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Chapter 9

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

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Additional information is available at the end of the chapter

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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 it pharmacological properties.

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

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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, photochemical 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-inflammatory, 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, saponins, phytosterols and sphingolipids were recorded in soybean [12–14]. Due to importance of

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this crop and its products, this study was amid at estimating the most active constitutes 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_25049.200 N 46_22012.500E) 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	29	Indo-I	Indonesia
8	X2 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 invistigated 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 Eppndorf 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% $NaNO_2$ (15:1, v/v). After 6 min, 150 μl of 10% $AlCl_3$ 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	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-cisoctadecadienoate 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	Antioxidant	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	M-Pyrol	Ketone		11.86	99
42	2,5-Dimethyl-4-hydroxy-3(2H)-furanone		Ketone		12.28	128
44	Phenol, 2-methoxy-		Phenolic compound	Antimicrobial, antioxidant, anti-inflammatory, analgesic	13.83	124
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
52	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one		Heterocyclic compound	Antimicrobial, anti-inflammatory	16.61	144
57	Phenol, 4-ethenyl-, acetate	4-Vinylphenyl acetate	Phenolic compound	Antimicrobial, antioxidant, anti-inflammatory	19.29	162
60	Benzofuran, 2,3-dihydro	Coumaran	Heterocyclic compound	Antihelminthic, anti-inflammatory, antidiarrheal	20.16	120
62	Benzeneacetaldehyde, 3-methyl	m-Tolualdehyde	Aldehyde	Antimicrobial	20.34	120

	Compound	Other names	Nature	Activity	RT	MW
61	1,2-Benzenediol,3-methoxy-	Pyrocatechol, 3-methoxy	Phenolic compound	Antioxidant	21.01	140
64	2-Methoxy-4-vinylphenol	Phenol, 4-ethenyl-2-methoxy-	Phenolic compound	Antimicrobial, antioxidant, anti-inflammatory	23.35	150
68	Phenol, 2,6-dimethoxy-	Pyrogallol 1,3-dimethyl ether	Phenolic compound	Antimicrobial, antioxidant, anti-inflammatory	24.99	154
70	Phenol,2,6-bis(1,1-dimethylethyl)-4-methyl-	Butylated hydroxytoluene	Phenolic compound	Antimicrobial, antioxidant, anti-inflammatory, analgesic	30.99	220
71	Phenol, 2,4-bis(1,1-dimethylethyl)-	Phenol, 2,4-di-tert-butyl-	Phenolic compound	Antimicrobial, antioxidant, anti-inflammatory	31.19	206
72	5-tert-Butyl-1,2,3-benzenetriol	5-Tert-butylpyrogallol	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	Palmitic 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.

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 [30]. These chemicals are also active in many biological activities that were listed (**Table S2**). Phytochemicals also possess antioxidant activities, anti-cancer, anticarcinogenic, antibacterial, antiviral, or anti-inflammatory activities and play an important role for plant metabolism [30, 31]. 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 electronegative arrangement of conjugated group C=C double bond [32], as the electronegativity increase, antimicrobial activity also increases in those genotypes [33, 34]. These compounds react with vital nitrogen components such as protein and nucleic acid, consequently inhibit microorganism. Thirteen ketone related compounds were identified, i.e., 1-(dimethylamino)-,

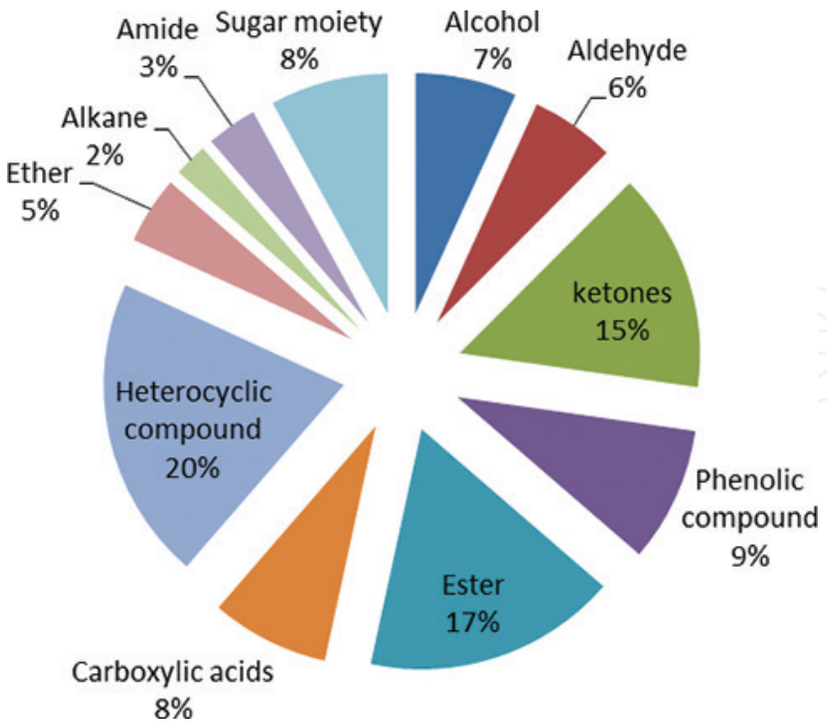


Figure 1. Pie diagram showing the percentage of phytochemical groups identified in 24 soybean genotypes.

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-pyrrolidinone, 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 6ketonic group each. Ketones might be formed by beta-oxidation of fatty acid and have some important flavor compounds [35]. During fatty

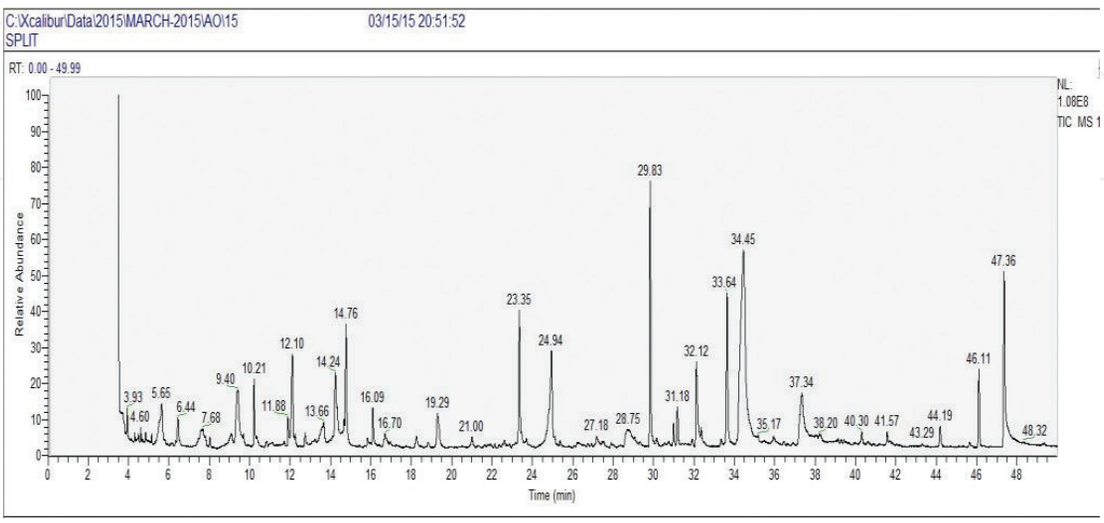


Figure 2. A typical GC-MS profile of seeds of soybean genotype.

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-ethanediyloxy)]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-methy 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-m annopyranoside, 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-diisoprpylnapthalene, 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.

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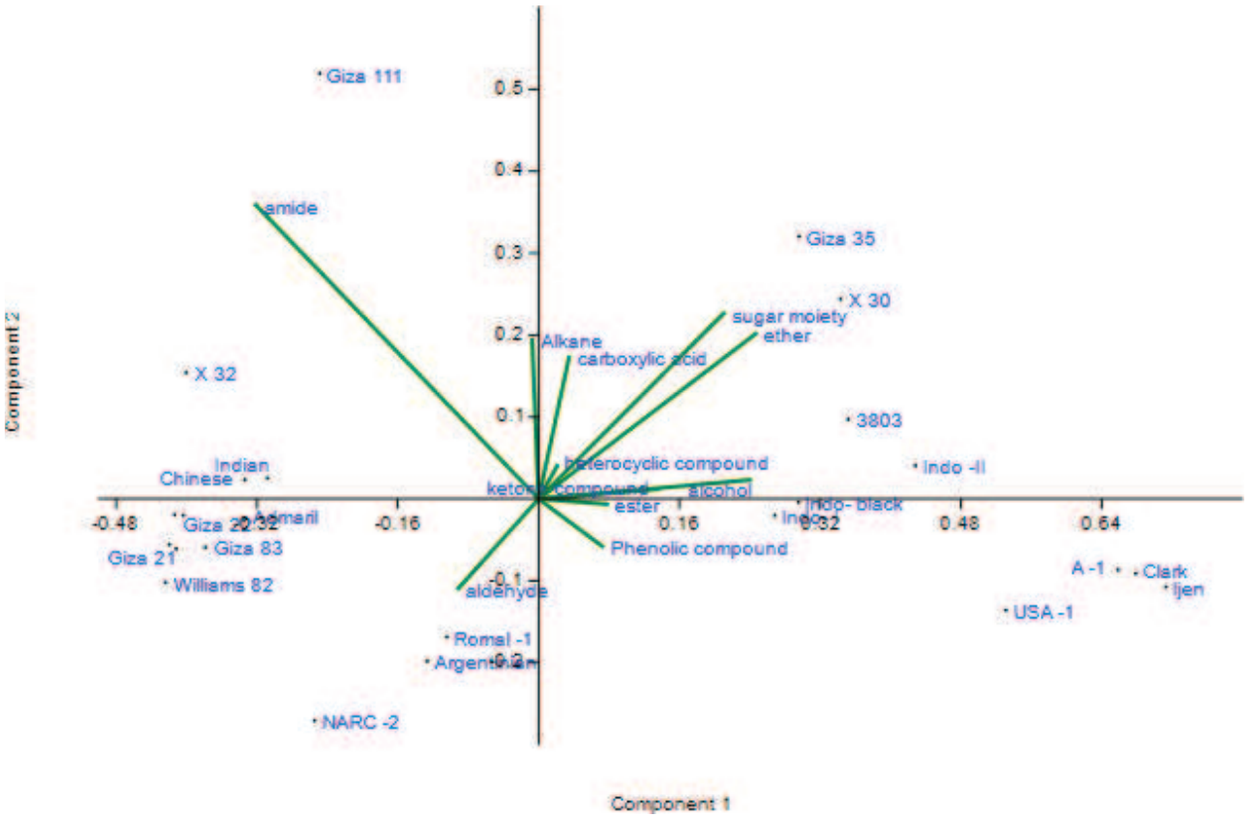


Figure 3. Two-dimensional biplot ordination of 24 soybean genotypes on principal component axes according to 11 phytochemical classes.

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-cyclopentanedione, 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

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 wdevelopment as a health supplement. This study also provides a platform for isolating and understanding the properties of each compound for it 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	Antimicribial	3.86	74
5	1,2-Naphthalenedione, 4 chloro		Heterocyclic compound		3.92	192
6	1,3-Dioxolane-4-methanol	Glycerol formal	Alcohol		3.93	104
7	1-Monolinoleoyglycerol trimethylsilyl ether		Ether		4.02	498
8	Acetic acid		Carboxylic acid		4.17	60
9	Acetic acid, 2,2-[oxybis(2,1-ethanediyloxy)]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*)]		Carboxylic acid		4.73	268
13	2-Pyridinecarboxylic acid	Picolinic 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-Propenamide	Acrylamide	Amide		6.43	71
20	1,2,4-Triazole, 4-(4-methoxybenzylidenamino)-5-methyl-3-(3,5-dimethylpyrazol-1-yl		Heterocyclic compound		7.54	310

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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-propenyloxy)methyl}-	Propane, 1-(allyloxy)2,3-epoxy-	Heterocyclic compound		10.26	114
37	HEPES[4-(2-Hydroxyethyl)-1-piperazineethanesuffonic acis)]		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

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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-isopropoxyphenyl ester	2-Isopropoxyphenyl pentanoate	Ester		18.26	236
57	Phenol, 4-ethenyl-, acetate	4-Vinylphenyl acetate	Phenolic compound	Antimicrobial, antioxidant, anti-inflammatory, analgesic	19.29	162
58	Benzaldehyde, 3,4-dimethyl-	3,4-dimethylbenzaldehyde	Aldehyde	Antibacterial	19.3	134
59	Benzene, (ethenyloxy)-	Ether, phenyl vinyl	Ether		19.31	120
60	Benzofuran, 2,3-dihydro	Coumaran	Heterocyclic compound	Antihelminthic, anti-inflammatory, anti-diarrhoeal	20.16	120
61	Benzeneacetaldehyde, 3-methyl	m-Tolualdehyde	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		22.61	146
64	2-Methoxy-4-vinylphenol	phenol, 4-ethenyl-2-methoxy-	Phenolic compound	Antimicrobial, antioxidant, anti-inflammatory, analgesic	23.35	150
65	(p-Hydroxyphenyl)glyoxal	Benzeneacetaldehyde, 4-hydroxy-a-0x0	Aldehyde	Antibacterial	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-dimethylethyl)-4-methyl-	Butylated hydroxytoluene	Phenolic compound	Antimicrobial, antioxidant, anti-inflammatory, analgesic	30.99	220
70	Phenol, 2,4-bis(1,1-dimethylethyl)-	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 compound	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-carboxyisopropyl,isobutyl ester		Ester		32.97	286
75	3,5-Dimethoxyacetophenone		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-Diisopropylnapthalene		Hetercyclic 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-butyl 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

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