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Research Article

COMPARATIVE PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION OF FIVE *GLYCINE MAX* (L.) MERRILL GENOTYPES

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ABSTRACT

Objective: Soybean (*Glycine max* L. Merrill) is the world's most important consumed seed legume. The objectives of the present study were to determine the variability in phytochemical composition and biological activities between five genotypes of *G. max*.

Methods: Lipoidal matters were determined using glucose (GLC). Amino acids were detected by the amino acid analyzer. The phytoconstituents present within each ethanol extract was investigated by gas chromatography-mass spectrometry. The amount of total phenolics, flavonoids, and tannins was analyzed using a spectrophotometric technique, based on Folin–Ciocalteu reagent, aluminum chloride colorimetric assay, and the modified vanillin hydrochloric acid method, respectively. Quercetin, catechin, and gallic acid were used as standard compounds, respectively. Isoflavones content were detected by high-performance liquid chromatography (HPLC)/photodiode array (PDA). The radical scavenging and antioxidant capacity of the genotypes using different *in vitro* analytical assays such as 2,2-diphenyl,1-picryl hydrazyl, 2,2'-Azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid, reducing power, metal chelating, and ferric reducing anti-oxidant power. Butyl hydroxyl toluene and trolox were used as the reference antioxidant radical scavenger compounds. Antitumor activity was evaluated by detecting the viability of Ehrlich ascites carcinoma cells on four different concentrations (1–5 mg/mL).

Results: GLC analysis showed the high value of total unsaturated fatty acids and 16 amino acids including glutamic acid with the highest concentration. The variation between genotypes according to their chemical composition of the aldehydes, esters, ketones, alcoholics, and carboxylic content were reported. HPLC/PDA referred to the presence of daidzein, genistein, and in all genotypes.

Conclusion: The results confirm the higher value of phytoconstituents of the genotype Giza 35 and Giza 21 as well as their better bioactivity.

Keywords: *Glycine max*, Antioxidant, Antitumor, Glucose, Amino acids, Gas chromatography-mass spectrometry, High-performance liquid Chromatography/photodiode array.

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INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) has been a part of traditional food for the human population in eastern part of the world and recently it has become popular in other parts of the world [1]. It is one the most widely researched and health-promoting cheapest food for human and animals. Glycine genus is a unique genus. There is an increasing interest for this genus due to its biological effects including estrogen-like activity, prevention of breast, prostate, and colon cancer [2,3]. Other activities as prevention of menopausal symptoms, anti-osteoporosis, and antioxidant were reported [4,5]. Soybean is an important crop following cotton, in Egypt. It is a fundamental storehouse of many chemical metabolites which are divided into two categories, primary and secondary metabolites. The secondary metabolites including lipids, flavonoids, phenols, and tannins are the compounds produced by the plant as defense chemicals [6]. These metabolites have important properties as anti-aging, anti-inflammatory, antioxidant, and anti-proliferative agents [4,5]. There are more than 25 genotypes of *G. max* used in Egypt including the common genotypes; Giza 22 (G_{22}), Giza 35 (G_{35}), Giza 82 (G_{82}), and Giza 111 (G_{111}). These genotypes were selected according to their harvest season in May 2014. It is recommended to cultivate genotype G_{82} in the delta of Egypt [[6]. G_{22} is advised to be cultivated in upper and middle Egypt regions. G_{35} and G_{111} are recommended to cultivate in a new land in Nuba ria. The present study aimed to detect the variation between the five used genotypes of G. max. Four genotypes (G_{22}, G_{35}, G_{82}, and G_{111}) are locally grown in Egypt, and the fifth one (Crawford) was imported from the USA..

MATERIALS AND METHODS

Plant materials

G. max (L.) Merrill seeds of five genotypes of (G_{22}, G_{35}, G_{82}, G_{111}, and Crawford) were purchased from Agriculture Research Centre, Giza, Egypt. The pedigree of soybean genotypes was as follows: Giza 22 (G_{22}) was Forrest x Crawford, Giza 35 (G_{35}) was Crawford x Celest, Giza 82 (G_{82}) was Crawford x Maplepresto, Giza 111 (G_{111}) was Crawford x Celest, and Crawford was Williams x Columbus. The genotypes were collected in the cultivation season of May 2014. The samples were collected in sterile polyethylene bags. The seeds were dried under shade.

Preparation of ethanol extracts

Fine air-dried powder of each genotype (500 g) was mixed with 80% ethanol (2 L) and stirred. It was filtered and the residues were discarded. The resulting extract was evaporated to dryness using rotatory evaporator and stored at 4°C.

Proximate analyses

All the following tests were carried out on powdered seeds according to A.O.A.C. [7]. Proximate analyses of moisture, ash, carbohydrates, and proteins content were investigated.

Chemical composition

Lipoidal matters of five genotypes

Saponification of *n*-hexane extract

The powder of the air-dried seeds of each genotype (100 g) was extracted with *n*-hexane in a continuous extraction apparatus. The solvents were evaporated to dryness under reduced pressure at 40°C. The residue was subjected to saponification by refluxing with 0.5 M alcoholic KOH in a water bath for 2 h [8]. After cooling, 50 mL of water was added, and the solution was extracted with chloroform. The organic phase was washed with water until it became alkali-free and was then dried over anhydrous sodium sulfate.

Preparation of fatty acid methyl esters (FAME)

The free fatty acids obtained from saponification were subjected to methylation (MeOH, 4%-5% dry H_2SO_4) for 2 h, extracted with ether, then evaporated and analyzed by glucose (GLC) [9].

Determination of total amino acids content

Powdered seed (65 mg for each genotype) was put into a hydrolysis tube. Hydrochloric acid (HCL) (6 N, 1 mL) was added. The solution was hydrolyzed with HCl at 110°C for 24 h. After removing the acid by evaporation in a rotary evaporator, dilute citrate buffer at pH 2.2 was added and the sample was filtered through Millipore Membranes (USA) with pores of 0.22 µm. The sample was then injected into the Eppendorf - Germany LC 3000 amino acid analyzer. The following conditions were used. The flow rate was 0.2 mL/min; the pressure of buffer was from 0 to 50 bars; the pressure of reagent was from 0 to 150 bars, and the reaction temperature was 123°C.

Gas chromatography-mass spectroscopy (GC/MS)

The GC-MS analysis of an ethanolic extract of five genotypes was carried out using a Thermo scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30 nm×0.251 mm, 0.1 mm film thickness) for GC/MS detector. An electron ionization system with ionization energy of 70 e V was used. Helium gas was used as the carrier gas at a constant flow rate of 1 mL/min. The injector was operated at 280°C and the oven temperature was programmed at an initial temperature 40°C (hold 4 min) to 280°C as a final temperature at an increasing rate of 5°C/min (hold 5 min). The peaks in the chromatogram were identified according to their mass spectra. The constituents were identified by comparison of their spectral fragmentation patterns with those of the available database libraries, Wiley (Wiley International, USA) and NIST (Nat. Inst. St. Technol., USA), and/or published data [10,11].

Determination of total polyphenolics, flavonoids, and tannins content

The total polyphenols content of each seed genotype was determined according to the method described by Meda *et al.* [12]. The total flavonoids content (TFC) was estimated using the method of Ordoñez *et al.* [13]. While tannins content were determined using the modified vanillin HCL method as reported by Maxon and Rooney [14].

Detection of isoflavones

Isoflavones were detected in the five genotypes according to Klejdus *et al*. [15]. The grounded seeds (50 g) were mixed with 150 mL of extraction solvent (80% MeOH in water with 0.1% HCl). The extraction continued for 60 min at room temperature in an ultrasonic bath. After extraction, the mixture was filtered on paper filter connected to vacuum and form the filtrate. The MeOH was separated result an aqueous extract. The remaining solution was re-extracted for an additional 60 min, then re-filtrated, and concentrated as before. The two layers of extracts were joined and hydrolyzed to free isoflavone aglycones. Hydrolysis of the extract was performed by reflux with concentrated HCl at pH=zero. The isoflavone aglycone forms were re-extracted with diethyl ether, 4–5 times. The diethyl ether extracts were joined, concentrated and transferred to an Eppendorf tube and stored at 5°C. The samples were centrifuged for 10 min at 13.500 rpm at 10°C temperature (Centrifuge Eppendorf mod. 5417 R). After centrifugation, 100 mL was injected in high-performance liquid Chromatography (HPLC).

Biological activities

2,2-diphenyl,1-picrylhydrazyl (DPPH) free-radical scavenging assay

The free-radical scavenging activity using DPPH reagent was determined according to Brand-Williams *et al*. [16]. Each genotype extract was soluble with 85:15 v/v methanol:water. To 0.5 mL of different concentrations of the extract sample, 1.0 mL of freshly prepared ethanolic DPPH solution (20 µg/mL^{-1}) was added and stirred. The decolorizing process was recorded after 5 min of reaction at 517 nm and compared with a blank control. Butyl hydroxyl toluene (BHT) was used as a positive control. All samples were analyzed in triplicate. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging activity (%)=([Abs. control−Abs. sample]÷Abs. control)×100

Where, Abs. control is the absorbance reading of control and Abs. sample is the absorbance reading of the sample.

Reducing power assay

The method of Oyaizu [17] was used to estimate the reducing power of each genotype *G. max* seeds. Extract (0.5 mL of different concentrations) was added to phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 mL, 10%) were added to the mixture, which was centrifuged at 1000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL 0.1 %). The intensity of the blue-green color was measured at 700 nm. In reducing power assay, the yellow color of the test solution changes to be green depending on the reducing power of test specimen. The presence of reductants in the solution causes the reduction of the ferric/ferricyanide complex to the ferrous form. Therefore, ferrous can be monitored by the measurement of the absorbance at 700 nm. Increased absorbance of their action mixture indicated increased reducing power.

Metal chelating activity assay

The chelation of ferrous ions by extract of each genotype of *G. max* was estimated according to the method of Dinis *et al*. [18]. Each genotype extract (0.5 mL of different concentrations of extract) was added to a solution of 50 µL $FeCl_2$ (2 mM). The reaction was initiated by the addition of 200 µL ferrozine (5 mM). The mixture was shaken vigorously and left at room temperature for 10 min. After equilibrium had been reached, the absorbance of the solution was measured spectrophotometrically at 562 nm. The following formula was used to calculate the percentage of inhibition of ferrozine-ferrous complex of each sample:

Inhibition %=([Abs. control−Abs. sample]÷Abs. control)×100

Where, Abs. control is the absorbance reading of control and Abs. sample is the absorbance reading of the sample.

2,2'-Azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity

ABTS radical scavenging activity was measured by the ABTS cation decolorization assay as described by Re *et al.* [19], with some modifications. The stock solutions included 7 mM ABTS solutions and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS radical solution with 60 mL methanol to obtain an absorbance of 0.706±0.001 units at 734 nm using the spectrophotometer. ABTS radical solution was freshly prepared for each assay. The extract of each soybean genotype (0.5 mL of different concentrations of extract) was reacted with 2.5 mL of the ABTS reagent, and the absorbance was taken

at 734 nm after 7 min by spectrophotometer. The ABTS radical cation decolorization assay capacity of the extract and percentage inhibition calculated as ABTS radical scavenging activity.

ABTS (%)=([Abs. control−Abs. sample])÷[Abs. control])×100

Where, Abs. control is the absorbance reading of control and Abs. sample is the absorbance reading of the sample.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to Benzie and Strain [20], with some modifications. Trolox was used as positive control. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM of 2, 4, 6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution and then warmed at 37°C before use. Each genotype extract (500 µL of different concentrations) was allowed to react with 2500 µL of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were then taken at 593 nm. Results were expressed in µmol Trolox/100 g dry matter. Additional dilution was required if the FRAP value measured was over the linear range of the standard curve.

***In vitro* antitumor activity**

To detect the cell viability, blue exclusion test was applied. The suspension of the tumor cells was attained from peritoneal cavities of tumor-bearing mice and then diluted with phosphate buffered saline (PBS, pH 7) so that the final preparation comprised 2.5×10^5 cells/0.1 mL. Briefly, in a set of sterile test tubes, aliquots (0.1 mL/tube) of the cell preparation were distributed followed by addition of aliquots (0.8 mL/tube) of PBS. The investigated samples (dissolved in PBS) were then applied to the tubes in aliquots (0.1 mL/tube) at different concentrations of each genotypwe extract. The tubes were incubated at 37°C for 2h under 5% CO_2. The tubes were centrifuged at 1000 rpm for 5 min, and separated cells were suspended in saline. For each examined tube and control, a new clean, dry small test tube was used, and 0.1 mL of cells suspension, 0.8 mL saline, and 0.1 mL try pan-blue were added and mixed, and then the number of living cells was calculated by hemocytometer slide. Viable cells appeared as unstained bodies while non-viable cells stained blue [21].

RESULTS

Proximate analyses

It can observe from Table 1 that genotype G_{111} had the highest value of total proteins, carbohydrates, and moisture while genotype G_{22} had the highest value of total ash.

Lipoidal matters

In the present study, investigation of lipid content of each *G. max* genotype was carried out using GLC. The unsaponified matters of the five genotypes have hydrocarbons, sterols, and triterpenes. The highest value of hydrocarbons was in genotype Crawford called *n*-hexacosane, 33.83%. The lowest one was in genotype G_{111} (*n*-hexadecane, 0.42 %). The ascending order of total hydrocarbons content was Crawford (59.49 %)>G_{111} (58.49 %)>G_{22} (56.85 %)>G_{82} (52.83%)>G_{35} (52.83%). The highest value for sterols was β-sitosterol in genotype Crawford (15.65 %) as shown in Fig. 1. The lowest value was campesterol (1.12 %) in genotype Crawford. The ascending order of total sterol content was G_{82} (28.04 %)>G_{22} (25.35 %)>Crawford (22.45 %)>G_{35} (21.42 %)>G_{111} (17.02%). There was a-amyrin was detected as the only represented triterpene in all five genotypes. The highest value was in genotype G_{35} (12.31%), and the lowest one was in genotype G_{22} (1.08%).

Free fatty acids were classified as saturated such as myristic (C14:0), palmitic (C16:0), stearic acid (C18:0), and unsaturated such as oleic (C18:1), linoleic (C18:2), and linolenic acid (C18:3). The range of fatty

Table 1: Proximate analyses of the five genotypes of *G. max*

Parameter	G_{22}	G_{35}	G_{82}	G_{111}	Crawford
Moisture	6.74	6.71	6.83	6.90	6.82
Total ash	5.82	5.52	5.41	4.70	5.20
Proteins	30.51	32.62	32.50	33.91	31.10
Total carbohydrates	24.62	25.01	26.21	27.10	24.52

G. max: Glycine max

Table 2: GLC of fatty acid methyl esters of five genotypes of *G. max*

Compound	Area % of genotypes				
	G_{82}	G_{22}	G_{35}	G_{111}	Crawford
Palmitic acid	18.61	36.72	35.40	35.60	32.79
Palmitoleic acid	-	9.01	-	0.98	-
Myristic acid	1.20	-	2.41	-	-
Stearic acid	2.15	7.52	6.88	7.25	3.59
Oleic acid	33.10	36.72	13.83	7.24	45.12
Linoleic acid	36.99	-	38.57	44.80	-
Linolenic acid	-	-	1.12	0.92	-
Arachidonic acid	-	-	-	0.82	-
Total SFA	21.96	44.24	44.69	42.85	36.38
Total USFA	70.09	45.73	53.52	54.76	45.12

SFA: Saturated fatty acids, USFA: Unsaturated fatty acids, *G. max: Glycine max*, GLC: Gas-liquid chromatography

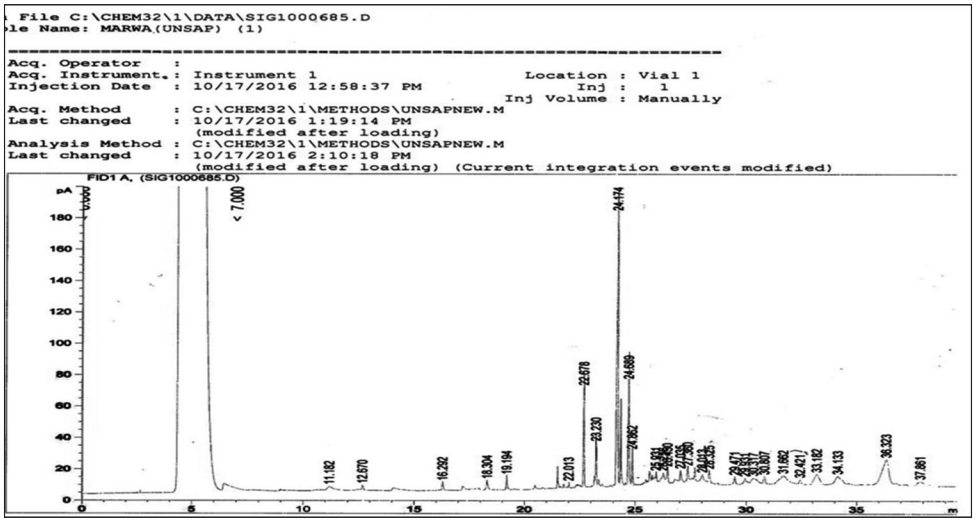

Fig. 1: GLC chromatogram of USM of G_{35} represented the highest value of sterols

acids contents in five genotypes of *G. max* as clarified in Table 2. They were myristic (1.20–2.41), palmitic (18.61–36.72), stearic (2.15–7.52), linoleic (36.99–44.80), linolenic (0.92–1.12), and oleic acid (7.24–45.12%). The highest value of total unsaturated fatty acids in the investigated genotypes was in G_{82} (70.09 %) as shown in Fig. 2.

Amino acids analysis

The content of the five genotypes of *G. max* seeds revealed the identification of 16 amino acids. Eight essential amino acids and eight nonessential amino acids, in addition to $NH4^+$, were detected. The concentration of glutamic acid in the five genotypes was the highest value in all the identified amino acids. The range of glutamic acid was within 23.387–13.396%. The order of the concentration of glutamic acid in genotypes was G_{35} (23.387)>G_{22} (21.562)>G_{111} (19.135)>Crawford (18.407)>G_{82} (13.396 %). Table 3 showed the content of each detected amino acids in the five genotypes.

GC/MS analysis

A large number of phytochemical were identified in the ethanolic extract of the five genotypes of *G. max* using (GC–MS). Collectively, 195 compounds of bioactive phytochemicals were identified in the five genotypes based on peak area, retention time (R_t), and molecular formula. The total numbers of the identified compounds were 47 (G_{35}), 42 (G_{82}), 40 (G_{22}), 35 (Crawford), and 31 (G_{111}) and were identified using GC/MS analysis as shown in Tables 4-8. The GC/MS analyses revealed that the predominantly composition of esters (64 compounds), phenolics (23), alcoholics (21), ketones (21), aldehydes (19), heterocyclics (18), alkanes (17), and carboxylic (12 compounds), respectively, that were collectively detected in all genotypes. Hence, esters group represented the highest class percentage in the five genotypes (32.82 %). The order of composition detected by GC/MS was esters (32.82%)>phenolics (11.79%)>alcoholics, ketones (10.77%)>aldehydes (9.74%)>heterocyclics (9.23%)>alkanes (8.72%)>and carboxylic (6.15%), respectively (Fig. 3).

Giza 35 had the highest number of aldehydes, phenolics, ketones, esters, alcoholics, and carboxylic. Fig. 4 showed a typical GC-MS profile of Giza 35 as the highest genotype having a number of phytochemicals group.

Detection of isoflavones by HPLC with photo-diode-array detector HPLC-photodiode array (PDA)

HPLC with photo-diode-array detector was used to analyze isoflavones in aglycones form in two genotypes of *G. max* seeds. One of them was local detected in Egypt (G_{35}), and the other was imported from USA (Crawford). The comparison between detected compounds made according to R_t, area percentage and their known molecular weight. G_{35} was selected because it represents that the local genotypes were collected from Egypt, and it had the highest value of total phenolics, flavonoids, antioxidant, and antitumor activity. While Crawford is foreign genotype imported from the USA. The molecular weight of daidzein is 254 g/mol,

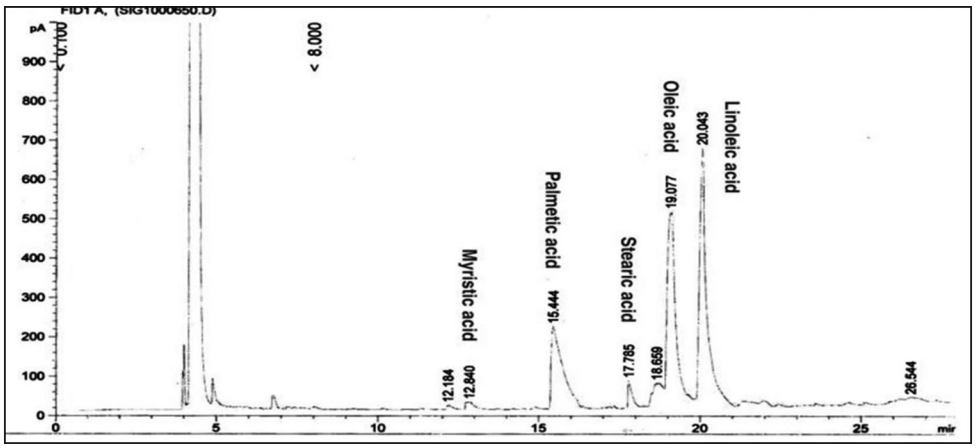

Fig. 2: GLC of FAME of G_{82} represented the highest value of total unsaturated fatty acids

Table 3: Total amino acids content of the five *G. max* genotypes

Amino acids	*G. max* genotypes									
	G_{82}		G_{22}		Crawford		G_{111}		G_{35}	
	Time (min)	Conc. %	Time (min)	Conc. %	Time (min)	Conc. %	Time (min)	Conc. %	Time (min)	Conc. %
Essential amino acids										
Phenylalanine	42.95	1.821	42.47	4.127	42.58	2.208	42.70	3.102	43.13	7.352
Threonine	15.27	0.357	15.38	0.692	15.28	0.531	15.78	0.585	15.00	0.719
Leucine	-	-	-	-	37.35	1.869	37.38	2.774	38.58	1.896
Isoleucine	37.25	1.261	37.27	2.226	36.37	0.226	-	-	37.57	0.179
Histidine	50.82	1.902	50.95	2.568	51.13	2.335	51.12	2.937	49.90	2.786
Lysine	53.88	1.886	54.08	2.097	54.15	1.863	54.20	2.257	53.10	4.103
Valine	31.92	0.397	32.07	0.652	32.17	0.684	32.37	0.920	32.62	0.576
Methionine	34.65	0.034	34.68	0.265	34.92	0.390	34.93	0.718	35.83	0.032
None essential amino acids										
Alanine	26.73	1.502	27.08	2.243	27.03	2.179	27.47	2.341	26.62	2.947
Aspartic acid	12.25	1.996	12.05	3.281	11.98	2.470	12.53	2.691	11.62	3.393
Glutamic acid	17.93	13.396	18.65	21.562	18.58	18.407	19.08	19.135	18.20	23.387
Glycine	25.57	0.558	25.93	0.747	25.85	0.632	26.28	0.731	25.43	0.953
Serine	16.30	0.741.	16.68	1.511	16.60	1.185	17.05	1.400	16.28	1.647
Tyrosine	40.95	0.300	40.90	0.600	40.93	0.164	41.08	1.108	-	-
Arginine	61.60	2.698	62.18	3.265	62.50	2.641	62.60	2.682	60.47	4.197
Proline	20.75	0.281	21.25	0.434	21.13	0.405	21.60	0.479	20.78	0.781

G. max: Glycine max

Table 4: GC/MS analysis of local genotype Giza 35 of *G. max* seed

Name of identified compounds	R_t (min)	Nature of compounds	Area%	M.w_t	Molecular formula
Acetic acid, 2-propenyl ester	10.59	Ester	1.20	100	$C_5H_8O_2$
Acetic acid, phenyl ester	10.70	Ester	0.64	136	$C_8H_8O_2$
Pentane, 1-thoxy	11.08	Alkane	0.57	116	$C_7H_{16}O$
2,5-Hexanediol, 2,5-dimethyl	11.11	Alcoholic	0.57	146	$C_8H_{18}O_2$
Hydroperoxy diethyl ether	11.21	Ester	1.52	106	$C_4H_{10}O_3$
Acetic acid, mercapto, 1,2ethanediyl ester	11.69	Ester	0.59	210	$C_6H_{10}O_4$
Phenol, 2-methoxy	24.73	Phenolic	0.98	124	$C_7H_8O_2$
Phenol, 4-ethenyl-, acetate	19.29	Phenolic	0.99	162	$C_7H_8O_2$
Cyclohexane methanol- 4-1methylethyl	21.71	Alcoholic	1.30	156	$C_{10}H_{20}O$
Menthol	21.73	Alcoholic	5.77	157	$C_{10}H_{20}O$
2-Methoxy-4-vinylphenol	23.35	Phenolic	0.89	150	$C_9H_{10}O_2$
Phenol, 2,6-dimethoxy	24.99	Phenolic	0.97	154	$C_8H_{10}O_3$
2,4-dodecadienal	25.94	Alkane	1.24	182	$C_{12}H_{20}O$
13-Heptadecyn-1-ol	25.95	Alcoholic	1.36	252	$C_{17}H_{32}$
2,4-Dodecadienal	26.70	Aldehyde	2.08	180	$C_{12}H_{20}O$
2,4-Undecadienal	26.75	Aldehyde	2.08	166	$C_{11}H_{18}O$
3-Ethoxy-2-butanone	28.10	Ketone	2.46	116	$C_6H_{12}O_2$
13-Tetradecenal	28.24	Aldehyde	1.34	210	$C_{14}H_{26}O$
11-Pentadecenal	28.30	Aldehyde	1.34	224	$C_{15}H_{28}O$
11-Hexadecenal	28.32	Aldehyde	1.34	238	$C_{16}H_{30}O$
1,2-Benzenediol, 3-methoxy	21.01	Phenolic	0.89	140	$C_7H_8O_3$
Phenol, 2,6-*bis*-1,1-dimethylethyl-4-methyl	30.99	Phenolic	0.95	220	$C_{16}H_{26}O_2$
Phenol, 2,4-*bis*-1,1-dimethylethyl	31.19	Phenolic	0.90	200	$C_{42}H_{63}O_3$
2,6-*di*-(t-Butyl) 4-hydroxy-4-methyl-2,5-cyclo hexadien-1-one	31.70	Ketone	0.70	236	$C_{15}H_{24}O_2$
5,8,11,14-Eicosatetraynoic acid	31.73	Carboxylic acids	0.70	296	$C_{20}H_{24}O_2$
2,2,7,7-Tetramethyltri cyclo-4-en-3-one	32.01	Ketone	0.70	218	$C_{15}H_{22}O$
1-Acetyl1hydroxy 6,8-dimethyoxynaphthalene-2-(1H)-one	33.03	Ketone	0.69	262	$C_{14}H_{14}O_5$
Methyl4,4,7trimethyl4,7dihydroindan-6-carboxylate	33.04	Carboxylic acids	35.85	220	$C_{14}H_{20}O_2$
3 (2,2 Dimethylpropylidene) bicycle-3,3,1-nonane 2,4-dione	33.05	Ketone	35.90	221	$C_{14}H_{20}O_2$
Pyran-4-carboxylic acid, 4-4-methoxyphenyl- tetrahydro	33.70	Heterocyclic	0.88	236	$C_{13}H_{16}O_4$
2H-Pyran-2,6 (3H)-dione	33.81	Alcoholic	0.85	112	$C_5H_6O_3$
1,2-Benzenedicarboxylic acid, di-thyl ester	35.49	Ester	0.74	222	$C_{12}H_{14}O_4$
2-Furanmethanol, tetrahydro-5-tri-methyl-5-4-methyl-3-cyclohexen-1-yl	39.81	Alcoholic	1.34	238	$C_{15}H_{26}O_2$
2HPyran-3-ol, tetrahydro-2,2,6-tri-methyl-6-4methyl-3-cyclohexen-1-yl	39.86	Alcoholic	1.34	238	$C_{15}H_{26}O_2$
Octadecane	40.01	Alkane	0.34	254	$C_{18}H_{38}$
Pentadecanoic acid, 14-methyl, methyl ester	40.06	Ester	2.36	270	$C_{17}H_{34}O_2$
Octadecane, 3-ethyl-5-2ethylbutyl	40.15	Alkane	0.56	366	$C_{26}H_{54}$
Methyl-1,3dihydro2Hisobenzofuran4carboxylate	40.16	Carboxylic acids	0.96	178	$C_{10}H_{10}O_3$
3-Heptyl2ethylhexanoate	40.20	Ester	1.25	242	$C_{15}H_{30}O_2$
Fumaric acid, ethyl-4-heptyl ester	40.21	Ester	1.25	242	$C_{13}H_{22}O_4$
8,11-Octadecadienoicacid, methyl ester	40.22	Ester	2.97	294	$C_{19}H_{34}O_2$
9,12-Octadecadienoicacid, methyl ester	40.23	Ester	2.97	295	$C_{19}H_{34}O_2$
13-Octadecenoicacid, methyl ester	40.24	Ester	4.00	296	$C_{19}H_{36}O_2$
9-Octadecenoicacid, methyl ester	40.26	Ester	400	295	$C_{19}H_{36}O_2$
Hexadecanoic acid, methyl ester	41.23	Ester	0.92	270	$C_{17}H_{34}O_2$

R_t (min): Retention time in minutes,M.w_t: Molecular weight, *G. max*: *Glycine max*, GC/MS: Gas chromatography-mass spectroscopy

while genistein is 270 g/mol and glycitein is 284 g/mol. In this study, HPLC-PDA analysis showed the presence of isoflavone aglycones, daidzein, glycitein, and genistein in both genotypes G_{35} and Crawford. Daidzein has the highest value. Daidzein was detected in G_{35} had a higher value than daidzein in Crawford as clarified in Fig. 5. It followed by genistein which had the higher value in G_{35} than Crawford. There was glycitein which had the lowest value in three detected aglycones. G_{35} has a higher value of glycitein than Crawford as shown in Table 9.

Determination of total polyphenolics, flavonoids, and tannins content

Table 10 summarized the results of the total content of secondary metabolites in the five genotypes of *G. max*. Total phenolics content, TFC, and total tannins content were determined using equivalents to gallic acid, quercetin, and catechin, respectively.

The present study showed that G_{35} recorded the highest value of total phenolic, flavonoid and tannin contents, followed by Crawford, $G_{111,}$ G_{22}, and G_{82} respectively. It showed descending value of total phenolics (mg gallic acid equivalent [GAE]/g) and tannins (mg catechin equivalent /g) content. The descending order of total flavonoid content (mg Quercetin equivalent (QE)/g) was G35 followed by G_{111}, Crawford, G_{22} and G_{82}, that might be responsible for the highest antioxidant activity of G_{35}.

Biological activity

Antioxidant activity

Many phytochemical components, especially polyphenols (such as flavonoids, phenolic acids, and tannins) are known to be accountable of the free radical scavenging and antioxidant activities of plants. The antioxidant activity was evaluated using five different assays (DPPH free-radical scavenging, reducing power ability, ABTS radical scavenging, metal chelating, and FRAP). The results were illustrated in Table 11.

The radical scavenging and antioxidant capacity of the genotypes using five analytical assays were evaluated. BHT and Trolox were used as the

Table 5: GC/MS analysis of local genotype Giza 82 of *G. max*

Name of identified compounds	Rt (min)	Nature of compound	Area%	M.w$_t$	Molecular formula
Acetic acid, ethyl ester	18.57	Ester	3.55	88	$C_9H_8O_4$
4-Hydroxyphenyl pyruvic acid	18.62	Carboxylic acids	3.55	181	$C_{10}H_{10}O_2$
Phenol, 4-ethenyl acetate	19.29	Phenolic	0.99	162	$C_{10}H_{18}O$
4-methyl-2-haptanol	21.01	Alcoholic	0.66	152	$C_{10}H_{18}O$
Cyclohexanone, 5-methyl-2-methylethyl	21.11	Ketone	0.70	155	$C_{10}H_{20}O$
Cyclohexanol, 5-methyl-2-methylethyl	21.70	Alcoholic	5.73	156	$C_{10}H_{20}O$
Menthol	21.73	Alcoholic	5.77	157	$C_{10}H_{20}O$
Isomenthol	21.76	Alcoholic	5.97	159	$C_{10}H_{20}O$
Phenol, 2-methoxy	24.73	Phenolic	0.98	124	$C_7H_8O_2$
8-Methylenecyclooctene3,4-*di*-ol	24.81	Alcoholic	0.81	154	$C_9H_{14}O_2$
1,6-Cyclodecanediol	24.83	Alcoholic	0.81	172	$C_{10}H_{20}O_2$
Phenol, 2,6-dimethoxy	24.99	Phenolic	0.97	154	$C_8H_{10}O_3$
13-Heptadecyn-1-ol	25.95	Alcoholic	1.36	252	$C_{17}H_{32}$
2,4-Dodecadienal	26.70	Aldehyde	2.08	182	$C_{12}H_{20}O$
2,4-Undecadienal	26.79	Aldehyde	2.08	166	$C_{11}H_{18}O$
1,2,3-Propanetriol, triacetate	27.83	Ester	0.77	218	$C_9H_{14}O_6$
1,2,3-Propanetriol, diacetate	27.87	Ester	0.87	176	$C_7H_{12}O_5$
Triacetin	27.89	Ester	0.79	220	$C_9H_{14}O_6$
13,14-Epoxy-tetradec1-1-en-1-ol-acetate	28.25	Ester	0.84	268	$C_{16}H_{28}O_3$
Cyclopropaneoctanal, 2-octyl	28.30	Aldehyde	0.94	280	$C_{19}H_{36}O$
Undecenal	28.31	Aldehyde	0.84	168	$C_{11}H_{20}O$
Phenol, 2,6-*bis*1,1-dimethylethyl-4-methyl	30.99	Phenolic	0.95	220	$C_{16}H_{26}O_2$
1,6-Methanofluorene	31.73	Heterocyclic	0.59	183	$C_{14}H_{12}$
2,6-*di*-t-Butyl-4hydroxy-4-methyl-2,5-cyclohexadien-1-one	31.75	Ketone	0.59	236	$C_{15}H_{24}O_2$
2,5-Dimethyl-4-hydroxy-3 (2H) -furanone	31.77	Ketone	0.87	128	$C_6H_8O_3$
1,4-Dimethoxy2,3,6-tri-methylbenzene	31.79	Heterocyclic	0.62	180	$C_{11}H_{16}O_2$
Pyran-4-Carboxylic acid, 4-4-methoxyphenyl-tetrahydro	33.70	Heterocyclic	0.88	236	$C_{13}H_{16}O_4$
2H-Pyran-2,6 (3H)-dione	33.81	Heterocyclic	0.85	112	$C_6H_8O_3$
Benzofuran, 2,3-dihydro	34.07	Heterocyclic	0.94	120	C_8H_8O
Bicyclo-4.4.0-Dec-1-en-2-isopropyl-5-methyl-9-methylene	36.89	Heterocyclic	0.88	244	$C_{15}H_{24}$
Benzyl benzoate	40.28	Ester	3.21	212	$C_{14}H_{12}O_2$
Octadecane	40.82	Alkane	0.38	254	$C_{18}H_{38}$
Docosane	40.85	Alkane	0.38	310	$C_{22}H_{46}$
Pentadecanoic acid, 14-methyl, methyl ester	41.06	Ester	1.67	270	$C_{17}H_{34}O_2$
Hexadecanoic acid, methyl ester	41.12	Ester	1.69	273	$C_{17}H_{34}O_3$
1,2-Benzenedicarboxylic acid, butyl octyl	41.13	Carboxylic acids	0.77	310	$C_{20}H_{30}O_4$
n-Propyl-9,12-octadecadienoate	41.15	Ester	0.85	322	$C_{21}H_{38}O_2$
Hexadecanoic acid, methyl ester	41.23	Ester	0.92	270	$C_{17}H_{34}O_2$
13-Octadecenoicacid, methyl ester	41.50	Ester	0.80	295	$C_{19}H_{36}O_2$
12-Octadecenoic acid, methyl ester	42.20	Ester	0.80	296	$C_{19}H_{36}O_2$
Octadecanoic acid, methyl ester	42.36	Ester	0.83	298	$C_{19}H_{38}O_2$
1,3-Myristin	42.80	Phenolic	1.17	315	$C_{21}H_{38}O_6$

Rt (min): Retention time in minutes, M.w$_t$: Molecular weight, GC/MS: Gas chromatography-mass spectroscopy, *G. max: Glycine max*

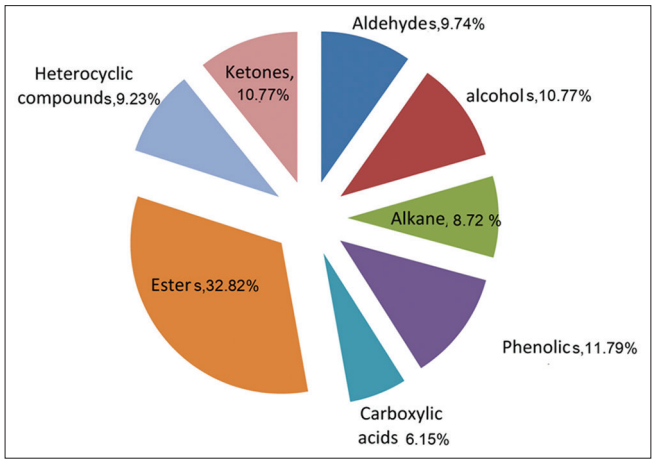

Fig. 3: Pie diagram showing the percentage of phytochemical groups identified in five *Glycine max* genotypes

reference antioxidant radical scavenger compounds. G_{35} showed highest antioxidant activity using the five assays as clarified in Table 11, DPPH (35.95±0.32%), ABTS radical scavenging (54.59±0.50%), and metal chelating ability (61.54±0.57%). Furthermore, the high reducing power ability (0.509±0.008) and FRAP (2913±26.80 µmol Trolox/100 g DW) were recorded, followed by G_{22} in two methods; reducing power ability and ABTS radical scavenging activity. G_{82} had recorded the lowest values in three methods (reducing power ability, metal chelating activity, and FRAP). Furthermore, G_{111} had recorded the lowest ABTS radical scavenging activity while Crawford had recorded lowest DPPH activity. The results showed that genotype G_{35} had the highest content of phenolics and flavonoids as well as the antioxidant activity.

In vitro antitumor activity

Different concentrations of the five genotypes of *G. max* seeds extract were applied on Ehrlich ascites carcinoma cells.

Table 12 illustrated that G_{35} exhibited the highest antitumor activity (14.25±0.38%) among the five genotypes of *G. max* followed by Crawford (13.18±0.15%). The lowest antitumor activity recorded by the genotype G_{22} (5.8±0.16%) at the concentration 5 mg/mL. Fig. 6 showed the effect of different concentrations (1–5 mg/mL) of genotype G_{35} seed extract on the viability of Ehrlich ascites carcinoma cells. Data are means of triplicate experiments±standard deviation. The range of percentage of dead cells of 1 mg/mL of the five genotypes of *G.*

Table 6: GC/MS analysis of local genotype Giza 22 of *G. max*

Name of identified compounds	R_t (min)	Nature of compound	Area%	M.w_t	Molecular formula
3-Ethoxy-2-butanone	24.35	Ketone	1.25	116	$C_6H_6O_2$
Phenol, 2-methoxy	24.73	Phenolic	0.98	124	$C_7H_8O_2$
Menthol	24.83	Alcoholic	5.77	157	$C_{10}H_{20}O$
13-Octadecenoic acid	24.84	Carboxylic acids	0.47	282	$C_{18}H_{34}O_2$
2-Decenal	24.85	Aldehyde	0.47	154	$C_{10}H_{18}O$
Tridecanol	25.85	Alcoholic	0.47	200	$C_{13}H_{28}O$
2,4-Decadienal	26.69	Aldehyde	3.67	152	$C_{10}H_{16}O$
2-Undecenal	28.23	Aldehyde	1.02	168	$C_{11}H_{20}O$
Undecenal	28.26	Aldehyde	1.02	168	$C_{11}H_{20}O$
Phenol, 2,6-*bis*1,1-dimethylethyl-4-methyl	30.99	Phenolic	0.95	220	$C_{16}H_{26}O_2$
2,6-*di*-t-Butyl-4-hydroxy4methyl2,5-cyclo hexadien-1-one	31.72	Ketone	0.82	236	$C_{15}H_{24}O_2$
2-Heptanone-6 (3-acety-l2-methyl1cyclopropen-1-yl)-6-mEthyl	31.75	Ketone	0.52	222	$C_{14}H_{22}O_2$
2,5-Dimethyl-4-hydroxy-3 (2H)-furanone	31.77	Ketone	0.87	128	$C_6H_8O_3$
Pyran-4-Carboxylic acid, 4-(4-methoxyphenyl)- tetrahydro	33.70	Heterocyclic	0.88	236	$C_{13}H_{16}O_4$
1,4-Dimethoxy2,3,6-*tri*-methylbenzene	33.79	Heterocyclic	0.62	180	$C_{11}H_{16}O_2$
2H-Pyran-2,6 (3H)-dione	33.81	Heterocyclic	0.85	112	$C_5H_6O_3$
Diethyl Phthalate	35.49	Ester	0.55	222	$C_6H_{14}O_4$
1,2-Benzenedicarboxylic acid, diethyl ester	35.50	Ester	0.55	222	$C_6H_{14}O_4$
2H-Pyran-3-ol, tetrahydro2,2,6-trimethyl6 (4methyl3cyclohexen1yl)	39.80	Heterocyclic	0.75	238	$C_{15}H_{26}O_2$
2-Furanmethanol, tetrahydro-5-trimethyl-5 (4methyl3cyclohexen1yl)	39.83	Alcoholic	0.75	238	$C_{15}H_{26}O_2$
Benzyl benzoate	40.28	Ester	0.48	212	$C_{14}H_6O_2$
Octadecane	40.82	Alkane	0.38	254	$C_{18}H_{38}$
Docosane	40.85	Alkane	0.38	310	$C_{22}H_{46}$
Hexadecanoic acid, methyl ester	41.23	Ester	0.92	270	$C_{17}H_{34}O_2$
Phthalic acid, butylhex-3-yl ester	42.8	Ester	0.45	306	$C_{18}H_{26}O_4$
Phthalic acid, 5-methylhex-2-yl isobutyl ester	42.84	Ester	0.45	320	$C_{19}H_{28}O_4$
Pentadecanoic acid, 13-methyl, methyl ester	44.06	Ester	2.01	270	$C_{17}H_{34}O_2$
Pentadecanoic acid, 14-methyl, methyl ester	44.06	Ester	2.01	270	$C_{17}H_{34}O_2$
Nonacosane	44.98	Alkane	0.56	311	$C_{29}H_{60}$
Hexadecanoic acid	45.01	Carboxylic acids	2.14	256	$C_{16}H_{32}O_2$
1,2-Benzenedicarboxylic acid, dibutyl ester	45.12	Ester	1.24	278	$C_{16}H_{22}O_4$
3,7-Dimethyl4,6-nonandione	45.27	Ketone	0.87	184	$C_{11}H_{20}O_2$
Octanoic acid, 1-methyltridecylester	45.30	Ester	0.87	296	$C_{22}H_{44}O_2$
8,11-Octadecadienoic acid, methyl ester	45.38	Ester	2.46	294	$C_{19}H_{34}O_2$
13-Octadecenoicacid, methyl ester	45.40	Ester	0.8	295	$C_{19}H_{36}O_2$
7,10-Octadecadienoic acid, methyl ester	45.46	Ester	2.46	294	$C_{19}H_{34}O_2$
9,12-Octadecadienoic acid	45.48	Carboxylic acids	5.06	280	$C_{18}H_{32}O_2$
Octadecane, 3-ethyl-5 (2ethylbutyl)	45.49	Alkane	0.5	366	$C_{26}H_{54}$
9-Octadecenoic acid	45.88	Carboxylic acids	3.05	282	$C_{18}H_{34}O_2$
Nonadecane, 9-methyl	45.96	Alkane	0.91	282	$C_{20}H_{42}$

R_t (min): Retention time in minutes, M.w_t: Molecular weight, GC/MS: Gas chromatography-mass spectroscopy, *G. max: Glycine max*

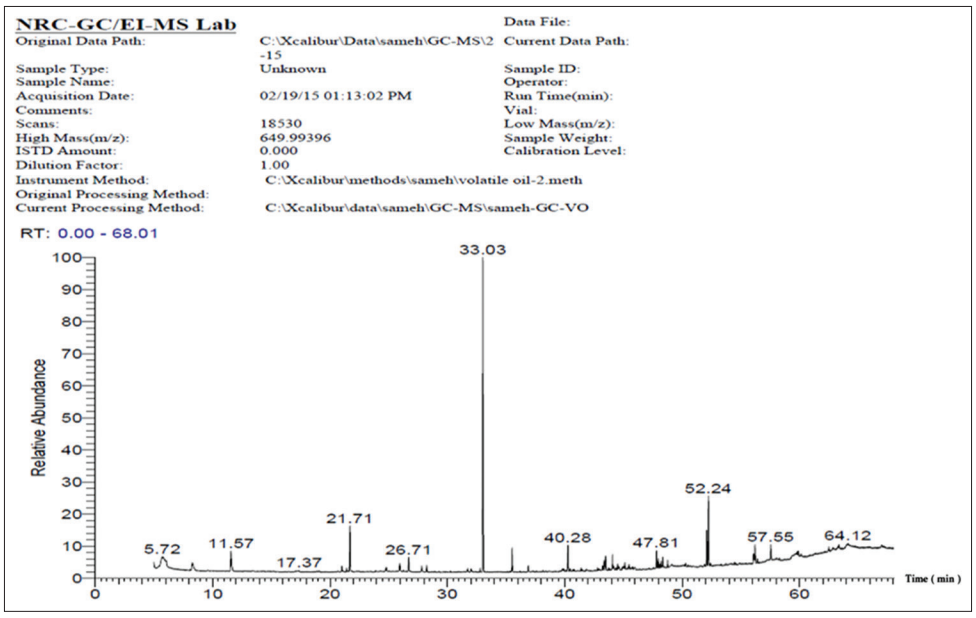

Fig. 4: A typical gas chromatography-mass spectroscopy profile of Giza 35 as the highest genotype having number of phytochemicals

Table 7: GC/MS analysis of foreign genotype Crawford of *G. max* seed

Name of identified compounds	R_t (min)	Nature of compounds	Area%	M.w_t	Molecular formula
2,4-Pentanedione	17.60	Ketone	1.80	100	$C_5H_8O_2$
Bicyclo-3.2.2- non-8-en-6-ol	17.71	Alcholic	0.76	138	$C_9H_{14}O$
1,3-Propanediol	18.56	Alcoholic	2.29	76	$C_3H_8O_2$
Propanoic acid, 2-oxo	18.69	Carboxylic acids	0.86	88	$C_3H_4O_3$
Lavandulyl acetate	18.91	Ester	0.44	196	$C_{12}H_{20}O_2$
Phenol, 4-ethenyl, acetate	19.29	Phenolic	0.99	162	$C_{10}H_{10}O_2$
2-Methoxy-4-vinylphenol	23.35	Phenolic	0.88	150	$C_9H_{10}O_2$
Phenol, 2-methoxy	24.73	Phenolic	0.98	124	$C_7H_8O_2$
Benzyl benzoate	24.80	Ester	0.60	212	$C_{14}H_{12}O_2$
2-Decenal	24.81	Aldehyde	1.5	154	$C_{10}H_{18}O$
Phenol, 2,3,4,6-tetramethyl	25.93	Phenolic	1.56	150	$C_{10}H_{14}O$
2,4-Decadienal	26.69	Aldehyde	3.67	152	$C_{10}H_{16}O$
1-Propanol, 3-Methoxy	28.11	Alcoholic	2.44	90	$C_4H_{10}O_2$
Undecenal	28.23	Aldehyde	1.02	168	$C_{11}H_{20}O$
Phenol, 2,6-bis1,1-dimethylethyl-4-methyl	30.99	Phenolic	0.95	220	$C_{16}H_{26}O_2$
2,6-*di*-t-Butyl-4-hydr oxy4methyl2,5-cyclo hexadien-1-one	31.72	Ketone	0.89	236	$C_{15}H_{24}O_2$
1,6-Methanofluorene	31.75	Heterocyclic	0.89	183	$C_{14}H_{12}$
Tertbutyl2methoxy Phenol	31.79	Phenolic	0.89	180	$C_{11}H_{16}O_2$
2,2,7,7-Tetramethyl-tri cyclo-4-en-3-one	32.01	Ketone	0.70	218	$C_{15}H_{22}O$
1-Acetyl1hydroxy6,8-dimethyoxynaphthalene-2-(1H)-one	33.03	Ketone	0.69	262	$C_{14}H_{14}O_5$
Pentyl -3-oxobutanoate	33.12	Ester	0.61	172	$C_9H_{16}O_3$
2H-Pyran-2,6 (3H)-dione	33.81	Heterocyclic	0.85	112	$C_5H_6O_3$
1,2-Benzenedicarboxylic acid, diethyl ester	35.49	Ester	1.06	222	$C_{12}H_{14}O_4$
2-H-Pyran-3-ol, tetrahydro2,2,6-*tri*-methyl6-4-methyl-3-cyclohexen-1-yl	39.80	Heterocyclic	0.74	238	$C_{15}H_{26}O_2$
2-Furanmethanol, tetrahydro, 5-trimethyl-5-4-methyl-3-cyclohexen-1-yl	39.85	Alcoholic	0.74	238	$C_{15}H_{26}O_2$
Hexadecanoic acid, methyl ester	41.23	Ester	0.92	270	$C_{17}H_{34}O_2$
9,9-Dimethyl8,10-dioxapentacyclo-decane	42.87	Ester	0.51	166	$C_{10}H_{14}O_2$
1,2-Benzenedicarboxylic acid, dibutyl ester	42.89	Ester	0.51	278	$C_{16}H_{22}O_4$
Pentadecanoic acid, 14-methyl, methyl ester	44.02	Ester	1.60	270	$C_{17}H_{34}O_2$
Nonacosane	44.98	Alkane	0.56	311	$C_{29}H_{60}$
6-Formylazulene	45.57	Heterocyclic	0.74	156	$C_{11}H_8O$
7,10-Octadecadienoic acid, methyl ester	45.60	Ester	1.82	294	$C_{19}H_{34}O_2$
9,12-Octadecadienoicacid , methyl ester	45.63	Ester	1.82	294	$C_{19}H_{34}O_2$
8,11-Octadecadienoic acid, methyl ester	45.65	Ester	1.82	194	$C_{19}H_{34}O_2$
8-Octadecenoic acid, methyl ester	45.68	Ester	2.67	296	$C_{19}H_{36}O_2$

R_t (min): Retention time in minutes, M.w_t: Molecular weight, GC/MS: Gas chromatography-mass spectroscopy, *G. max: Glycine max*

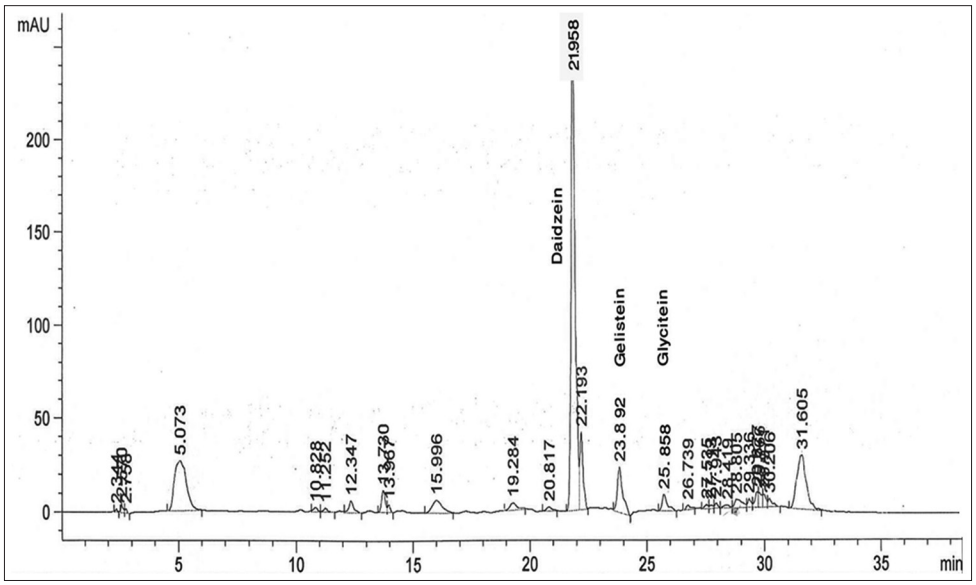

Fig. 5: High-performance liquid chromatography chromatogram of G_{35} hydrolyzed extract

max seeds was 3.27±0.12–12.00 ± 0.40% (Table 12). The range of the concentration of dead cells of 2 mg/mL was 3.55±0.14–12.35±0.14%. The concentration of the dead cells of 3 mg/mL was within 3.64±0.15–12.83±0.24%. The range of the concentration of dead cells of 4 mg/mL was 4.07±0.09–13.53±0.13% and the range of the concentration of dead cells 5 of mg/mL was 5.80±0.16–14.25±0.38% (Table 12). It was observed that the antitumor activity of the examined genotypes increased gradually by increasing the concentration of the extract.

Table 8: Phytocompound identified in of G_{111} by GC–MS

Name of identified compounds	R_t (min)	Nature of compounds	Area%	Mw_t	Molecular formula
Propanoic acid, 2-oxo	21.69	Carboxylic acids	0.86	88	$C3H_4O3$
Phenol, 2-methoxy	24.73	Phenolic	0.98	124	$C7H_8O_2$
Docosane	24.76	Alkane	0.69	310	$C_{22}H_{46}$
Phenol, 5-methyl-2-1-methylethyl	25.92	Phenolic	1.33	150	$C_{10}H_{14}O$
Thymol	25.95	Alcoholic	1.34	152	$C_{10}H_{14}O$
2-Undecenal	28.23	Aldehyde	0.85	168	$C_{11}H_{20}O$
Phenol, 2,6-*bis*1,1-dimethylethyl-4-methyl	30.99	Phenolic	0.95	220	$C_{16}H_{26}O_2$
2,6-*di*-t-Butyl-4-hydroxy4methyl2,5-cyclo hexadien-1-one	31.72	Ketone	0.82	236	$C_15H_{24}O_2$
2,2,7,7-Tetramethyl-*tri* cyclo-undec-4-en-3-one	32.00	Ketone	0.70	218	$C_15H_{22}O$
2-Propen1one, 1-cyclohexyl	32.07	Ketone	0.70	219	$C_15H_{22}O$
2H-Pyran-2,6 (3H)-dione	33.81	Heterocyclic	0.85	112	$C5H_6O3$
Benzene1,2,3,4-tetramethyl-5-1-methylethyl	36.89	Heterocyclic	0.39	176	C_13H_{20}
Cyclohexane, 1,4-dimethyl-2-octade	38.15	Alkane	0.38	312	$C_{26}H5_2$
Acetic acid, 10,11-dihydroxy3,7,11-trimethyldodeca2,6-dienyl ester	39.8	Ester	0.86	298	$C_17H3_0O_4$
2-H-Pyran-3-ol, tetrahydro2,2,6-trimethyl6-4-methyl-3-cyclohexen-1-yl	39.86	Heterocyclic	0.86	238	$C_15H_{26}O_2$
Benzyl benzoate	40.27	Ester	0.62	212	$C_{14}H_{12}O_2$
Octadecane	40.81	Alkane	0.34	254	$C_{18}H3_8$
Hexadecanoic acid, methyl ester	41.23	Ester	0.92	270	$C_17H_{34}O_2$
Phthalic acid, hept-4-yl isobutyl ester	42.8	Ester	0.63	320	$C_{19}H_{28}O_4$
1,2-Benzenedicarboxylic acid, bis-2-methylpropyl- ester	42.86	Ester	0.63	278	$C_{16}H_{22}O_4$
Hexadecanoic acid, methyl ester	44.06	Ester	5.62	294	$C_{19}H_{34}O_2$
9,12-Octadecadienoicacid, methyl ester	44.12	Ester	5.62	296	$C_{19}H3_6O_2$
7,9-Ditertbutyl-1-oxaspiro-4,5-deca-6,9-diene-2,8-dione	44.14	Ketone	0.41	276	$C_17H_{24}O3$
3Ethyl3methylnona- decane	44.99	Alkane	0.45	310	$C_{22}H_{46}$
1,2-Benzenedicarboxylic acid, di-butyl ester	45.12	Ester	1.58	278	$C_{16}H_{22}O_4$
8,11-Octadecadienoic acid, methyl ester	47.08	Ester	5.62	294	$C_{19}H_{34}O_2$
8-Octadecenoicacid, methyl ester	47.20	Ester	5.27	296	$C_{19}H3_6O_2$
Octadecanoic acid, methyl ester	47.75	Ester	1.79	298	$C_{19}H3_8O_2$
Octadecane, 3-ethyl-5-2-ethylbutyl	47.80	Alkane	0.50	311	$C_{26}H5_4$
1,2-Benzenedicarboxylic acid, *bis*-2-ethylhexyl ester	47.85	Ester	3.39	310	$C_{24}H3_8O_4$
1,2-Benzenedicarboxylic acid, *di*-isooctyl ester	47.90	Ester	3.39	319	$C_{24}H3_8O_4$

R_t (min) : Retention time in minutes, $M.w_t$: Molecular weight, GC/MS: Gas chromatography-mass spectroscopy

Table 9: HPLC-PDA characteristics (R_t and area %) of the hydrolyzed extract of G_{35} and Crawford

Isoflavones	G_{35}		Crawford	
	R_t (min.)	Area %	R_t (min.)	Area %
Daidzein	21.958	14.057	21.936	3.922
Genistein	23.892	2.248	23.905	1.794
Glycitein	25.858	1.439	25.865	0.122

R_t (min): Retention time in minutes, HPLC: High-performance liquid chromatography, PDA: Photodiode array

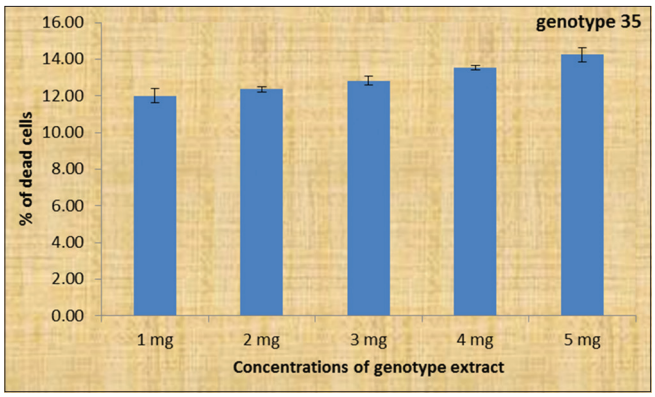

Fig. 6: Effect of different concentrations of genotype G_{35} seed extract on the viability of Ehrlich ascites carcinoma cells

DISCUSSION

Significant differences were observed between the genotypes with respect to moisture content, ash, proteins, and carbohydrates content (Table 1). The moisture ranged from 6.71 to 6.90%. G_{111} had the highest moisture content, but G_{22} and G_{35} had the lowest content. Silva *et al.* [22] reported the moisture percentage of *G. max* (State of Goias), and it was 5.60%. These differences in moisture content may be due to the conditions of drying the grain after harvest, storage period, and the ability of the grains to lose moisture [23]. Protein content was ranged from 30.5 to 33.9%. G_{111} had the highest protein content, while G_{22} had the lowest content. Silva *et al.* [22] found that soybean with yellow seed coat (in the State of Goiás) had protein content 40.4%. The total carbohydrates content was ranged from 24.5 to 27.1%. Vieira *et al.* [24] evaluated amino acids composition of six genotypes grown in Campinas and they ranged from 29.81% to 33.33%. In the current work, genotype G_{111} has the highest percentage of total proteins, carbohydrates, and moisture. Concerning to lipoidal matters, the total identified hydrocarbons were 52.83–59.49%, total identified sterols 17.02–28.04%, and the total identified triterpenes 1.08–12.31%. Unsaturated fats are considered the healthy fats and they are important to include as part of a healthy diet. There are two main forms of unsaturated fatty acids, polyunsaturated such as linoleic acid, linolenic acid, and monounsaturated such as oleic acid. GLC analysis of FAME of the five genotypes of *G. max* showed that the total unsaturated fatty acid has higher values than a total saturated fatty acid. The highest value of total unsaturated fatty acids in the investigated genotypes was in G_{82} (70.09%). The ascending order of total unsaturated fatty acid was G_{82} (70.09%)>G_{111} (54.76%)>G_{35} (53.52%)>G_{22} (45.74 %)>Crawford (45.12%). The percentages of these fatty acids in soybean are considered to be unstable [25]. Two main types of polyunsaturated fats are omega-3 fats and omega-6 fats. There are three types of omega-3 fatty acids involved in human physiology (linolenic, eicosapentaenoic, and docosahexaenoic acid). Linolenic acid was found in G_{35} and G_{111}. The inhibition effect of omega-3 fatty acids on tumor angiogenesis was reported [26]. Furthemore, omega-6 (ω-6s) fatty acids such

Table 10: Total content of secondary metabolites in the five genotypes extract of (*G. max*)

Genotypes	Determination		
	Total phenolics (mg GAE/g DW)	Total flavonoids (mg QE)/g DW)	Total tannins (mg CE)/g DW)
Genotypes			
G_{82}	7.35±0.19	2.95±0.09	0.91±0.04
G_{22}	8.48±0.15	3.81±0.13	1.00±0.06
G_{111}	9.38±0.22	4.83±0.12	1.07±0.10
Crawford	9.99±0.19	4.25±0.08	1.19±0.07
G_{35}	11.90±0.19	5.26±0.08	1.49±0.17

GAE: Gallic acid equivalent, QE: Quercetin equivalent, CE: Catechin equivalent, *G. max: Glycine max*

Table 11: Summary of five different assays of antioxidant activity of *G. max* genotypes

Genotype	DPPH%	Absorbance at 700 nm	ABTS%	Metal chelating %	FRAP (µmol Trolox/100 g DW)
G_{82}	30.98±0.42	0.297±0.005	37.72±0.57	42.87±0.27	1740±18.50
G_{22}	29.59±0.50	0.342±0.012	39.34±0.37	47.89±0.46	2118±30.30
G_{111}	30.27±0.88	0.320±0.007	37.27±0.35	50.34±0.21	2201±11.50
Crawford	28.24±0.69	0.327±0.008	37.80±0.22	51.23±0.20	1977±17.20
G_{35}	35.95±0.32	0.509±0.008	54.59±0.50	61.54±0.57	2913±26.80

Each value are mean of three replicates±standard deviation, *G. max: Glycine max*, DPPH: 2,2-diphenyl, 1-picryl hydrazyl, FRAP: Ferric reducing antioxidant power, ABTS: 2,2'-Azino-*bis* (3-ethylbenzothiazoline-6-sulphonic acid

Table 12: Effect of different concentrations of the five genotypes on the viability of EACC

Genotypes	Dead cells %				
	1 mg	2 mg	3 mg	4 mg	5 mg
G_{82}	3.79±0.13	4.96±0.17	5.15±0.15	6.02±0.11	6.64±0.14
G_{22}	3.27±0.12	3.55±0.14	3.64±0.15	4.07±0.09	5.80±0.16
G_{111}	7.47±0.24	8.37±0.30	8.80±0.14	9.79±0.15	10.36±0.19
Crawford	11.83±0.14	11.97±0.09	12.15±0.09	12.49±0.19	13.18±0.15
G_{35}	12.00±0.40	12.35±0.14	12.83±0.24	13.53±0.13	14.25±0.38

Data are means of triplicate experiments±standard deviation, EACC: Ehrlich ascites carcinoma cells

as linoleic and arachidonic acid were recorded in our work. The ω-6s have been shown to exert anticancer proliferation effects by influencing gene and protein expression by disrupting cell cycle progression and inducing apoptosis. Linoleic acid was present in G_{35}, G_{82}, and G_{111} (r: 36.99–44.80%) Arachidonic acid which presents only in G_{111} (0.82%). Omega-6 fatty acids have possessed diverse bioactivities and are associated with many beneficial effects on human health including cancer development [27]. Recently [28], omega-3 (ω-3) polyunsaturated fatty acids were reported to protect Sertoli cells apoptosis at the physiologically relevant levels [28]. In our present work, the glutamic acid concentration in all genotypes is the highest values in all identified amino acids. The range of glutamic acid was within 23.387–13.396%. Glutamic acid, a non-essential amino acid, is highly abundant amino acid found in most foodstuffs and comprise between 5 and 15% of dietary protein [29]. It is a non-essential amino acid. The non-essential amino acid is produced in the body from other substances, so the body does not require a dietary intake of glutamic acid [30]. Glutamic acid is mostly metabolized in the brain, and this substance is an essential neurotransmitter which is essential for normal healthy brain function [31].

There was wide variation in the composition of phytochemicals in the five *G.max* genotypes detected by GC/MS. G_{35} had the highest number of aldehydes, phenolics, ketones, esters, alcoholics, and carboxylic. G_{22} had the highest number of esters, carboxylic, and alkanes. G_{82} had the highest number of heterocyclic and ester compounds. G_{111} had the highest number of aldehydes, alkanes, esters, and other valuable compounds possessing powerful radical scavenging activity, suggesting the correlation with antiproliferative activity [32,33]. Phenolics inhibit carcinogenesis by influence on the molecular events in the initiation, promotion, and progression stages [34]. HPLC analysis of isoflavone aglycones is the most performing technique for qualitative analysis of *G. max* isoflavones. It can make a fingerprint of soybean extracts thought the content of isoflavone aglycones. The photo-diode-array detection was used over a wavelength range 260 nm to collect spectral data. The major form of flavonoids in soybean seeds is isoflavone; it has many chemical actions such as antioxidative and anticancer agents [35]. In our comparative study, G_{35} which is local genotype had a higher value of detected isoflavone aglycones than genotype Crawford which was imported from the USA. The impact of dietary isoflavones, daidzein, and genistein, on the health of adults, and infants is well known [36]. Isoflavones are categorized chemically by their functional groups. Recently [37], a study reported that the genotype G_{111} showed the lowest phenolic content (1.15 mg/g) while the genotype Romal-1 was of the highest value (1.7 mg/g) compared with the other hybrids. The range of total flavonoids (0.68–2.13 mg QE/g) and total phenolics (1.15–1.77 mg GAE/g) showed great variability between the investigated genotypes [32,37]. Many studies suggested that the majority of chemical components significantly depend on both genetic and environmental factors, with a large influence derived from temperature, water, nutrient supply, and other factors [32,37]. In our study, genotype G_{35} showed a large class of secondary plant metabolites of different structures such as aldehydes, phenolics, ketones, esters, alcoholics, and carboxylic. Other phtyoconstituents as unsaturated fatty acids, sterols and/or triterpenes, and polyphenols were inconsiderable amount in all the studied genotypes. These detected compounds have valuable effect as antitumor and antioxidant agents [38,39]. The consumption of plants rich in polyphenol compounds has been linked with inhibitory and preventive effects in various human cancers and cardiovascular diseases, which may be related to the antioxidant activity of polyphenols [40]. In the present paper, HPLC-PDA analysis showed the presence of isoflavone aglycones; daidzein, glycitein, and genistein in both genotypes. Genistein had been reported that it can act by both hormonal and non-hormonal action in the inhibition of cancer and act as

antioxidant compound [41,42]. G_{35} showed high chemical composition of phenolics, flavonoids, and tannins in comparison with other genotypes and that might be responsible for their comparatively higher antioxidant activity [39,42]. In our study, DPPH and $ABTS^+$ scavenging methods have been used to evaluate the antioxidant activity of extracts due to the simple, rapid, sensitive, and reproducible procedure [43]. G_{35} showed the highest radical scavenging and antioxidant capacity using five analytical assays (Table 11) compared with the reference antioxidant radical scavenger compounds BHT and Trolox. It followed by G_{22} in two methods; reducing power ability and ABTS radical scavenging activity. The results showed that G_{35} had the highest content of phenolics and flavonoids as well as the antioxidant activity. A positive result was observed between the antioxidant activity potential and total flavonoids level of the genotype extracts. The results of this study explore the potential of G_{35} and G_{22} as a rich source of natural antioxidants for the development of functional foods and nutraceutical applications. The strong correlation was observed between antioxidant capacities and their total phenolic contents. In parallel with our results, Prakash *et al.* [44] suggested the high antioxidant activity (50.5–74.7%) may be linked to high polyphenols content (52.7–81.7 mg GAE/g GAE) of soybean Hara genotypes. The previous study reported the structure-activity relationship of antioxidant and antitumor polyphenols [45]. Inhibitions of tumor initiation and promotion, which are affected by antioxidant and binding activities, are exhibited in various potencies, being influenced by the structure and molecular weight of polyphenols as tannins [45]. Concerning the antitumor activity of *G. max* genotypes, all the samples especially G_{35} have showed remarked antitumor activity against carcinogenic cells. Soybean isoflavone intake exerts a protective effect against postmenopausal breast cancer, the overexpression of human epidermal growth factor receptor 2 promotes the malignant transformation of breast epithelial cells [46]. Sugiyama *et al*. [47] demonstrated that daidzein converted to a metabolite called equol by intestinal bacteria leads to a significantly reduced risk of prostate cancer.

Our results indicated that the tested genotypes showed substantial differences in terms of chemical composition, antitumor, and antioxidant activities. The results confirm the higher value of phytoconstituents of the genotype G_{35} and G_{21} as well as their better bioactivity. G_{35} which is local genotype had the highest value of total phenolics, flavonoids, and tannins also this genotype showed highest antioxidant activity evaluated by the five assays and antitumor activity which found to be 14.25±0.38 at concentration 5 mg/mL.

CONCLUSION

The results confirm the higher value of phytoconstituents of the genotype Giza 35 and Giza 21 as well as their better bioactivity. Hence, we recommended to use these genotypes as they had healthy fats and can be included as part of a healthy diet.

AUTHOR'S CONTRIBUTIONS

Howaida I. Abd-Alla: Plan of work, chemical composition study, writing, revision, and corresponding author. Hanan A. Taie: Plan of work, biological activities study, writing, and revision. Marwa M. Abd-Elmotaleb: Samples preparation and share of the practical study.

CONFLICTS OF INTEREST

The authors have none to declare.

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