4B)   | S29              | black        | +    | +           | 4B                     |            |
| BW BLACK(4TH-4D)    | BW49880          | black        | +    | +           | 4D                     |            |
| E22 BLACK(4TH-4D)   | Element 22       | black        | +    | +           | 4D                     |            |

4.2. Chemicals and Reagents

HPLC grade acetonitrile was purchased from Fisher Scientific (Southborough, UK), and MS grade formic acid from Sigma-Aldrich (Steinheim, Germany). Ultra-pure water was prepared by means of a SIEMENS ULTRA clear (SIEMENS Water Technologies, Munich, Germany), and all other chemicals were of analytical grade.

4.3. Fractional Maceration

To obtain highly concentrated extracts, fractional maceration was employed. In this technique, the total amount of an extractant (reagent grade ethyl alcohol) is divided into three parts and is sequentially applied to grains (first, the first part, then with the second and third). The infusion time for each part of the extractant was 14 days.

4.4. Liquid Chromatography

HPLC was performed on a Shimadzu LC-20 Prominence HPLC system (Shimadzu, Tokyo, Japan) equipped with a UV sensor and a Shodex ODP-40 4E reverse-phase column for the separation of multicomponent mixtures. The gradient elution program was as follows: from time point 0.01 min to 4.00 min, 100% A; from 4 to 60 min, 100–25% A; from 60 to 75 min, 25–0% A; then, a control wash from 75 to 120 min at 0% A. The entire HPLC analysis was carried out with an ESI detector at wavelengths of 230 and 330 nm; the temperature was set to 17 °C, and the injection volume was 1 mL.

4.5. MS

This analysis was performed on an ion trap amaZon SL instrument (Bruker Daltonics, Bremen, Germany) equipped with an electrospray ionization source, in negative ion mode. The following optimal parameters were found and applied: ionization source temperature 70 °C, gas flow 4 L/min, nebulizer gas (atomizer) 7.3 psi, capillary voltage 4500 V, end plate bend voltage 1500 V, fragmentation voltage 280 V, and collision energy 60 eV. The ion trap was used in the scan range m/z 100–1700 for MS and MS/MS. The capture rate was 1 spectrum/s for MS and 3 spectra/s for MS/MS. Data collection was controlled by Hystar Data Analysis 4.1 software (Bruker Daltonics, Bremen, Germany). All the measurements were performed in triplicate. The combination of both ionization modes (positive and negative) in MS full scan mode provided extra confidence of the molecular mass determination. A comprehensive table of molecular masses of the target analytes.
isolated from the EtOH extracts of *T. aestivum* grains was compiled by comparing the m/z values, retention times, and the fragmentation patterns with the MS/MS spectral data from the literature [28,29,31,34,49–57], and other sources or from searches of databases (MS2T, MassBank, and HMDB).

4.6. Data Analysis

A nonparametric test (Spearman’s rank correlation analysis) was performed to compare the wheat lines having different grain colors; for estimation of differences between two groups, we used the two-sided version of the test. We also carried out the Kruskal–Wallis H test (one-way ANOVA on ranks), the Fisher F test (two-way ANOVA), and multiple pairwise analysis (Duncan test) in the STATISTICA 10.0 software [58]. To visualize the obtained data, a dendrogram based on Euclidean distances was drawn by the UPGMA.

5. Conclusions

As shown by a number of pharmacological studies, single-component drugs cannot be sufficiently effective in the treatment of multifactorial diseases. The mixtures of biologically active compounds that possess an ability to interact with each other often turn out to be more effective against a disease as compared to individual components of the mixture. Bioactive natural products containing a wide variety of compounds are considered more attractive for the production of functional foods and pharmacological research than formulations containing only a few components. Currently, the search for raw materials with a wide variety of biologically active compounds is an urgent task. In the present study, diversity of such compounds was investigated in anthocyanin-rich wheat grains by HPLC-MS/MS. Aside from anthocyanin, the study was focused on identifying other families of compounds of a phenolic and nonphenolic nature. A total of 125 biologically active compounds were identified, and among them, 87 were found in wheat grains for the first time. Statistically significantly higher diversity of the compounds was noted in colored grains of wheat in comparison with a control line, whereas between blue- and black-grained groups of lines, no differences were found. The unique chemical profiles with line-specific compounds were determined for each anthocyanin-rich line. The results make these lines promising sources of functional-food ingredients with a wide spectrum of biological activities.

Supplementary Materials: The following are available online, Figure S1: Dendrograms for seven *T. aestivum* lines. The trees were built using the UPGMA and Euclidean distance from data on different groups of chemicals, Figure S2: The breeding scheme for the development of the blue- and black-grained wheat lines used in this study, Table S1: The presence of biologically active compounds in the wheat lines grouped by chromosome substitution, grain color, or both.

Author Contributions: Conceptualization, E.K.K., M.P.R. and K.S.G.; methodology, M.P.R.; resources, E.K.K., A.M.Z., E.I.G. and K.S.G.; metabolome investigation and interpretation, M.P.R. and K.S.P.; metabolomic assay supervision, K.S.G.; metabolomic assay assistance, L.A.K.; data analysis and interpretation of genotype contributions, O.Y.S. and E.V.A.; writing—original draft preparation, M.P.R.; writing—review and editing, E.K.K., A.M.Z., O.Y.S., E.V.A. and K.S.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Russian Science Foundation, grant number 21-66-00012. Wheat lines for chemical analysis of grain were propagated using resources of the Greenhouse Core Facility supported by ICG, project number 0259-2021-0012.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding authors.

Acknowledgments: We thank Nikolai Shevchuk for linguistic advices and proofreading of the manuscript.
Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. The list of compounds identified in EtOH extracts of *T. aestivum* grains.

| ID | Identified Compound | Molecular Formula | Calculated Mass | Observed Mass | Fragment Ions, m/z | References |
|----|---------------------|-------------------|-----------------|---------------|--------------------|------------|
|    | Anthocyanin         |                   |                 |               |                    |            |
| 1  | Cyanidin 3-(2′′-galloylglucoside) | C_{26}H_{32}O_{15} | 601.4891        | 602           | 592, 556, 429, 349, 287, 231 | [59]       |
| 2  | Cyanidin-3-O-3′,6′-O-Dimalonylglucoside | C_{27}H_{32}O_{17} | 621.4772        | 622           | 612, 395, 328, 287, 221 | [31]       |
| 3  | Cyanidin-3-O-glucoside | C_{21}H_{21}O_{11}+ | 449.3848        | 450           | 287; 213; 169; 115 | [31,54,60] |
| 4  | Malvidin 3-O-rutinoside | C_{29}H_{35}O_{16} | 639.5786        | 640           | 493; 331, 315 | [31,61]    |
| 5  | Malvidin 3-O-rutinoside-5-O-glucoside | C_{35}H_{45}O_{21} | 801.7192        | 802           | 639; 493; 331 | [31,61]    |
| 6  | Peonidin 3-O-rutinoside | C_{28}H_{33}O_{15} | 609.5526        | 610           | 463; 343; 301; 285; 258 | [31,59,62] |
| 7  | Peonidin 3-rutinoside-5-glucoside | C_{34}H_{43}O_{20} | 771.6932        | 772           | 705; 463; 367; 301 | [31]       |
| 8  | Peonidin-3-O-glucoside | C_{22}H_{21}O_{11}+ | 463.4114        | 464           | 301; 445; 286; 258 | [31,59,63] |
| 9  | Petunidin | C_{16}H_{13}O_{7} | 317.2702        | 318           | 300; 256; 238; 198 | [31,61]    |
| 10 | Petunidin 3-O-rutinoside-5-O-glucoside | C_{34}H_{43}O_{21} | 787.6926        | 788           | 625; 479; 317 | [31,61]    |
|    | Cinnamic Acid Derivative |                   |                 |               |                    |            |
| 11 | Ferulic acid methyl ester | C_{11}H_{12}O_{4} | 208.2106        | 207           | 179; 135 | [64]       |
|    | Hydroxycinnamic Acid |                   |                 |               |                    |            |
| 12 | 1-Caffeoyl-β-O-glucose | C_{15}H_{18}O_{9} | 342.298         | 341           | 161; 143 | [34,63]    |
| 13 | 1-O-Sinapoyl-β-O-glucose | C_{17}H_{22}O_{10} | 386.3576        | 387           | 205 | [63]       |
| 14 | Caffeic acid derivative | C_{16}H_{15}O_{9}Na | 377.2985        | 377           | 341; 178; 143 | [65]       |
| 15 | Caftaric acid [C6-Caftaric acid; 2-Caffeoyl-1-Tartaric acid] | C_{13}H_{12}O_{9} | 312.23          | 311           | 267; 293; 249; 193; 183; 167 | [37,49,63,66] |
| 16 | Chlorogenic acid [3-O-Caffeoylquinic acid] | C_{16}H_{18}O_{9} | 354.3087        | 355           | 337; 319; 301; 239; 222; 227 | [54,67]    |
| 17 | Ferulic acid | C_{10}H_{10}O_{4} | 194.184         | 195           | 137 | [54,68]    |
|    | Coumarin |                   |                 |               |                    |            |
| 18 | Fraxetin | C_{10}H_{8}O_{5} | 208.1675        | 207           | 179; 161; 135; 117 | [69–71]   |
| 19 | Fraxetin-7-O-sulfate | C_{10}H_{8}O_{5}S | 288.2307        | 287           | 207; 163; 119 | [69]       |
|    | Dihydrochalcone |                   |                 |               |                    |            |
| 20 | Phlorizin [Phloridzin; Phlorizoside; Floridzin; Phloretin 2′-Glucoside] | C_{21}H_{24}O_{10} | 436.4093        | 437           | 275; 257; 203; 173; 150 | [37,53,55,72] |
Table A1. Cont.

| ID | Identified Compound | Molecular Formula | Calculated Mass | Observed Mass | Fragment Ions, m/z | References |
|----|---------------------|-------------------|-----------------|---------------|-------------------|------------|
|    |                     |                   |                 |               | [M − H]^− | [M + H]^+ |
| 21 | Catechin [0-Catechol] | C_{15}H_{14}O_{6} | 290.2681        | 291           | 245; 203; 145     | [53,55,66,68,73–76] |
| 22 | Epicatechin          | C_{15}H_{14}O_{6} | 290.2681        | 291           | 273; 255; 213; 147; 127 | [37,53,66,75–77] |
| 23 | Galloカテchin [+(−)Gallocatechin] | C_{15}H_{14}O_{7} | 306.2675        | 305           | 287; 249; 205; 151; 138; 125 | [37,66,73] |
| 24 | Naringenin [Naringetol] | C_{15}H_{12}O_{3} | 272.5228        | 273           | 156; 191; 112     | [53,57,63,73,78] |
| 25 | 6-C-hexosyl-chrysoeriol O-rhamnoside-O-hexoside | C_{33}H_{38}O_{21} | 770.6422        | 771           | 753; 704; 687; 585; 529; 499; 427; 422; 385; 337; 207 | [27] |
| 26 | Acacetin C-glucoside methylmalonylated | C_{26}H_{26}O_{13} | 546.4758        | 547           | 529; 511; 427; 301; 253; 172 | [79] |
| 27 | Apigenin C_{15}H_{10}O_{5} | 270.2369         | 271             | 271           | 252; 239; 226; 211 | [34] |
| 28 | Apigenin 2′′-O-sinapoyl, C-hexosyl, C-pentosyl | C_{27}H_{38}O_{18} | 770.6868        | 768           | 545; 425; 365; 335; 185 | [29] |
| 29 | Apigenin 6,8-di-C-pentoside | C_{25}H_{26}O_{13} | 534.4661        | 535           | 499; 481; 415; 409; 307; 291 | [34,49,80] |
| 30 | Apigenin 6-C-deoxyhexoside-8-C-pentoside | C_{26}H_{26}O_{13} | 548.4927        | 549           | 531; 465; 369; 519; 248 | [34] |
| 31 | Apigenin-6-C-β-galactosyl-8-C-β-glycosyl-O-glycuronopyranoside | C_{33}H_{38}O_{21} | 770.6422        | 771           | 679; 651; 561; 511; 457; 367; 313; 297; 267; 249; 215; 207; 177; 121 | [28] |
| 32 | Apigenin 8-C-hexoside-6-C-pentoside | C_{26}H_{25}O_{14} | 564.4921        | 565           | 547; 529; 511; 481; 427; 349; 325; 313 | [28,29,35] |
| 33 | Apigenin 8-C-pentoside-6-C-hexoside | C_{26}H_{25}O_{14} | 564.4921        | 565           | 547; 529; 511; 427; 391; 325; 291; | [28,29,35] |
| 34 | Chrysoeriol C_{16}H_{12}O_{6} | 300.2629         | 301             | 286; 258; 229 | [33,60,81] |
| 35 | Chrysoeriol C-hexoside-C-pentoside | C_{27}H_{30}O_{13} | 594.5181        | 595           | 577; 558; 499; 481; 427; 379; 327; 287 | [27,29,32,35] |
| 36 | Cirsiliol C_{17}H_{14}O_{7} | 330.2889         | 329             |               | 229; 211; 171; 155 | [52] |
| 37 | Dihydroxytetramethoxyflavanone | C_{19}H_{20}O_{6} | 376.3573        | 377           | 361; 323; 265; 179 | [37] |
| 38 | Diosmetin [Luteolin 4′-Methyl Ether; Salinigriflavonol] | C_{16}H_{12}O_{6} | 300.2629        | 301           | 286; 258; 177; 138 | [81–83] |
| 39 | Genistein C-glucosylglucoside | C_{27}H_{30}O_{13} | 594.5181        | 595           | 577; 529; 457; 427; 302 | [79] |
| 40 | Hydroxydimethoxyflavone hexoside | C_{23}H_{24}O_{10} | 460.4307        | 461           | 301; 286; 258; 243 | [37] |
| 41 | Luteolin C_{15}H_{10}O_{6} | 286.2363         | 285             |               | 199; 151 | [33,36,50,52,73,84] |
| ID | Identified Compound                                      | Molecular Formula | Calculated Mass | Observed Mass | Fragment Ions, m/z | References |
|----|--------------------------------------------------------|-------------------|-----------------|--------------|-------------------|------------|
| 42 | Luteolin 8-C-Glucoside [Orientin; Orientin (Flavone); Lutexin] | C_{21}H_{20}O_{11} | 448.3769        | 449          | 431; 413; 356; 333; 290; 267; 233; 227 | [28,36,49,55] |
| 43 | Luteolin 8-C-hexoside-6-C-pentoside                     | C_{26}H_{26}O_{15} | 580.4915        | 581          | 563; 515; 485; 413; 377; 342; 205     | [27,29,32,35] |
| 44 | Luteolin 8-C-pentoside-6-C-hexoside                    | C_{26}H_{26}O_{15} | 580.4915        | 581          | 563; 528; 515; 496; 443; 413; 314; 335; 323; 181 | [28,29,33] |
| 45 | Myricetin                                              | C_{15}H_{10}O_{6}  | 318.2351        | 317          | 273; 260; 238     | [37,38,63,73,85] |
| 46 | Orientin 7-O-deoxyhexoside [Luteolin 8-C-glucoside 7-O-deoxyhexoside] | C_{27}H_{30}O_{15} | 594.5181        | 595          | 577; 528; 510; 438; 427; 325 | [34] |
| 47 | Pentahydroxy dimethoxyflavone                          | C_{17}H_{14}O_{6}  | 362.2877        | 363          | 345; 326; 247; 201; 155 | [37] |
| 48 | Pentahydroxy dimethoxyflavone hexoside                 | C_{23}H_{24}O_{14} | 524.4283        | 525          | 463; 363; 257     | [37] |
| 49 | Pentahydroxy trimethoxy flavone                        | C_{18}H_{16}O_{10} | 392.3136        | 393          | 375; 357; 328; 269; 230; 218 | [37] |
| 50 | Tricin                                                 | C_{17}H_{14}O_{7}  | 330.2889        | 331          | 315; 287; 285; 270; 229 | [28,33,36,54] |
| 51 | Tetrahydroxy-dimethoxyflavone-hexoside [Syringetin-hexoside; dimethyl-myricetin-hexoside] | C_{23}H_{24}O_{13} | 508.4289        | 509          | 347; 329; 316; 265; 185; 181 | [38,49,80] |
| 52 | Trihydroxy methoxyflavone triacetate                   | C_{18}H_{18}O_{9}  | 378.3301        | 379          | 361; 321; 287; 234; 223; 167 | [37] |
| 53 | Vicenin-2 [Apigenin-6,8-Di-C-Glucoside]                | C_{27}H_{30}O_{15} | 594.5181        | 595          | 577; 559; 541; 529; 523; 499; 469; 439; 427; 391 | [28–30,34,35,50,55] |
| 54 | Vitexin 2′-O-glucoside [Apigenin 6-C-glucoside 2′-O-glucoside] | C_{27}H_{30}O_{15} | 594.5181        | 595          | 577; 514; 457; 288 | [34] |
| 55 | Vitexin 6′-O-glucoside [Apigenin 8-C-glucoside 6′-O-glucoside] | C_{27}H_{30}O_{15} | 594.5181        | 595          | 577; 559; 528; 511; 499; 493; 487; 445; 427 | [34] |
| 56 | Wighteone-O-glucoside                                  | C_{26}H_{25}O_{10} | 500.4945        | 501          | 339; 262; 185; 167 | [79] |
| ID | Identified Compound | Molecular Formula | Calculated Mass | Observed Mass [M – H]$^*$ | [M + H]$^+$ | Fragment Ions, m/z | References |
|----|---------------------|------------------|----------------|-----------------------------|-------------|-------------------|-----------|
| 57 | Ampelopsin          | C$_{15}$H$_{12}$O$_{8}$ | 320.251        | 321                         | 303; 285; 163 | [86,87]           |
| 58 | Isorhamnetin        | C$_{16}$H$_{12}$O$_{7}$ | 316.2623       | 315                         | 300; 272; 243; 145 | [38,53]           |
| 59 | Kaempferide         | C$_{16}$H$_{12}$O$_{6}$ | 300.2629       | 301                         | 286; 258; 229; 174; 153 | [52,88]           |
| 60 | Kaempferol          | C$_{15}$H$_{10}$O$_{6}$ | 286.2363       | 287                         | 268; 214; 196; 160; 123 | [52,63,73,89]     |
| 61 | Quercetin           | C$_{15}$H$_{10}$O$_{7}$ | 302.2357       | 301                         | 283; 255; 227 | [38,53,54,57,63,68,73,76] |
| 62 | Rhamnetin I         | C$_{16}$H$_{12}$O$_{7}$ | 316.2623       | 317                         | 255; 197; 139; 122 | [86]          |
| 63 | Rhamnetin II        | C$_{16}$H$_{12}$O$_{7}$ | 316.2623       | 317                         | 256; 121; 228; 111 | [86]          |
| 64 | Selgin              | C$_{16}$H$_{12}$O$_{7}$ | 316.2623       | 317                         | 315; 256; 161 | [90]          |
| 65 | Taxifolin-3-O-glucoside | C$_{21}$H$_{22}$O$_{12}$ | 466.3922       | 467                         | 449; 373; 258; 199; 177 | [63]          |
| 66 | Taxifolin-O-pentoside | C$_{20}$H$_{20}$O$_{11}$ | 436.371        | 437                         | 303; 259; 177; 169 | [37]          |
| 67 | β-Glucogallin       | C$_{13}$H$_{16}$O$_{10}$ | 332.2601       | 331                         | 313; 295; 277; 171; 140; 127 | [53,91]     |
| 68 | 4-Hydroxybenzoic acid | C$_{2}$H$_{6}$O$_{3}$ | 138.1207       | 139                         | 137; 121 | [35,49,63,74]   |
| 69 | Cis-salvianolic acid J | C$_{27}$H$_{22}$O$_{12}$ | 538.4564       | 539                         | 523; 481; 393; 360; 319; 247; 204; 191; 120 | [81]          |
| 70 | Hydroxy methoxy dimethylbenzoic acid | C$_{10}$H$_{12}$O$_{4}$ | 196.1999       | 197                         | 179; 160; 133 | [37]          |
| 71 | Salvianolic acid D  | C$_{20}$H$_{14}$O$_{10}$ | 418.3509       | 417                         | 373; 329; 287 | [49,92]      |
| 72 | Salvianolic acid F  | C$_{17}$H$_{14}$O$_{6}$ | 314.2895       | 313                         | 295; 277; 223; 171; 155 | [49,92]      |
| 73 | Salvianolic acid G  | C$_{18}$H$_{12}$O$_{7}$ | 340.2837       | 341                         | 323; 260; 199; 168 | [81,92]      |

**Table A1. Cont.**

**Flavonol**

**Hydroxybenzoic Acid**

**Gallotannin**
Table A1. Cont.

| ID | Identified Compound                           | Molecular Formula | Calculated Mass | Observed Mass | Fragment Ions, m/z | References |
|----|----------------------------------------------|-------------------|-----------------|---------------|-------------------|------------|
|    |                                              |                   |                 | [M − H]⁺       |                   |            |
| 74 | Dimethyl-secoisolariciresinol               | C_{22}H_{30}O_{6} | 390.4700        | 391           | 355; 336; 308; 218; 149 | [93]       |
| 75 | Hinokinin                                    | C_{20}H_{18}O_{6} | 354.3533        | 355           | 336; 318; 300; 207; 181; 177 | [28,30,54] |
| 76 | Pinoresinol                                  | C_{20}H_{22}O_{6} | 358.3851        | 357           | 339; 311; 267; 213; 197; 171; 155; 139 | [28,30,34] |
| 77 | Podophyllotoxin [Podofilox; Condylox; Condyline; Podophyllinic acid lactone] | C_{22}H_{12}O_{6} | 414.4053        | 415           | 397; 379; 310; 275; 250; 182 | [93]       |
| 78 | Syringaresinol                               | C_{22}H_{26}O_{6} | 418.4436        | 419           | 357; 327; 275; 185; 158 | [54]       |
|    |                                              |                   |                 | [M + H]⁺       |                   |            |
| 79 | 1-O-cafeoyl-5-O-feruloylquinic acid         | C_{26}H_{26}O_{12} | 530.4774        | 531           | 513; 415; 337; 195; 176; 115 | [52]       |
| 80 | 4-O-Caffeoyl-5-O-p-coumaroylquinic acid     | C_{25}H_{21}O_{11} | 500.4515        | 501           | 339; 244; 189; 140 | [55,84]    |
| 81 | Feruloyl sulfate                             | C_{10}H_{10}O_{5} | 274.2472        | 273           | 193; 192; 149 | [94]       |
| 82 | Gallic acid hexoside                         | C_{13}H_{16}O_{10} | 332.2601        | 333           | 242; 212; 182; 159 | [95]       |
|    |                                              |                   |                 |               |                   |            |
| 83 | Pinosylvin [3,5-Stilbenedio; Trans-3,5-Dihydroxystilbene] | C_{14}H_{12}O_{2} | 212.2439        | 213           | 197; 183; 166; 124 | [96]       |
| 84 | Polydatin [Piceid; trans-Piceid]            | C_{20}H_{22}O_{8} | 390.3839        | 391           | 355; 333; 265; 227; 209; 145 | [73,77]    |
| 85 | Resveratrol [trans-Resveratrol; 3,4′,5-Trihydroxystilbene; Stilbentriol] | C_{14}H_{12}O_{3} | 228.2433        | 229           | 228; 142; 114 | [37,73]    |
|    |                                              |                   |                 |               |                   |            |
| 86 | Undecanedioic acid                           | C_{11}H_{20}O_{4} | 216.2741        | 217           | 173; 157; 142; 118; 115 | [34]       |
| 87 | Myristoleic acid [Cis-9-Tetradecanoic acid] | C_{14}H_{26}O_{2} | 226.3550        | 227           | 209; 138; 127; 110 | [34]       |
| 88 | 11-Hydroperoxy-octadecatrienoic acid        | C_{18}H_{30}O_{4} | 310.4284        | 309           | 291; 209; 207; 125 | [97]       |
| 89 | 9,10-Dihydroxy-8-oxooctadec-12-enoic acid [oxo-DHODE; oxo-Dihydroxy-octadecenoic acid] | C_{18}H_{32}O_{3} | 328.4437        | 327           | 229; 211; 171; 135; 125 | [35,36]    |
| 90 | Dihydroxy docosanoic acid                   | C_{22}H_{44}O_{4} | 372.5824        | 371           | 327; 297; 282; 251; 187; 125 | [34]       |
| 91 | Docosenoic acid [2-Docosenoic acid]         | C_{22}H_{42}O_{2} | 338.5677        | 339           | 322; 295; 256; 215; 163 | [34]       |
| 92 | Hydroxy methoxy dimethylbenzoic acid        | C_{10}H_{12}O_{4} | 196.1999        | 195           | 177; 129 | [34]       |
| ID | Identified Compound                        | Molecular Formula | Calculated Mass | Observed Mass | Fragment Ions, m/z | References |
|----|------------------------------------------|-------------------|----------------|---------------|-------------------|------------|
| 93 | Pentacosenoic acid                        | C<sub>35</sub>H<sub>48</sub>O<sub>2</sub> | 380.6474       | 381           | 363; 293; 173; 135 | [34]       |
| 94 | Salvianic acid C                          | C<sub>18</sub>H<sub>18</sub>O<sub>9</sub> | 378.3301       | 379           | 361; 343; 335; 326; 247; 237; 205; 151; 129 | [92]       |
| 95 | Vebonol                                  | C<sub>35</sub>H<sub>44</sub>O<sub>3</sub> | 452.6686       | 453           | 435; 336; 209     | [86]       |
| 96 | Cyclopassifloic acid glucoside            | C<sub>37</sub>H<sub>52</sub>O<sub>12</sub> | 698.8810       | 699           | 537; 421; 348; 203 | [31]       |
| 97 | (3S, 3′S, all-E)-zeaxanthin               |                   |                |               |                   |            |
|    | [Zeaxanthin; (35,3′S)-Zeaxanthin]         | C<sub>40</sub>H<sub>56</sub>O<sub>2</sub> | 568.8714       | 569           | 551; 375; 329; 279; 235; 210; 153 | [51]       |
| 98 | Cryptoxanthin [β-cryptoxanthin]           |                   |                |               |                   |            |
| 99 | Isocryptotanshinone II                    |                   |                |               |                   |            |
| 100| Tanshinone IIIB [S(6)-(Hydroxymethyl)-1,6-Dimethyl-6,7,8,9-Tetrahydrophenanthro[1,2-b]Furan-10,11-Dione] | C<sub>19</sub>H<sub>18</sub>O<sub>4</sub> | 310.3438       | 309           | 291; 273; 251; 235; 209; 207; 122 | [98]       |
| 101| β-Amyrin [β-Amyrenol; Amyrin; Olean-12-en-3β-ol] | C<sub>30</sub>H<sub>50</sub>O | 426.7174       | 427           | 409; 391; 373; 292; 269; 240; 190; 145; 137 | [34]       |
| 102| Gibberellic acid                          | C<sub>19</sub>H<sub>22</sub>O<sub>6</sub> | 346.3744       | 347           | 301; 282; 263; 242; 201; 185; 139 | [99]       |
| 103| Betunolic acid                            | C<sub>30</sub>H<sub>46</sub>O<sub>3</sub> | 454.3446       | 455           | 436; 355; 236; 226 | [86]       |
| 104| Ursolic acid                              | C<sub>30</sub>H<sub>48</sub>O<sub>3</sub> | 456.7003       | 457           | 439; 263; 177; 145 | [52,56,81,100] |
| 105| Squalene (Trans-Squalene; Spinacene; Supraene) | C<sub>30</sub>H<sub>50</sub> | 410.718        | 411           | 235; 218; 177; 147 | [101,102] |
| 106| Uvaol                                    | C<sub>30</sub>H<sub>50</sub>O<sub>2</sub> | 442.7168       | 443           | 425; 407; 315; 304; 287; 230; 154; 137 | [34]       |
| 107| L-Histidine                              | C<sub>6</sub>H<sub>11</sub>N<sub>2</sub>O<sub>2</sub> | 155.1546       | 156           | …                | [103]      |
| 108| L-Tryptophan [Tryptophan; (S)-Tryptophan] | C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub> | 204.2252       | 205           | 188; 146; 118     | [34,91,104] |
| 109| L-Valine                                 | C<sub>6</sub>H<sub>11</sub>NO<sub>2</sub> | 117.1463       | 118           | …                | [103]      |
| 110| Tyrosine [2S)-2-Amino-3-(4-Hydroxyphenyl)Propanoic acid] | C<sub>9</sub>H<sub>11</sub>NO<sub>3</sub> | 181.19         | 182           | 155; 127; 116     | [104]      |
| 111| Sesperdole                               | C<sub>31</sub>H<sub>53</sub>NO<sub>4</sub> | 519.7147       | 520           | 184; 125         | [86]       |
| 112| Berberine [Berberin; Umbelletine; Berbericine] | C<sub>26</sub>H<sub>38</sub>NO<sub>4</sub> | 336.3612       | 337           | 320; 303; 207; 206; 115 | [105]      |
| 113| GA8-hexose gibberellin                   | C<sub>25</sub>H<sub>32</sub>O<sub>12</sub> | 526.5303       | 527           | 365; 305; 275; 245; 203; 143 | [91]       |
| 114| Abscisic acid [Dormin; Abscisin II; (S)+-Abscisic acid] | C<sub>15</sub>H<sub>20</sub>O<sub>4</sub> | 264.3169       | 265           | 247; 122         | [99]       |
| 115| Ketoprofen [Orudis; 2-(3-Benzoylphenyl)Propionic acid; Profenid] | C<sub>16</sub>H<sub>14</sub>O<sub>3</sub> | 254.2806       | 253           | 209; 191; 165; 121 | [106]      |
Table A1. Cont.

| ID  | Identified Compound                                      | Molecular Formula | Calculated Mass | Observed Mass [M – H]$^+$ | [M + H]$^+$ | Fragment Ions, $m/z$ | References |
|-----|---------------------------------------------------------|-------------------|-----------------|---------------------------|-------------|----------------------|------------|
| 116 | Adenosine                                               | C$_{10}$H$_{13}$N$_5$O$_4$ | 267.2413        | 268                       | 136         | 379; 361; 309; 282; 239; 189; 125    | [105]      |
| 117 | Ergosterol [Provitamin D2; Ergosterin]                  | C$_{28}$H$_{41}$O | 396.6484        | 397                       |             | 395; 376; 358; 336; 325; 271; 269; 251; 229; 224; 201; 165; 159; 124  | [34]       |
| 118 | Avenasterol [Delta7-Avenasterol; 7-Dehydroavenasterol] | C$_{29}$H$_{48}$O | 412.6908        | 413                       |             | 493; 375; 358; 269; 261; 235; 152; 147 | [34]       |
| 119 | β-Sitostenone [Stigmast-4-En-3-One; Sitostenone]       | C$_{29}$H$_{48}$O | 412.6908        | 413                       |             | 493; 375; 297; 268; 213; 163; 133; | [34]       |
| 120 | β-Sitosterin [β-Sitosterol]                             | C$_{29}$H$_{50}$O | 414.7067        | 415                       |             | 337; 319; 311; 266; 239; 189; 182; 127 | [37,100]   |
| 121 | Campestenone                                            | C$_{28}$H$_{46}$O | 398.6642        | 399                       |             | 395; 375; 358; 340; 303; 267; 201; 195; 167; 121 | [34]       |
| 122 | Fucosterol [Fucostein; Trans-24-Ethylidenecholesterol] | C$_{29}$H$_{48}$O | 412.6908        | 413                       |             | 427; 385; 319; 205; 165; 164; 137  | [34]       |
| 123 | Oxo-hydroxy sitosterol                                   | C$_{29}$H$_{48}$O$_3$ | 444.6896        | 445                       |             | 337; 121; 263         | [86]       |
| 124 | Vapiprost                                               | C$_{30}$H$_{39}$NO$_4$ | 477.6350        | 478                       |             | 250.3764  | 251; 233; 204; 147  | [34]       |
| 125 | Hexadecatrienoic acid                                   | C$_{16}$H$_{26}$O$_2$ | 250.3764        | 251                       |             |                               | [34]       |

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