Phytochemical Profiling of Soybean (*Glycine max* (L.) Merr.) Genotypes Using GC-MS Analysis

Salem Alghamdi, Hussein Migdadi, Muhammad Khan, Ehab H. El-Harty, Megahed Ammar, Muhammad Farooq and Muhammad Afzal

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Abstract

Twenty-four soybean genotypes collected from different regions and origin were evaluated for their quality performance to explore their nutritional and medicinal values. The proximate compositions showed considerable variations among soybean genotypes. The USA genotypes recorded the highest values for protein (43.1 g/100 g), total fat (23.61 g/100 g), phenolic content and flavonoids (1.77 and 2.13 mg/g). Using GC-MS analyses of methanolic extracts, a total of 88 compounds were identified in the genotypes and were classified to: 19 heterocyclic compounds, 13 compounds for ketones and esters, 9 for phenolic compound, 7 compounds for carboxylic acids and sugar moiety, 5 compounds for aldehydes and alcohols, 4 ether compounds, 3 amide, 2 alkanes and one alkene and one fatty acid ester. Indonesian genotypes recorded the highest number of phenolic and the Australian genotype A-1 had the maximum number of esters. Genotypes showed high levels of proximate compositions and pharmaceutical components, offering potential candidates for improving those traits in adapted genotypes through breeding program, as well as serving as a good source of mass production of pharmaceutical and medicinal components either through classical or in vitro production. Furthermore, platform was set for isolating and understanding the characteristics of each compound for its pharmacological properties.

Keywords: soybean, phenolic compounds, GC-MS, flavonoids, nutritional value
1. Introduction

Soybean (*Glycine max* (L.) Merr) considered among ancient cultivated crops, it was domesticated in the 11th century BC around Northeast of China. It is one of the most widely grown leguminous crops in the world. Its cultivated area was recorded in 95 countries more than 121 million hectare that produced 335 million tons of dry seeds [1] (FAOSTAT, 2016). Soybean had a wide variability, the USDA alone maintains more than 15 thousand soybean accession grouped into 13 maturity classes including both determinate and indeterminate soybean. Early maturing groups are adapted to short summer growing seasons in North USA and Canada while late maturity groups are adapted to southern or coastal plain counties [2].

Soybean occupies an advanced position among agricultural crops, being the most important source of proteins and vegetable oils [3]. Its seeds provided abundant and high quality protein and oil for human diet and animal feed. Its seeds contain more than 36% protein, 30% carbohydrates in addition to fiber, vitamins, and minerals [3]. It also contains about 20% oil, which makes soybean one of the most important edible oil crops. Soy oil has used as binding additives in manufacturing of papers, inks, paints, varnishes, cosmetics, and plastics. It was used also in production of farming pesticides and pharmaceuticals products [4]. Nowadays, biodiesel utilizing soy oil become a new industrial renewable sources of energy. Additionally, soybean as a nitrogen-fixing legume crop helps in reducing the chemical source of nitrogen fertilizers production [4].

Furthermore, tofu, soy milk, soy sauce, miso, etc., have been developed for human consumption, while soya meal (oil extraction by-product) is used as a nutritious animal feed [5]. Moreover, soybean is now regarded as a model legume crop owing to the availability of genome sequence information [6]. Keeping in mind its vast uses, there is huge number of justifications for crop improvement programs throughout the world. Having 53% global production share of all oilseed crops, USA, China, Brazil, Argentina and India gave soybean much attention in the agricultural production systems. Yield and total production of soybean increased over the last two decades due to genetic improvement of this crop [7].

In comparison with conventional legume and animal feed sources, soybean is considered one of the cheapest food resources with medicinal properties due to their highest protein content and no cholesterol due to its contents of Genistein, phytochemical and isoflavones [8]. It can help in disease fighting due to its pharmacological properties and its phytochemicals constitutes, including antioxidant, estrogenic, antidiabetic, anti-hypercholesterolemic, anti-hyperlipidemic, anti-obesity, antihypertensive, anticancer, anti-mutagenic, hepatoprotective, anti-osteoporotic, antiviral, bifidogenic, anti-inflamatory, immunomodulatory, neuroprotective, wound healing, antimicrobial, goitrogenic anti-skin aging, anti-photoaging activity and the effects of anti-nutritional factors [3]. A 111 volatile compounds in fermented soybean curds were reported by Chung [9] and an 83 in commercial plain sufu [10]. Messina [11] reported that the presence of isoflavones in soybean is behind the pharmacological attributes of this crop. Chemical composition included Phenolic acids, flavonoids, isoflavones, sapo- nins, phytosterols and sphingolipids were recorded in soybean [12–14]. Due to importance of
this crop and its products, this study was aimed at estimating the most active constituents of 24 soybean genotypes including total phenolic, flavonoid and protein content and phytochemicals using GC-MS.

2. Materials and methods

2.1. Plant materials

Twenty-four soybean genotypes were grown in Dirab Agriculture Research Station, King Saud University, Riyadh, Kingdom of Saudi Arabia (24°25'49.200N 46°22'01.500E) on August, 2014 and were collected from nine countries (Argentina, Australia, China, Egypt, India, Indonesia, USA, and Pakistan). The name and geographical origin of these genotypes are presented in the Table 1.

2.2. Chemical analysis

2.2.1. Proximate composition

Triplicate sample is used to determine the proximate analysis of soybean genotypes for crude proteins, moisture, total ash, fat and carbohydrate by using the methods described in AOAC, [15]. Protein content was estimated using Kjeldahl method with titration and percent nitrogen was determined using [16] equation.

| Entry no. | Genotype name | Source/origin | Entry no. | Genotype name | Source/origin |
|-----------|---------------|---------------|-----------|---------------|---------------|
| 1         | Admaril       | Pakistan      | 13        | Giza 111      | Egypt         |
| 2         | Romal-1       | Pakistan      | 14        | Clark         | USA           |
| 3         | NARC-2        | Pakistan      | 15        | 3803          | Syria         |
| 4         | Williams 82   | USA           | 15        | A-1           | Australia     |
| 5         | X 32          | Egypt         | 17        | Ijen          | Indonesia     |
| 6         | Giza 22       | Egypt         | 18        | Indo-black    | Indonesia     |
| 7         | Giza 21       | Egypt         | 19        | Indo-I        | Indonesia     |
| 8         | X 2 L 12      | Egypt         | 20        | Indo-II       | Indonesia     |
| 9         | Giza 83       | Egypt         | 21        | USA-1         | USA           |
| 10        | Crawford      | USA           | 22        | Indian        | India         |
| 11        | Giza 35       | Egypt         | 23        | Chinese       | China         |
| 12        | X 30          | Egypt         | 24        | Argentinian   | Argentina     |

Table 1. Name and source of the 24 soybean genotypes investigated in the study.
2.2.2. Antioxidants determination

Soybean samples approximately (1 g) were powdered and homogenized in 10 ml 80% methanol. The mixture was shaken at 300 rpm at room temperature for 3 h. Then the extract was centrifuged for 10 min at 3000 rpm and upper aqueous phase were transferred to new Eppendorf tubes. Moreover, the residues were again extracted with 5 ml 80% methanol overnight. The extraction was performed in three replicates, later on extracts combined and stored in dark at 4°C. The Folin-Ciocalteu reagent was used to determine the total phenolic compounds from the extracts using gallic acid calibration curve as standard. The total phenolics were expressed as mg/g gallic acid equivalents (GAE). An extract was aliquot (50 μl) and mixed with Folin-Ciocalteu reagent of 250 μl and 7.5% sodium carbonate of 750 μl. The volume was increased to 5 ml with water and sample was incubated for 2 h. The absorbance was measured at 765 nm against distilled water as blank. The flavonoid determination was measure by aluminum chloride method with the help of Quercetin equivalent as standard. An aliquot of extract (250 μl) was mixed with ddH2O and 5% NaNO2 (15:1, v/v). After 6 min, 150 μl of 10% AlCl3 was added to the mixture. A 500 μl of 1 M NaOH was added to the mixture at the 5th min, and volume made up to 2.5 ml with distills water and the absorbance was measured spectrophotometrically at 410 nm.

2.2.3. Gas chromatography-mass spectroscopy

The GC-MS analysis of fractions were performed using a TSQ™ 8000 Evo Triple Quadrupole GC-MS/MS (Thermo Fisher Scientific) equipped with an Elite-5 capillary column (length 30 nm and inner diameter 0.25 mm and film thickness 0.25 μm) and mass detector was operated in electron impact (EI) mode with full scan (50–550 amu). Helium was used as the carrier gas at constant flow rate 1 mL/min and an injection volume of 1 μL. The oven injector temperature was programmed from 50°C with an increase of 8°C/min to 200°C, then 7°C/min to 290°C/min. The results were compared using the database of National Institute Standard and Technology (NIST).

2.3. Data analysis

The data were subjected to descriptive statistics (mean, standard deviation, coefficient of variability, minimum and maximum values) and principal component analysis (PCA) using statistical software Past3 program [17].

3. Results and discussion

3.1. Proximate analysis

The proximate analysis values of 24 soybean genotypes (crude protein, ash fat, carbohydrate, and moisture contents) values and total phenolic and flavonoid contents are shown in Table 2, and the detailed proximate analysis estimates are presented in Table S1. The minimum crude protein value was recorded for Argentinian (35.63%), while maximum recorded for Clark genotypes (43.13%). The genotypes, i.e., Clark, Indo-1, Indo-black, Ijen, Romal-1, X 30 and 3803 recorded higher than 40% crude protein. The significant variations for crude proteins
among genotypes were recorded and that might observed due to differences in genetic background and/or origin. The higher protein content in the genotypes is also reported previously which ranged from 43 to 45% [18]. These results are also in line with Zarkadas et al. [19, 20] who reported crude protein contents in soybean ranging from 33.67 to 42.11%. The minimum moisture contents were recorded in Giza 83 (3.08%) while maximum was recorded for Indo-1 (5.88%) with an average (4.90%) mean value showing non-significant difference. Ash contents ranged from 4.55 to 6.28% with an average of 5.44%. The maximum was recorded for Giza 111 (6.28%) genotype while Romal-1 genotype had the lowest (4.55%) of ash contents. The moisture and ash contents values were recorded lower than that reported by [21]. Total fat ranged from 16.92 to 22.94% with a mean value of 21.16%. The genotype Indo-black contained the lowest while the genotype 3803 recorded the highest content. Soybean is considered about 47% of its energy value in fat content [22, 23], which is compared to other legumes. Our results regarding total fat were in line with that of [24] who reported that that total fat value ranged 18 and 22 g/100 g in soybean genotypes. The minimum carbohydrate content in Clark (26.11%) while maximum in Argentinian (33.18%), with an average (29.48%) was recorded among soybean genotypes.

3.2. Flavonoid and phenolic contents

Flavonoid and phenolic compounds are the important phytochemicals and natural antioxidants founds in fruits, vegetable and cereals grains. It serves as multiple biological functions, i.e., defense against cardiovascular disease, cancer and aging [25]. The results regarding total phenolic and flavonoids contents for 24 soybean genotypes are presented in Table S1, and significant differences were recorded for all soybean genotypes. The seed extracted results indicated that the maximum phenolic contents was recorded in Romal-1 (1.7 mg/g) while minimum in Giza 111 (1.15 mg/g) with an average 1.45 GAE/g mg/g (Table 2). However, total flavonoid content ranged 0.68 to 2.13 mg QE/g (Table 2). Phenolic content is strongly linked with antioxidant capacity [26, 27] and can contribute towards antioxidants activities [28]. The use and demands of phenolic are increasing rapidly in food industry to enhance nutritional value and quality of food [29].

| Crude protein (g/100 g) | Moisture (g/100 g) | Ash (g/100 g) | Total fat (g/100 g) | Carbohydrate (g/100 g) | Total phenolic content (TPC) | Total flavonoid content (TFC) |
|-------------------------|-------------------|---------------|---------------------|------------------------|-----------------------------|-------------------------------|
| N                       | 24                | 24            | 24                  | 24                     | 24                          | 24                            |
| Min                     | 35.63             | 3.08          | 4.55                | 16.92                  | 26.11                       | 1.15                          | 0.68                          |
| Max                     | 43.13             | 5.88          | 6.28                | 23.61                  | 33.18                       | 1.77                          | 2.13                          |
| Mean                    | 39.02             | 4.90          | 5.44                | 21.16                  | 29.48                       | 1.45                          | 1.24                          |
| Stand. dev.             | 2.09              | 0.65          | 0.33                | 1.41                   | 1.86                        | 0.16                          | 0.36                          |
| Coeff. Var.             | 5.35              | 13.26         | 6.11                | 6.68                   | 6.30                        | 11.58                         | 29.32                         |

Table 2. Descriptive statistics of chemical composition in 24 soybean genotypes.
3.3. GC-MS analysis

Methanolic extracts of 24 soybean genotypes using GC-MS analysis were used to identify a large number of phytochemical. Based on peak area, retention time and molecular formula, about 88 compounds were recognized. A large number of bioactive phytochemicals including flavonoids, phenolic acids, saponins, isoflavones, sphingolipids and phytosterols were also reported previously for soybean [12–14]. The carbamide was the first compound that identified at 3.67 min retention time, whereas, last compound identified at 48.53 min retention time was methyl 10 Trans, 12-cis octadecadienoate recognized at 48.53 min retention time (Table S2). A wide difference was recorded for composition of phytochemical in 24 soybean genotypes. The phytocompounds and their biological activities in soybean genotypes were presented in Table 3. The phytocompounds of the studied soybean genotypes divided into different groups (Figure 1). The resulted 88 compounds were categorized into heterocyclic compounds (19), aldehydes (5), alcohols (5), esters (13), amide (3), sugar moiety (7), ether (4),

| Compound | Other names | Nature            | Activity                                  | RT  | MW  |
|----------|-------------|------------------|-------------------------------------------|-----|-----|
| 22       | 2H-1-Benzopyran,3,5,6,8a-tetrahydro-2,5,5,8a-tetramethyl-(2S-cis)- | Edulan II | Heterocyclic compound                     | 7.58 | 192 |
| 27       | 1,2-Cyclopentanedione |          | Ketone                                   | 7.98 | 98  |
| 28       | Pyran-4-Carboxylic acid, 4-(4-methoxyphenyl)-tetrahydro- |          | Heterocyclic compound                     | 8.02 | 236 |
| 34       | 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one |          | Ketone                                   | 9.74 | 144 |
| 36       | 2H-Pyran-2,6(3H)-dione Glutaconic anhydride |          | Heterocyclic compound                     | 10.75 | 112 |
| 39       | 2-Pyrrolidinone, 1-methyl |          | Ketone                                   | 11.86 | 99  |
| 42       | 2,5-Dimethyl-4-hydroxy-3(2H)-furanone |          | Ketone                                   | 12.28 | 128 |
| 44       | Phenol, 2-methoxy- |          | Phenolic compound                        | 13.83 | 124 |
| 49       | 4H-Pyran-4-one, 3-hydroxy-2-methyl- |          | Heterocyclic compound                     | 14.78 | 126 |
| 50       | 5-Hepten-3-one, 5-methyl- |          | Ketone compound                          | 15.09 | 126 |
| 52       | 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one |          | Heterocyclic compound                     | 16.61 | 144 |
| 57       | Phenol, 4-ethenyl-, acetate 4-Vinylphenyl acetate |          | Phenolic compound                        | 19.29 | 162 |
| 60       | Benzofuran, 2,3-dihydro Coumaran |          | Heterocyclic compound                     | 20.16 | 120 |
| 62       | Benzeneacetaldehyde, 3-methyl m-Tolualdehyde |          | Aldehyde                                  | 20.34 | 120 |
phenolic compound (9), carboxylic acids (7), ketones (13), alkanes (2), one fatty acid ester and one Alkene. A typical chromatogram of one soybean genotype was shown in Figure 2. The GC-MS analyses showed that the methanolic extract is largely composed of heterocyclic compound, ester and phenolic compound. Hexadecanoic acid, methyl ester, 2,6-dimethoxy, 3,5-dimethoxyacetophenone, 2-methoxy-4-vinylphenol, phenol and 1,2-cyclopentanedione were noticed in most of the genotypes. These phytochemicals are involved in various pharmacological actions, i.e., antioxidants and antimicrobial activities. These chemicals are also active in many biological activities that were listed (Table S2). Phytochemicals also possess antioxidant activities, anti-cancer, anticarcinogenic, antibacterial, antiviral, and anti-inflammatory activities and play an important role for plant metabolism. The five compounds belong to aldehyde group (benzeneacetaldehyde, 3,4-dimethylbenzaldehyde, methoxypropanal, p-hydroxyphenyl, glyoxal and propanal, 2-(benzoyloxy), benzeneacetaldehyde), were detected in 10 genotypes (Table S2). Admiral and Williams 82 contains 3-methoxypropanal while indo-black, Indo-1 and Indo-II contains 3,4-dimethylbenzaldehyde, whereas Giza 35 and X30 contains p-hydroxyphenyl) glyoxal and propanal, 2-benzoyloxy, respectively. The highest number of aldehyde compounds is present in William 82 genotype (2). It is also reported that; aldehyde possess powerful antimicrobial activity due to their highly electron-negative arrangement of conjugated group C=C double bond, as the electronegativity increase, antimicrobial activity also increases in those genotypes. Thirteen ketone related compounds were identified, i.e., 1-(dimethylamino)-

| Compound | Other names | Nature | Activity | RT | MW |
|----------|-------------|--------|----------|----|----|
| 61       | 1,2-Benzenediol,3-methoxy... | Phenolic compound | Antioxidant | 21.01 | 140 |
| 64       | 2-Methoxy-4-vinylphenol | Phenolic compound | Antimicrobial, antioxidant, anti-inflammatory | 23.35 | 150 |
| 68       | Phenol, 2,6-dimethoxy-... | Phenolic compound | Antimicrobial, antioxidant, anti-inflammatory | 24.99 | 154 |
| 70       | Phenol,2,6-bis(1,1-dimethylethyl)-4-methyl-... | Phenolic compound | Antimicrobial, antioxidant, anti-inflammatory, analgesic | 30.99 | 220 |
| 71       | Phenol, 2,4-bis(1,1-dimethylethyl)-... | Phenolic compound | Antimicrobial, antioxidant, anti-inflammatory | 31.19 | 206 |
| 72       | 5-tert-Butyl-1,2,3-benzenetriol | Phenolic compound | Antioxidant, antiseptic, antibacterial, anti-dermatitic fungicide, pesticide | 31.91 | 182 |
| 76       | 3,5-Dimethoxyacetophenone | Ketone | Antioxidant | 33.65 | 180 |
| 85       | Hexadecanoic acid, methyl ester | Ester | Antioxidant, flavor, hypocholesterolemic, nematicide | 46.13 | 270 |

Table 3. List of important phytocompounds identified in the methanolic seed extract of soybean genotypes by GC-MS.
1,2-propanone, 1,2-cyclopentanedione and 6-Oxa-bicyclo [3.1.0] hexan-3-one, 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine, butyrolactone, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 5-hepten-3-one, 5-methyl-, dihydroxyacetone, 2-pyrroolidinone, 1-methyl, 2,4,6-cycloheptatrien-1-one, 4-methyl-, 3,5-dimethoxyacetophenone. The indo-11 and 3803 genotypes recorded highest ketonic compounds (8) followed by present in Giza 35 and USA-1 genotypes that contained 6 ketonic group each. Ketones might be formed by beta-oxidation of fatty acid and have some important flavor compounds [35].

Figure 1. Pie diagram showing the percentage of phytochemical groups identified in 24 soybean genotypes.

Figure 2. A typical GC-MS profile of seeds of soybean genotype.
acid metabolism, many volatile compounds are also formed, producing alcohols, acids and esters. Many alcoholic compounds are derived from bioremediation of unsaturated fatty acids and are prerequisite for the formation of long chain esters. These identified compounds in soybean genotypes are 4-methyl-2-haptanol, 1,2,3-propanetriol, isosorbide (D-glucitol, 1,4,3,6-dianhydro), 1-undecanol alcohol, and 1,3-dioxolane-4-methanol (glycerol formal).

4-Methyl-2-haptanol was present in Genotype Giza 35 while 1,2,3-propanetriol was present in nine genotypes and isosorbide was detected in three soybean genotypes. The highest alcoholic compounds (3) were detected in Clark genotype as compared to other genotypes. Alcohols also possess antibacterial activity against vegetative cell. Glycerol and derivatives also show bacterial inhibiting effect [36]. The following seven carboxylic acids namely acetic acid, 2-pyridinecarboxylic acid (also called picolinic acid), 2,2-[oxybis(2,1-ethanediyoxy)]bis, butanoic acid, 4-hydroxy-, propyl-(also called 2-propylmalonic acid), propanedioic acid, benzoic acid, butanoic acid, 4,4-dithiobis[2-amino-,(S-(R//R//)] were detected (Table S2). Five genotypes were having acetic acid and 2-pyridinecarboxylic acid was present in five genotypes. Three genotypes have butanoic acid and 4-hydroxy- was appeared in three genotypes while one genotype has benzoic acid. Giza 35, X30, Argentinian and Chinese compassed the maximum numbers of carboxylic acids compounds. Thirteen esters were identified. The butyrolactone, acetic acid, 2-(dimethylamino)ethyl ester, formic acid, 3-methylbut-2-yl ester, pentanoic acid, 2-isopropoxyphenyl ester, phthalic acid, hex-3-yl-isobutyl ester, hexadecanoic acid, methyl ester, phthalic acid, butyl undecyl ester, 5,8,11-heptadecatriynoic acid methyl ester, methyl 10-trans, 12-cis-octadecadienoate, 9,12-octadecadienoic acid(Z,Z)-methyl ester, benzoic acid, 4-ethoxy-, ethyl ester, 1,2-benzenedicarboxylic acid, dibutyl ester, and pentanoic acid, 2,2-4-trimethyl-3-carboxyisopropyl, isobutyl, ester were identified. The genotype A-1 had maximum six esters compounds followed by others genotypes (Giza 83, Romal-1, Clark, Argentinian and 3803) having five (5) esters compounds. Hexadecanoic acid ethyl ester shows antioxidant, nematicidal activities and hypocholesterolemic [37]. Regarding phenolic compound, a total of nine compounds were identified. 1,2-benzenediol,3-methoxy-, 5-tert-butyl-1,2,3-benzenetriol, phenol, 4-ethenyl-, acetate, phenol, 2,6-dimethoxy-, 2-methoxy-4-vinylphenol, phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl l-, phenol, 2,4-bis(1,1-dimethylethyl)-, and phenol, 2-methoxy. The genotypes Indo-1 and Ijen and recorded the highest number of phenolic compounds which is five while the genotypes Clark, NARC-2, Giza 35, USA-1 and Indo-11 contained the four (4) phenolic compounds each. The plant phenolics compounds are of great interest to human due to their anti-oxidative and possible anticarcinogenic activities. The dietary phenolics are considered anti-carcinogens because of antioxidants, but there is no clear proof supporting this supposition [38]. Phenolic may inhibit carcinogenesis by interfering the molecular events in initiation, promotion, and progression stages. Isoflavones and lignans from soybean may distract tumor formation by mediating estrogen-related activities and also modulate the growth of benign and malignant prostatic epithelial cells in vitro [39]. The following sugar moiety, L-galactose, 6-deoxy-, 3,4-0-isopropylidene-d-galactose, a-methyl-D-mannopyrano-side, 3-O-methyl-d-glucose a-D-galactopyranoside, methyl were appeared among soybean studied genotypes. The relatively notable amounts of heterocyclic compounds were identified including 3,5-dihydroxy-6-methyl-2, 2,6-diisopropyl-naphthalene, 4H-pyran-4-one, 3-dihydro-4Hpyran-4-one, 3-hydroxy-2-methyl-, pyrazine, ethyl-, oxirane, 2-ethyl-2-methyl, 1H-indazole, 4,5,6,7-tetrahydro, N-aminomorpholine, and benzofuran, 2,3-dihydro. The genotype X30 had
four sugars compounds while genotypes USA-1, Indo-1, and Indo-11 had three sugars compounds each. Benzofurans are considered to possess anti-oxidant, antimicrobial effect and anti-inflammatory [40]. The compounds detected in this study have reported to have potentials as therapeutic agents, antioxidant, antimicrobial, and anti-inflammatory compounds and demonstrating that different compounds can exhibit similar activity and this might be due to presence of similar functional groups (Table S2). Antioxidant properties of soybean extract could be the basis for the presence of various antioxidant and anti-inflammatory compounds.

3.4. Principal component analysis (PCA)

The first three principal components explained 78.64% of total variations among genotypes (Table 4 and Figure 3). The first component described 59.65% of total variation, and positively correlated with phytochemical classes of ether, alcohol, sugar moiety ketone and phenolic compounds. Genotypes Ijen, Clark, A-1, USA-1, Indo-II, 3803, X 30, Giza 35, Indo-black and Indo-I showed the most variability according to these components and can be selected for these classes. PC2 illustrated 10.63% of the total variance, and the amide, sugar moiety, ether, alkane, ketone and carboxylic acid positively correlated with this component. The genotypes showed most variability were Giza 111, Giza 35, X 30, X 32, Indo-II and 3803. Alkane, Aldehyde, Carboxylic acid and Phenolic compound were positively correlated with the third component. The genotypes Giza 35, X 32 showed most variability based on this component. In this study, genotypes Giza 35, X 30, Indo-II and genotype 3803 showed positive loading in at least two out of the three PCs, which can be utilized in breeding for ceratin class of phytochemical. Utilizing PCA effectively reduces the number of variables needed to classify cultivars

|                      | PC 1  | PC 2   | PC 3  |
|----------------------|-------|--------|-------|
| Eigen values         | 0.17  | 0.03   | 0.02  |
| Percent of variance  | 59.65 | 10.63  | 8.36  |
| Cumulative percentage| 59.65 | 70.28  | 78.64 |
| Alcohol              | 0.42  | 0.11   | −0.12 |
| Aldehyde             | −0.14 | 0.00   | 0.24  |
| Alkane               | −0.01 | 0.29   | 0.75  |
| Amide                | −0.59 | 0.67   | −0.28 |
| Sugar moiety         | 0.39  | 0.44   | −0.06 |
| Carboxylic acid      | 0.03  | 0.06   | 0.31  |
| Ester                | 0.12  | −0.25  | −0.26 |
| Ether                | 0.45  | 0.34   | 0.02  |
| Heterocyclic compound| 0.05  | 0.14   | −0.16 |
| Ketone               | 0.27  | 0.21   | −0.18 |
| Phenolic compound    | 0.11  | −0.09  | 0.24  |

Table 4. Eigen values and proportion of the variance explained for the three principal components of the 24 soybean genotypes based on phytochemical components.
and permitted soybean researchers to more easily develop significant relationships between important soybean characteristics. Soybean cultivars have been classified using (PCA) of the fatty acid data [41]. The first four principal components generated in total 81.49% of the variance, where PC1 positively correlated with oleic, linoleic, and gondoic acids, PC2 with stearic, linolenic and arachidic acids, PC3 behenic and lignoceric acids, and PC4 by palmitic acid. Moreover, due to the ability of PCA to manage and interpret large data sets, it has been used in studying relationships that exist in fatty acid characterization [42]. Although soybean oil has been included in some chemometric studies comparing vegetable oils, soybean cultivars have yet to be extensively classified using multivariate techniques [43, 44].

4. Conclusion

The results revealed that soybean genotypes cover variable patterns of total proteins flavonoids, phenolic and various bioactive volatile compounds. The mass spectrometry analysis results demonstrated that, majority of soybean genotypes are a source bioactive compounds with antioxidant, anti-inflammatory, antimicrobial and other functions. 2-Methoxy-4-vinylphenol, phenol, 2,6-dimethoxy-, 3,5-dimethoxyacetophenone, hexadecanoic acid methyl ester, 1,2-cyclopentanediene, and 3,5-dihy droxy-6-methyl-2,3-dihydro-4H-pyran-4-one were present in majority of genotypes. However, the genotypes Ijen and Indo-1 contributed more phenolic compound than others genotype. Genotype A-1 has the maximum compound in esters compounds. The genotypes Indo-11 and 3803 contribute

Figure 3. Two-dimensional biplot ordination of 24 soybean genotypes on principal component axes according to 11 phytochemical classes.
maximum ketone compounds while Giza 111 contributes more in heterocyclic compounds. Some genotypes may have good therapeutic potential and could be served as a potential source in drug development as a health supplement. This study also provides a platform for isolating and understanding the properties of each compound for its pharmacological properties.

A. Appendix (supplementary materials)

| Genotype name | Crude protein (g/100 g) | Moisture (g/100 g) | Ash (g/100 g) | Total fat (g/100 g) | Carbohydrate (g/100 g) | Total phenolic content mg/g | Total flavonoid content mg/g |
|---------------|------------------------|-------------------|--------------|---------------------|------------------------|-----------------------------|-----------------------------|
| Admaril       | 37.84                  | 4.56              | 5.27         | 21.65               | 30.68                  | 1.30                        | 0.975                       |
| Romal-1       | 40.93                  | 4.79              | 4.55         | 20.35               | 29.38                  | 1.75                        | 1                           |
| NARC-2        | 38.01                  | 4.84              | 5.79         | 21.16               | 30.2                   | 1.50                        | 1.25                        |
| Williams 82   | 38.23                  | 4.97              | 5.65         | 22.79               | 28.36                  | 1.42                        | 1.05                        |
| X 32          | 39.8                   | 4.31              | 5.54         | 21.04               | 29.31                  | 1.25                        | 0.875                       |
| Holladay      | 37.04                  | 4.35              | 5.55         | 23.61               | 29.45                  | 1.35                        | 0.75                        |
| Giza 22       | 39.82                  | 4.45              | 5.55         | 21.91               | 28.27                  | 1.37                        | 0.675                       |
| Giza 21       | 39.84                  | 4.4               | 5.56         | 21.72               | 28.48                  | 1.40                        | 0.8                         |
| X2 L 12       | 38.26                  | 4.26              | 5.29         | 21.96               | 30.23                  | 1.42                        | 1.2                         |
| Giza 83       | 38.29                  | 3.08              | 5.39         | 21.42               | 31.82                  | 1.38                        | 0.925                       |
| Crawford      | 39.43                  | 4.84              | 5.58         | 22.38               | 27.77                  | 1.30                        | 1.125                       |
| Giza 35       | 38.8                   | 3.77              | 5.49         | 21.78               | 30.16                  | 1.32                        | 1.025                       |
| X 30          | 40.05                  | 4.99              | 5.64         | 21.78               | 27.54                  | 1.70                        | 1.0375                      |
| Giza 111      | 36.89                  | 5.34              | 6.28         | 22.07               | 29.42                  | 1.15                        | 1.75                        |
| Clark         | 43.13                  | 5.41              | 5.77         | 19.58               | 26.11                  | 1.35                        | 1.375                       |
| 3803          | 40                     | 5.12              | 5.45         | 22.94               | 26.49                  | 1.33                        | 1.625                       |
| A – 1         | 39.01                  | 5.19              | 4.8          | 20.69               | 30.31                  | 1.37                        | 1.45                        |
| Ijen          | 41.7                   | 5.54              | 5.54         | 18.66               | 28.56                  | 1.65                        | 1.25                        |
| Indo-black    | 42.71                  | 5.88              | 5.36         | 16.92               | 29.13                  | 1.65                        | 1.025                       |
| Indo-I        | 42.74                  | 5.88              | 5.7          | 19.33               | 26.35                  | 1.62                        | 1.775                       |
| Indo-II       | 37.87                  | 5.51              | 5.14         | 21.17               | 30.31                  | 1.32                        | 1.375                       |
| USA-1         | 36.89                  | 5.34              | 5.24         | 21.34               | 31.19                  | 1.77                        | 2.125                       |
| Indian        | 36.59                  | 5.43              | 5.25         | 20.88               | 31.85                  | 1.65                        | 1.7625                      |
| Chinese       | 35.98                  | 5.19              | 5.26         | 21.06               | 32.51                  | 1.50                        | 1.35                        |
| Argentinian   | 35.63                  | 5.12              | 5.3          | 20.77               | 33.18                  | 1.37                        | 1.325                       |

Table S1. Proximate analysis, total phenolic and flavonoid in the seeds of 24 soybean genotypes seeds (on a dry weight basis).
| Sr. no | Compound                                      | Other name                                      | Nature   | Activity         | RT  | MW  |
|-------|-----------------------------------------------|-------------------------------------------------|----------|------------------|-----|-----|
| 1     | Carbamide                                     | Urea                                            | Amide    |                  | 3.67| 60  |
| 2     | Propanal, 3-methoxy                           | 3-Methoxy-propanal                              | Aldehyde | Antibacterial    | 3.75| 88  |
| 3     | n-Hexane                                      |                                                 | Alkane   | Antibacterial    | 3.8 | 86  |
| 4     | Acetamide, oxime                              |                                                 | Amide    | Antimicrobial    | 3.86| 74  |
| 5     | 1,2-Naphthalenedione, 4 chloro                |                                                 |         |                  | 3.9 | 192 |
| 6     | 1,3-Dioxolane-4-methanol                     | Glycerol formal                                 | Alcohol  |                  | 3.93| 104 |
| 7     | 1-Monolinoleoglycerol trimethylsilyl ether    |                                                 | Ether    |                  | 4.02| 498 |
| 8     | Acetic acid                                   |                                                 |         | Carboxylic acid  | 4.17| 60  |
| 9     | Acetic acid, 2,2-[oxybis(2,1-ethanediolxy)]bis | (2-[2-(Carboxymethoxy)ethoxy]ethoxy)acetic acid |         | Carboxylic acid  | 4.24| 222 |
| 10    | Ethyl(dimethyl)isopropoxysilane               | Ethyl(dimethyl)silyl isopropyl ether            | Ether    |                  | 4.54| 146 |
| 11    | Silane, triethylmethoxy-                      | Methyl trethylsilyl ether                       | Ether    |                  | 4.6 | 146 |
| 12    | Butanoic acid, 4,4-dithiobis[2-amino-,(S-(R*,R*)| Picolinic acid                                  | Carboxylic acid | Natural chelator | 4.73| 268 |
| 13    | 2-Pyridinecarboxylic acid                     |                                                 | Carboxylic acid | Natural chelator | 4.73| 123 |
| 14    | 2-Propanone, 1-(dimethylamino)-               | (Dimethylamino)acetone                          | Ketone compound |                  | 4.89| 101 |
| 15    | 2,2-Bioxirane                                 | Butane1,2,3,4-diepoxy-                          | Heterocyclic compound |      | 4.92| 86  |
| 16    | Cyclotrisiloxane, hexamethyl                  | Dimethylsiloxane cyclic trimer                  | Heterocyclic compound |      | 5.3 | 222 |
| 17    | Pyrimidine, 2-methyl-                         | 2-Methylpyrimidine                              | Heterocyclic compound |      | 5.61| 94  |
| 18    | L-Galactose, 6-deoxy-                         | 6-Deoxyhexose                                   | Sugar moiety | Preservative      | 6.39| 164 |
| 19    | 2-Propanamide                                 | Acrylamide                                      | Amide    |                  | 6.43| 71  |
| 20    | 1,2,4-Triazole, 4-(4-methoxybenzylidnamino)-5-|                                                 | Heterocyclic compound |      | 7.54| 310 |

**Phytochemical Profiling of Soybean (Glycine max (L.) Merr.) Genotypes Using GC-MS Analysis**

[i](http://dx.doi.org/10.5772/intechopen.78035)
| Sr. no | Compound | Other name | Nature | Activity | RT | MW |
|-------|----------|------------|--------|----------|----|----|
| 21    | Acetic acid, 2-(dimethylamino)ethyl ester | Dimethylaminoethanol acetate | Ester |         | 7.57 | 131 |
| 22    | 2H-1-Benzopyran, 3,5,6,8a-tetrahydro-2,5,5,8a-tetramethyl-(2S-cis)- | Edulan II | Heterocyclic compound |         | 7.58 | 192 |
| 23    | Pyrazine, ethyl- | Ethylpyrazine | Heterocyclic compound |         | 7.67 | 108 |
| 24    | Oxirane, 2-ethyl-2-methyl | Butane, 1,2-epoxy-2-methyl | Heterocyclic compound |         | 7.77 | 86 |
| 25    | Butyrolactone | | Ketone compound |         | 7.88 | 86 |
| 26    | 4-Methyl-2-haptanol | | Alcohol |         | 7.96 | 130 |
| 27    | 1,2-Cyclopentanedione | | Ketone compound | Antioxidant | 7.98 | 98 |
| 28    | Pyran-4-carboxylic acid, 4-(4-methoxyphenyl)-tetrahydro- | | Heterocyclic compound |         | 8.02 | 236 |
| 29    | 6-Oxa-bicyclo[3.1.0]hexan-3-one | | Ketone compound |         | 8.09 | 98 |
| 30    | Dihydroxyacetone | 2-Propanone, 1,3-dihydroxy- | Ketone compound |         | 8.18 | 90 |
| 31    | Butanoic acid, 4-hydroxy- | | Carboxylic acid |         | 8.54 | 104 |
| 32    | Propanedioic acid, Propyl- | 2-Propylmalonic acid | Carboxylic acid |         | 9.1 | 146 |
| 33    | 1,2,3-Propanetriol | Glycerin | Alcohol |         | 9.33 | 92 |
| 34    | 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one | | Ketone compound |         | 9.74 | 144 |
| 35    | Oxirane, [2-propenyl(2-propyl)oxy)methyl]- | Propane, 1-(allyloxy)2,3-epoxy- | Heterocyclic compound |         | 10.26 | 114 |
| 37    | HEPES[4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid] | | Heterocyclic compound |         | 10.26 | 238 |
| Sr. no | Compound | Other name | Nature | Activity | RT  | MW  |
|-------|-----------|------------|--------|----------|-----|-----|
| 37    | 2H-Pyran-2,6(3H)-dione | Glutaconic anhydride | Heterocyclic compound | | 10.75 | 112 |
| 38    | 1H-Indazole, 4, 5, 6, 7-tetrahydro | | Heterocyclic compound | | 11.54 | 122 |
| 39    | 2-Pyrrolidinone, 1-methyl | M-Pyrol | Ketone compound | | 11.86 | 99 |
| 40    | Benzeneacetaldehyde | | Aldehyde | Antibacterial | 12 | 120 |
| 41    | 2,4,6-Cycloheptatrien-1-one,4-methyl- | | Ketone compound | | 12.06 | 120 |
| 42    | 2,5-Dimethyl-4-hydroxy-3(2H)-furanone | | Ketone compound | | 12.28 | 128 |
| 43    | a-D-Glucopyranoside, O-a-D-glucopyranosyl-(1. fwdarw.3)-a-D-fructofuranosyl | | Sugar moiety | | 12.81 | 504 |
| 44    | Phenol, 2-methoxy- | | Phenolic compound | Antimicrobial, antioxidant, anti-inflammatory, analgesic | 13.83 | 124 |
| 46    | Formic acid, 3-methylbut-2-yl ester | | Ester | | 14.24 | 116 |
| 45    | 1-Butanol,3-methyl-, formate (isopentyl alcohol, formate) | Isopentyl alcohol, formate | Fatty acid ester | Antimicrobial activity | 14.24 | 116 |
| 47    | 1,5-Hexadien-3-ol | | Alkene | | 14.36 | 98 |
| 48    | Cyclopentane, (1,1-dimethylethyl)-(Tert-Butylcyclopentane) | Tert-Butylcyclopentane | Alkane | Antibacterial | 14.68 | 126 |
| 49    | 4H-Pyran-4-one,3-hydroxy-2-methyl- | Maltol | Heterocyclic compound | Flavor enhancer | 14.78 | 126 |
| 50    | 5-Hepten-3-one, 5-methyl- | | Ketone compound | | 15.09 | 126 |
| 51    | 2-Acetyl-2,3,5,6-tetrahydro-1,4-thiazine | 1-(3-Thiomorpholinylethanone | Ketone compound | | 15.85 | 145 |
| 52    | 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one | | Heterocyclic compound | Antimicrobial, anti-inflammatory, anti-proliferative | 16.61 | 144 |
| Sr. no | Compound | Other name | Nature | Activity | RT  | MW  |
|-------|----------|------------|--------|----------|-----|-----|
| 53    | Propanal, 2-(benzoyloxy)- | 1-Methyl-2-oxoethyl benzoate | Aldehyde |          | 16.69 | 178 |
| 54    | Benzoic Acid | | Carboxylic acid | | 16.76 | 122 |
| 55    | N-aminomorpholine | 4-Aminomorpholine | Heterocyclic compound | | 16.95 | 102 |
| 56    | Pentanoic acid, 2-isoproxyphenyl ester | 2-Isoproxyphenyl pentanoate | Ester | Antimicrobial, antioxidant, anti-inflammatory, analgesic | 18.26 | 236 |
| 57    | Phenol, 4-ethynyl-, acetate | 4-Vinylphenyl acetate | Phenolic compound | | 19.29 | 162 |
| 58    | Benzaldehyde, 3,4-dimethyl- | 3,4-dimethylbenzaldehyde | Aldehyde | Antibacterial | 19.3 | 134 |
| 59    | Benzene, (ethenoxy)- | Ether, phenyl vinyl | Ether | | 19.31 | 120 |
| 60    | Benzofuran, 2,3-dihydro | Coumaran | Heterocyclic compound | Antihelminthic, anti-inflammatory, anti-diarrhoeal | 20.16 | 120 |
| 61    | Benzenecetaldehyde, 3-methyl | m-Tolu aldehyde | Aldehyde | Antimicrobial | 20.34 | 120 |
| 62    | 1,2-Benzenediol, 3-methoxy | Pyrocatechol, 3-methoxy | Phenolic compound | Antioxidant | 21.01 | 140 |
| 63    | Isosorbide | D-Glucitol, 1,4,3,6-dianhydro | Alcohol | Antimicrobial, antioxidant, anti-inflammatory, analgesic | 22.61 | 146 |
| 64    | 2-Methoxy-4-vinylphenol | Phenol, 4-ethenyl-2-methoxy-phenol | Phenolic compound | Antioxidant | 23.35 | 150 |
| 65    | (p-Hydroxyphenyl)glyoxal | Benzeneacetaldehyde, 4-hydroxy-a-0x0 | Aldehyde | Antimicrobial | 23.71 | 150 |
| 66    | 2-Acetamido-2-deoxy-d-mannolactone | | Sugar moiety | Anti-bacterial | 24.8 | 217 |
| 67    | Phenol, 2,6-dimethoxy- | Pyrogallol 1,3-dimethyl ether | Phenolic compound | Antimicrobial, antioxidant, anti-inflammatory, analgesic | 24.99 | 154 |
| 68    | 1-Undecanol alcohol | Undecyl alcohol | Alcohol | | 29.83 | 172 |
| 69    | Phenol, 2,6-bis(1,1-dimethyl-ethyl)-4-methyl- | Butylated hydroxytoluene | Phenolic compound | Antimicrobial, antioxidant, anti-inflammatory, analgesic | 30.99 | 220 |
| 70    | Phenol, 2,4-bis(1,1-dimethyl-ethyl)- | Phenol, 2,4-di-tert-butyl- | Phenolic compound | Antimicrobial, antioxidant, anti-inflammatory, analgesic | 31.19 | 206 |
| Sr. no | Compound | Other name | Nature | Activity | RT  | MW |
|-------|-----------|------------|--------|----------|-----|----|
| 71    | 5-Tert-butyl-1,2,3-benzenetriol | 5-Tert-butylpyrogallol | Phenolic | Antioxidant, antiseptic antibacterial, anti-dermatitic fungicide, pesticide | 31.91 | 182 |
| 72    | Benzoic acid, 4-ethoxy-, ethyl ester |  | Ester |  | 32.12 | 194 |
| 73    | 3,4,0-Isopropylidene-d-galactose | 3,4,0-(1-Methylethylidene) hexopyranose | Sugar moiety | Preservative | 32.35 | 220 |
| 74    | Pentanoic acid, 2,2-4-trimethyl-3-carboxyisopropylisobutyl ester |  | Ester |  | 32.97 | 286 |
| 75    | 3,5-Dimethylacetophenone |  | Ketone compound | Antioxidant | 33.65 | 180 |
| 76    | a-Methyl-D-mannopyranoside |  | Sugar moiety | Preservative | 34.35 | 194 |
| 77    | a-D-Galactopyranoside, methyl | Galactopyranoside, methyl, a-D- | Sugar moiety | Preservative | 34.61 | 194 |
| 78    | 3-O-methyl-d-glucose | 3-O-methylhexose | Sugar moiety | Preservative | 37.68 | 194 |
| 79    | 2,6-Diisopropynaphthalene |  | Heterocyclic compound |  | 37.71 | 212 |
| 80    | Dodecyl acrylate | n-Lauryl acrylate | Ester |  | 38.17 | 240 |
| 81    | Cyclopenta [1,3][cyclopropa [1,2]cyclohepten-3(3ah)one, 1,2,3b,6,7,8-hexahydro-6,6-dimethyl- | Ketone compound |  |  | 40.31 | 190 |
| 82    | 5-Tert.butyloxy carboxamido-2,3,3-trimethyl-3H-indole | Tert-buty1 2, 3,3-trimethyl-3H-indole-5-ylcarbamate | Heterocyclic compound |  | 41.6 | 274 |
| 83    | Phthalic acid, hex-3-yl-isobutyl ester |  | Ester |  | 42.4 | 306 |
| 84    | Hexadecanoic acid, methyl ester | Palmitic acid, methyl ester | Ester | Antioxidant, flavor, hypocholesterolemic, nematicide | 46.13 | 270 |
| 85    | 5,8,11-Heptadecatriynoic acid methyl ester |  | Ester |  | 46.2 | 272 |
| 86    | Phthalic acid, butyl undecyl ester |  | Ester |  | 47.36 | 376 |
| 87    | 1,2-Benzenedicarboxylic acid, dibutyl ester | Dibutyl phthalate | Ester | Plasticizer, antimicrobial, antifouling | 47.37 | 278 |
| 88    | Methyl 10 trans, 12-cis-octadecadienoate |  | Ester |  | 48.53 | 294 |

**Table S2.** List and basic features of identified phytocomponents in the methanolic extract of soybean genotypes by GCMS analysis.
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Conflict of interest

The authors have declared that no conflict of interest exists.

Author details

Salem Alghamdi1, Hussein Migdadi∗, Muhammad Khan1, Ehab H. El-Harty1, Megahed Ammar2, Muhammad Farooq1,3,4 and Muhammad Afzal1

*Address all correspondence to: h.migdadi@gmail.com

1 Legume Research Group, Plant Production Department, Faculty of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia
2 Rice Research and Training Center, Kafr El Sheikh, Egypt
3 Department of Agronomy, University of Agriculture, Faisalabad, Pakistan
4 Department of Crop Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, Al-Khoud, Oman

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