RSC Advances

ROYAL SOCIETY OF CHEMISTRY

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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

Paper

Profiling of phenolic and other compounds from Egyptian cultivars of chickpea (*Cicer arietinum* L.) and antioxidant activity: a comparative study†

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Chickpeas are basic food in many countries with several cultivars distributed all over the world. However, little is known about their secondary metabolites. Thus, this work is focused on the study of the phenolic profiles of seven Egyptian cultivars of chickpea. Selecting the most appropriate extraction method and analytical conditions using reversed-phase high-performance liquid chromatography-diode array detection, with a core-shell column, and coupled with quadrupole-time-of-flight-mass spectrometry (MS), a total of 96 phenolic compounds were characterized based on their retention time, UV spectra, and accurate MS and MS^2 data. Among them, the major phenolic subclasses were hydroxybenzoic acids and flavonoids. Moreover, other minor and major metabolites including organic acids, amino acids, nucleosides, peptides and soyasaponins were characterized. Using standards, 22 compounds were unequivocally identified. Remarkably, 88 of these compounds were tentatively reported for the first time in chickpeas. The total phenol content of the cultivars was determined as well as the antioxidant activity by the trolox equivalent antioxidant capacity assay.

Introduction

"*Let food be thy medicine and medicine be thy food*" Hippocrates (460 BC – 377 BC). It is nearly a 2500 years aphorism, and still a topic of current interest. In fact, the characterization of beneficial food constituents and formulation of novel functional foods continue to attract scientific and industrial attention. Such foods contribute to prevention and mitigation of diseases, promotion of health and well-being with a reduction of health care costs. It is noteworthy that the intake of vegetables, fruits and legumes reduces the risks of cancers, diabetes mellitus, atherosclerosis, and cardiac diseases.[1,2] This may be attributed to their richness in secondary metabolites and, in particular, phenolic compounds.[1]

To provide evidence of the connection between health and food constituents, "omics" tools such as genomics, transcriptomics, proteomics, and metabolomics have emerged.[3] Among them, metabolomics is the study of the biochemical composition of living organisms making use of hyphenated techniques such as chromatographic separation coupled to mass spectrometry (MS).

In fact, the application of advanced analytical techniques and hybrid mass analyzers has contributed to discover and characterize new phytochemicals, especially, minor ones that could promote human well-being. Among others, these analytical techniques include gas chromatography, liquid chromatography (LC) and capillary electrophoresis.[3,4] As an example, LC coupled to quadruple time-of-flight (QTOF)-MS using columns with < 2 µm particle sizes[5] and core-shell columns[6] provide enough resolution and high sensitivity detection to permit metabolic profiling of plant extracts.

On the basis of the total pulse production, chickpeas (*Cicer arietinum* L.) are the second most important legume in the world.[7] This plant is cultivated in India, Pakistan, Mexico, the Mediterranean basin regions, and many other countries.[8] Chickpeas with respect to other legumes represent the fifth most important product in Egypt.[7] Their use dates from at least the "New Kingdom" (1580-1100 BC) and they obtained the name "falcon-face" in that period.[9] This pulse constitutes a well recognized source of dietary proteins, carbohydrates, minerals and trace elements.[10] To obtain functional ingredients from chickpeas, several studies have focused on the development of protein hydrolysates with biological activity, including antioxidant activity, *e.g.*[11] Moreover, chickpeas contain several phytochemical classes, such as phenolic compounds,[12-14] soyasaponins,[15,16] and volatile aliphatic hydrocarbons.[17] In general, there is relatively little information about the phytochemicals present in most of dietary legumes.[18] This is even more limited in the case of chickpeas.

Concerning extraction procedures for phenolic compounds from chickpeas, many authors applied a single solid-liquid extraction step with solvents such as methanol and acetone,[13,19] while others used mulitple solid-liquid extractions.[20,21] Solid-liquid extraction combined with solid-phase extraction using a

silica gel column was recently applied to concentrate isoflavones.[22] In the case of soyasaponins, Kerem *et al.*[15]applied microwave-assisted extraction. Among the analytical techniques to analyse this legume are: methods based on high-speed countercurrent chromatography and high-performance-LC (HPLC) coupled to ultraviolet/visible; diode array (DAD) detectors and MS using electrospray ionization (ESI).[12,20,23,24] However these studies generaly focused on a sort list of phenolic compounds.

The objective of this study is to develop a global approach to characterize phenolic compounds from the edible seeds of seven Egyptian cultivars of chickpea, namely 'Giza 1', 'Giza 2', 'Giza 3', 'Giza 4', 'Giza 195', 'Giza 531' and 'Solala 104'. To achieve this, solid-liquid extraction and the analytical conditions by reversed-phase (RP)-HPLC-DAD-ESI-QTOF-MS were evaluated. Moreover, the total phenol content (TPC) and the antioxidant activity of the seeds using the trolox equivalent antioxidant capacity (TEAC) assay were also assessed.

Results and discussion

Selection of the extraction procedure

Prior to the optimization of the analytical method, the characterization as well as the determination of the antioxidant potential of the chickpea cultivars, three solid-liquid extraction procedures were tested using the chickpea seeds of the cultivar 'Giza 1'. In this way, the TPC was assessed according to the Folin-Ciocalteu method and the yield was determined (Fig. 1). These results showed that the TPC value was significantly higher using the extraction method M3 (129.4 mg of gallic acid/100 g of chickpea seeds).

Moreover, the comparison was also made with the total integrated area of the base peak chromatogram (BPC) and UV chromatograms at 240, 280, 330 and 350 nm, according to Hurtado-Fernández and co-workers.[25] These UV channels (bandwidth of 10 nm) were selected bearing in mind the phenolic classes that were previously reported on chickpeas *viz.* hydroxybenzoic acids, hydroxycinnamic acids and flavonoids, mainly flavonols and isoflavones.[12,22,26] Our results with standards are shown in Fig. S1 (supporting information), being in agreement with several studies:[27,28] 240 and 280 nm (approximate) was related to phenolic compounds, 280 nm was particularly useful for determining phenolic acids, dihydroflavonoids and flavanols, 320-330 nm was a very suitable wavelength for hydroxycinnamic acids in concrete, and above 330 nm and 350 nm for isoflavones and flavonols, respectively. As an example, Fig. 2 shows the BPC in the negative ionization mode of 'Giza 1' chickpea extracts, the corresponding chromatograms at 280 nm, as well as the total area of each chromatogram in a bar chart. In general, the qualitative profiles were quite similar, especially those obtained with M1 and M3, explained by the fact that the extraction experiments were based on at least one step using aqueous solutions of methanol. On the other hand, the total area of the chromatograms were higher using the extraction procedure M3, especially those at 240 nm and 280 nm, at which most phenolic compounds absorb, as well as organic acids and amino acids also contribute.[29-33]

Therefore, taking all of these results into account, total time for the extraction and solvent requirements, M3 was the method of choice in order to extract the rest of chickpea cultivars. In this sense, the selection of the extraction method is a critical step to dissolve the maximum amount of the metabolites of interest in the extraction solvent, and so achieve a successful characterization work.[25,34] Using methanol/water as extraction solvent constituted a reproducible protocol, allowing the selective extraction of polar glycosides of phenolic compounds and as well their aglycones with more hydrophobic features.[35,36] In addition, a wide range of other polar and semi-polar metabolites from vegetable matrices are generally co-extracted at the same time. Moreover, a sonication step was introduced in order to favour the extraction of phenolic compounds according to previous studies on different vegetal matrices.[34,37,38]

Selection of the analytical conditions

The analytical conditions and the MS parameters were preliminarily checked in order to further characterize the chickpea phenolic constituents. In this way, several aqueous solutions of acetic acid from 0.25 to 1% (v/v) were tested as mobile phase A, methanol and acetonitrile as mobile phase B, 5 and 8 μL for injection volume, flow at 0.5 and 0.8 mL/min, as well as two C18 reversed-phase columns with the same dimension but different particle technology. In general, an adequate separation of the compounds from the aforementioned 'Giza 1' extract was achieved in 35 min using the core–shell column, water with 0.5% acetic acid and acetonitrile as mobile phases, which produces lower system back pressure than other solvents as methanol, and a flow of 0.5 mL/min.[6] The maximum pressure was lower than 165 bars, and so this method may be used in conventional HPLC systems. As an example, Fig. S2 (supporting information) shows the BPC of 'Giza 1' chickpea extract using different analytical conditions, including the selected ones, and two column types. Although the co-elution of the major compounds could not be avoided due to the complexity of the sample, a higher number of minor peaks could be adequately separated by the core-shell column and the elution gradient applied (Fig. S3).

Moreover, in the BPC the peak shape in terms of symmetry and the full width at half maximum were also better using the selected analytical conditions (Fig. S3). This fact is important since several of the peaks are related to minor metabolites, which could have gone unnoticed and so uncharacterized. In this sense, most of the studies on chickpeas were only focused on few target compounds. This could be because only the most abundant metabolites were characterized or the analytical methods presented lower sensitivity. Therefore, the use of a core-shell column enabled sufficient separation of the extracted compounds at a reasonable analysis time, complying with previous reports.[39,40]

Qualitative profiling of Egyptian chickpea cultivars

Characterization by RP-HPLC-DAD-QTOF-MS and $-MS^2$

The metabolic profiling of seven chickpea cultivars was performed using the above-mentioned extraction and analytical methods. Fig. 3 shows the BPC of the extracts obtained with the optimized analytical conditions. Furthermore, Table 1 and Table 2 show the overall results: retention time (RT), experimental *m/z* of negative molecular ions ($[M-H]^-$), molecular formula, mass

error, MS score, main MS^2 fragments and UV maximums.

The observed values were compared with those reported in literature and databases. In brief, a total of 140 compounds were characterized. Among them, 22 compounds were confirmed with standards. A total of 88 compounds were found in chickpeas for the first time to our knowledge, including 7 new phenolic compounds in Fabaceae and 8 unreported ones and a jasmonate with new proposed structures. The phenolic compounds (Table 1) were primarily classified as: hydroxybenzoic acids, hydroxycinnamic acids and flavonoids (flavonols, isoflavonoids and others). Other non phenolic compounds (Table 2) were also tentatively identified, namely, organic acids, amino acids, nucleosides, peptides, terpenoids, jasmonates and a maltol derivative. Overall, the UV data were in accordance with several studies.[12,27,28,41]

In addition, Table S1 (supporting information) and Table S2 (supporting information) show additional details of the characterization study, such as theoretical neutral mass, compound subclass, plant species and family as well as previous studies that have reported on each compound.

Phenolic compounds

Hydroxybenzoic acids

A total of 28 hydroxybenzoic acids were characterized in Egyptian chickpeas, being the main subclass of phenolic acids in all of the studied cultivars, qualitatively (Table 1, Fig S4). Among them gallic, *p*-hydroxybenzoic and vanillic acids were confirmed with standards. It is worth mentioning that 23 hydroxybenzoic acids were reported for the first time in chickpeas. The structure of five of them is new and was predicted according to the UV and MS data. In contrast, gallic acid, dihydroxybenzoic acid, *p*-hydroxybenzoic acid and vanillic acid were described before in chickpeas.[12,26,42]

The compounds were derivatives of hydroxybenzoic acid, dihydroxybenzoic acid, trihydroxybenzoic acid (like gallic acid), i.e. *O*-methylated (like vanillic acid) and/or conjugated with sugars (hexose, pentose) and malonic acid. These moieties were assigned based on their respective fragments and neutral losses established on the basis of the fragmentation pattern in MS^2, as previously reported.[5,12,35,41] As an example, Fig. 4a shows the MS^2 spectra of the isomer II of dihydroxybenzoic acid malonyl hexoside detected at RT 15.90. The major fragments were at *m/z* 357.0827, 315.0730, 152.0125 and 108.0230 being generated by the consecutive neutral losses of CO_2 and the acetyl rest (CH_2CO) from the malonyl group,[35] hexose plus H, and CO_2. The latter neutral loss is the typical decarboxylation of phenolic acids.[41]

Hydroxycinnamic acids

The occurrence of 13 hydroxycinnamic acids was observed in most of the cultivars, except the caffeoylquinic acid isomers, which varied among the cultivars (Table 1). Their fragmentation showed the neutral loss of the caffeoyl moiety, among other product ions, as previously described.[35] The isomer that eluted at 15.03 min was chlorogenic acid based on the analysis of the standard. In addition, the presence of *p*-coumaric, ferulic and sinapic acids was also confirmed with standards, while their glucosides were characterized according to their fragmentation patterns[35] and literature (Table S1).

Flavonols

Qualitatively, flavonols (a total of 29) represent the main flavonoid subclass in the Egyptian cultivars of chickpea. Among them, 20 compounds were reported for the first time in chickpeas. On the other hand, kaempferol, kaempferol 3-*O*-β-D-glucopyranoside, kaempferol 3-*O*-rutinoside, kaempferide, quercetin 3-*O*-β-D-glucopyranoside and rutin were confirmed with standards. Other kaempferol and quercetin derivatives, including methylated such as isorhamnetin (3'-methoxyquercetin) and myricetin-*O*-methyl ethers and glycosides, were also detected. Six of the kaempferol derivatives were malonated, which is a common feature of chickpea phenolic compounds[12,43-45] and Fabaceae in general.[46,47] The fragmentation patterns are in accordance with several works,[5,12,25,35] observing: neutral losses of the conjugated moieties as well as common fragments ions at *m/z* 178.9981 ($C_8H_3O_5$) ($^{1,2}A^-$) and 151.0035 ($C_7H_3O_4$) ($^{1,3}A^-$), released after retro Diels–Alder fission and retrocyclization. In the case of *O*-methyl ethers of flavonols, the neutral loss of CH_3 was also released from the precursor ions and/or aglycones, *e.g.* fragments at *m/z* 300.0275 and 316.0224 for quercetin and myricetin derivatives, respectively. As an example, Fig. 4b shows the fragmentation pattern of kaempferol malonyl dihexoside pentoside (isomer I), as a new proposed structure.

Isoflavonoids

This flavonoid subclass is widely distributed in Fabaceae and exhibits antioxidant and estrogenic activities.[48,49] A total of 12 isoflavones and two isoflavanones were detected (Table 1). Genistein (RT 26.02 min, *m/z* 269.0459) was confirmed by the comparison with a commercial standard. It was previously reported in the chickpea cultivar 'Kocbasi',[24] whereas biochanin A, the major isoflavonoid according to our chromatographic profiles, was found in cultivars 'Kocbasi',[24] 'Sinaloa' and 'Castellano'.[12] Orobol and two isomers of dalpanin were detected in this legume for the first time. In general, the fragment ions in MS^2 agreed with various studies.[23,24] For example, characteristic ions related to the fission at $^{0,3}B^-$ were observed, such as fragment ions with *m/z* values above 133.0294 (even ion) ($C_8H_5O_2$) and 132.0217 (odd ion) ($C_8H_4O_2$). Moreover, the fragmentation pattern of dalpanin is in agreement with Chamarthi *et al.*,[50] showing the characteristic loss of 60 Da from the hexose moiety as other *C*-glycosides.[51] Among other fragments, the subsequent losses of water (*m/z* 353.1062) and CH_3 (*m/z* 326.0762) derived from the aglycone backbone of dalpanin (Table 1).

Other flavonoids

The flavan-3-ols (+)-catechin and (-)-epicatechin and the flavanones naringenin and naringenin-7-*O*-β-D-glucopyranoside were previously reported in chickpeas.[42,52] Alternatively, most of the rest were found in Fabaceae[5,53-56] and described here for the first time in chickpeas: aromadendrin (flavanonol), and apigenin (flavone) and their derivatives, and as well (epi)afzelechin (flavan-3-ol).

Non phenolic compounds

Organic acids were noticeably observed, with about 11 compounds tentatively identified. The aromatic amino acids tyrosine, tryptophan and phenylalanine were also detected, as well as γ-glutamyl dipeptides containing tyrosine and phenylalanine residues. Moreover, four nucleosides and five jasmonic acid derivatives were characterized in this legume for the first time. The latter group included a new predicted structure: tuberonic acid (hydroxyjasmonic acid) hexoside pentoside. The fragmentation of these compounds agreed with other previous

studies.[5,35] Licoagroside B, which is a maltol derivative with *m/z* value of 431.1205,[57] and the nucleoside derivative succinyladenosine with *m/z* value of 382.1002[5] were assigned according to their MS^2 spectra.

Among the terpenoid class, saponins represent a diverse group with a structure consisting of triterpenoid aglycones and sugar moieties.[58] The tentatively characterized saponins belong to soyasaponins, which are widely distributed in Fabaceae.[59] This family of compounds eluted later according to their more hydrophobic feature.[60,61] Two isomers of soyasaponin I were detected at 27.04 and 27.92 min with *m/z* value of 941.5123. The presence of soyasaponin I in chickpeas was previously reported by Sagratini *et al.*[62] and the UV data agreed with Hubert *et al.*[63] Its MS^2 spectra showed the neutral loss of rhamnose, galactose and then glucuronic acid. Other soyasaponins were soyasaponin II and kaikasaponin II and III and their characterization was based on the findings of Lu *et al*.[64] It is worth mentioning that six soyasaponins, namely lablab saponin I, soyasaponin αg (isomers I-III) and soyasaponin βg (isomers I and II), are conjugated with 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), explaining the observed absorption close to 292 nm.[63] The two first above-mentioned soyasaponins are described for the first time in this legume. Other terpenoids were dihydrophaseic acid and dihydrophaseic acid 4'-*O*-β-D-glucopyranoside, previously reported in Fabaceae.[5]

Qualitative comparison of the chickpea cultivars

In general, the qualitative profiles of the studied cultivars were quite similar, as Fig. 3 illustrates, with differences in some individual compounds (Table 1 and Table 2). For a simpler comparison of the qualitative results, see Fig. S4. The richest one was the cultivar 'Giza 2', with a higher number of hydroxycinnamic acids and flavonols (in particular, quercetin derivatives), among others, whereas 'Giza 531' was the qualitatively poorest. In the case of non phenolic compounds, all of the cultivars were also quite similar, except 'Solala 104' that showed a lower number of organic acids.

Quantitative comparison and antioxidant activity

The TPC of the chickpea cultivars ranged between 69 and 129 mg of gallic acid/100 g of chickpea seeds (Fig. 5). From the aforementioned results, cultivars 'Giza 1' followed by 'Giza195' showed significantly the highest TPC values, whereas 'Giza 3' and 'Giza 4' showed the lowest TPC values. In the case of the antioxidant activity, the TEAC values ranged between 159 and 207 µmol of trolox/100 g of chickpea seeds. In accordance with the TPC data, cultivars 'Giza 1' and 'Giza195' (Fig. 5) showed the highest antioxidant activity. Overall, these results are in agreement with previous results, that is the TPC value ranged between 72 (cultivar 'Blanco Sinaloa 92') and 112 (cultivar 'Balksar 2000') mg of gallic acid/100 g[65,66] and the TEAC value between 150 (cultivar 'Dwelly') and 655 (cultivar 'Small brown chana') µmol of trolox/100 g.[67,68] Caffeic acid (positive control) showed a TEAC value of 1.29 ± 0.02 µmol of trolox/µmol of the compound, in accordance with Rice-Evans *et al.*[69]

In general, the chickpea seeds presented slight differences in the qualitative profiles, and thus quantitative differences could also explain those results obtained for TPC and TEAC. In this way, relative amounts of each metabolite class/subclass were estimated as total area obtained by MS (Fig. 6). On the base of this, cultivar 'Giza 1' contained the highest relative amounts of hydroxybenzoic acids, isoflavones, among other flavonoids, justifying the TPC and antioxidant activity results. Alternatively, the antioxidant activity of cultivars 'Giza 195' and 'Giza 3' compared to the other cultivars is more difficult to explain taking into account all results globally. In these cases, other compounds such as aromatic amino acids and dipeptides containing aromatic moieties (Fig. 6) could also participate according to several studies.[70-73]

Experimental

Chemicals and reagents

Methanol, ethyl acetate, diethyl ether, acetone, acetonitrile, glacial acetic acid and hydrochloric acid were purchased from Fisher Chemicals (ThermoFisher, Waltham, MA, USA). Solvents used for extraction and analysis were of analytical and HPLC-MS grade, respectively. Ultrapure water was obtained by a Milli-Q system (Millipore, Bedford, MA, USA). Folin & Ciocalteu's phenol reagent, sodium carbonate, ABTS [2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate)], trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), potassium persulfate, L-tyrosine, citric acid and phenolic standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-tryptophan and L-phenylalanine were purchased from Acros Organics (Morris Plains, NJ, USA) and kaempferide from Extrasynthèse (Genay, France). The degree of purity of the standards was around 95% (w/w).

Samples procurement and extraction procedures

Seeds from the aforementioned Egyptian chickpea cultivars, 'Giza 1', 'Giza 2', 'Giza 3', 'Giza 4', 'Giza 195', 'Giza 531' and 'Solala 104', were kindly provided and identified by Dr. Mostafa Abdel Moamen, Field Crops Research Institute, Agricultural Research Center (Giza, Egypt). Previously to the extraction, the seeds were ground (particle size around 1 mm) with an Ultra Centrifugal Mill ZM 200, Retsch (Haan, Germany). The extraction of phenolic compounds was based on three procedures reported in literature on chickpea or other Fabaceae seeds, with some modifications (see details in supporting information), and named M1,[5] M2[12] and M3.[74] In order to select one of these options, three repetitions of each extraction procedure were performed using 'Giza 1' chickpea seeds. For further analysis of the rest of chickpea seeds, two repetitions were performed for each cultivar.

Total phenol content

The TPC of the chickpea seeds extracts was determined by a colorimetric assay using Folin–Ciocalteu reagent,[75] modified according to Romero-de Soto and co-workers[76] in 96-well polystyrene microplates (ThermoFisher). A Synergy Mx Monochromator-Based Multi-Mode Microplate reader (Bio-Tek Instruments Inc, Winooski, VT, USA) was employed. Each extract was appropriately diluted and assayed at least three times. The absorbance at a wavelength of 760 nm was measured after incubation for 2 hours in dark and compared with a calibration curve of serially diluted gallic acid elaborated in the same manner. The results were expressed as equivalents of gallic acid.

Trolox equivalent antioxidant capacity assay

TEAC absorbance measurements were performed using the aforementioned microplate reader and following the procedure described by Morales-Soto and co-workers.[77] This antioxidant assay is based on the reduction of the radical cation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) by antioxidants. The ABTS radical cation ($ABTS^{+\bullet}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration). The mixture was kept in dark at room temperature for 24 hours. The $ABTS^{+\bullet}$ solution was diluted with water till reaching an absorbance value of 0.70 (±0.03) at 734 nm. Afterwards, 300 µL of this solution and 30 µL of the sample were mixed and measured at 734 nm and 25 °C. For this, each extract was appropriately diluted and assayed at least three times. Absorbance readings were compared to a standard calibration curve of trolox. The results were expressed in µmol of trolox equivalents. Caffeic acid was used as a positive control.

Analysis by RP-HPLC-DAD-ESI-QTOF-MS and -MS^2

Analyses were made with an Agilent 1200 series rapid resolution (Santa Clara, CA, USA) equipped with a binary pump, an autosampler and a DAD. Separation was carried out with the analytical column core-shell Halo C18 (150 mm × 4.6 mm, 2.7 µm particle size) or Zorbax Eclipse Plus C18 (150 mm × 4.6 mm, 1.8 µm particle size). The system was coupled to a 6540 Agilent Ultra-High-Definition (UHD) Accurate-Mass Q-TOF LC/MS (Palo Alto, CA, USA) equipped with an ESI interface.

The gradient elution was conducted with two mobile phases, acidified water (0.5% acetic acid, v/v) (phase A) and acetonitrile (phase B), with a constant flow rate of 0.5 mL/min. The gradient program was as follows: 0 min 99% A and 1% B, 5.50 min 93% A and 7% B, 11 min 86% A and 14% B, 17.5 min 76% A and 24% B, 22.50 min 60% A and 40% B, 27.50 min 0% A and 100% B, 28.5 min 0% A and 100% B, 29.5 min initial conditions, which were finally maintained for 5.50 min for column equilibration (total run 35 min). The injection volume was 8 µL and each extract was analyzed twice.

The operating conditions briefly were: drying nitrogen gas temperature 325 °C with a flow of 10 L/min; nebulizer pressure 20 psig; sheath gas temperature 400 °C with a flow 12 L/min; capillary voltage 4000 V; nozzle voltage 500 V; fragmentor voltage 130 V; skimmer voltage 45 V; octapole radiofrequency voltage 750 V. Data acquisition (2.5 Hz) in profile mode was governed via MassHunter Workstation software (Agilent technologies). The spectra were acquired in the negative ionization mode, over a mass-to-charge (*m/z*) range from 70 to 1100. The detection window was set to 100 ppm. Reference mass correction on each sample was performed with a continuous infusion of Agilent TOF biopolymer analysis mixture containing trifluoroacetic acid ammonium salt (*m/z* 112.9856) and hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine (*m/z* 980.0164 corresponding to the acetic adduct).

Data analysis was performed on MassHunter Qualitative Analysis B.06.00 (Agilent technologies). Characterization of compounds was performed by generation of the candidate formula with a mass accuracy limit of 5 ppm, and also considering RT, UV, MS^2 data and literature. The MS score related to the contribution to mass accuracy, isotope abundance and isotope spacing for the generated molecular formula was set at ≥80. For the retrieval of chemical structure information and data from published literature, the following databases were consulted: ChemSpider (http://www.chemspider.com), SciFinder Scholar (https://scifinder.cas.org), Reaxys (http://www.reaxys.com), PubChem (http://pubchem.ncbi.nlm.nih.gov), KNApSAcK Core System (http://kanaya.naist.jp/knapsack_jsp/top.html), MassBank (http://www.massbank.jp), METLIN Metabolite Database (http://metlin.scripps.edu) and Phenol-Explorer (www.phenol-explorer.eu). Confirmation was made through a comparison with standards, whenever these were available in-house.

Statistical analysis

Microsoft Excel 2007 (Redmond, WA, USA) was employed for statistical analysis, with the level of significance set at 95%. One-way analysis of variance (ANOVA), followed by a LSD post-hoc test, was performed with the software IBM SPSS Statistics 22 (Armonk, NY, USA).

Conclusions

The combination of solid-liquid extraction and RP-HPLC-DAD-ESI-QTOF-MS analysis, using a C18 core-shell column (2.7 µm), enabled to perform the comprehensive metabolic profiling of Egyptian cultivars of chickpea. A total of 140 compounds were characterized, including 96 phenolic compounds. Most of them were reported for the first time in this legume, as well as new nine structures were tentatively proposed. Furthermore, qualitative and quantitative differences between the cultivars were found. Among the studied cultivars, 'Giza 1' contained the highest total phenol content as well as relative amounts of phenolic compounds, specially hydroxybenzoic acids and isoflavonoids that may contribute to its antioxidant capacity. Overall, the applied methodology is suitable for the metabolic profiling of leguminous seeds that helps to explain their potential biological activities, as well as the results could be useful in further chemosystematics and quantitative studies.

Acknowledgements

The authors are grateful to the Andalusian Regional Government Council of Innovation and Science for the Excellence Project P11-CTS-7625. R. H. Mekky thanks the Erasmus Mundus – Al Idrisi II programme "scholarship scheme for exchange and cooperation between Europe and North Africa". M. del M. Contreras also thanks the Spanish Ministry of Economy and Competitiveness (MINECO) for the Juan de la Cierva contract. Many thanks are also due to Rania H. Mekky for her support. We also appreciate Prof. J. H. Scarrow (Department of Mineralogy and Petrology/University of Granada) for her English revision of the introduction and writing suggestions.

Conflicts of interest

The authors declare no competing financial interest.

Notes and references

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† Electronic Supplementary Information (ESI) available: [detailed extraction procedures, tables S1 and S2 and figures S1-S4]. See DOI: 10.1039/b000000x/

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Table 1. Phenolic compounds characterized in seven Egyptian cultivars of chickpea: 'Giza 1' (1), 'Giza 2' (2), 'Giza 3' (3), 'Giza 4' (4), 'Giza 195' (5), 'Giza 531' (6) and 'Solala 104' (7).

RT (min)	Exp. *m/z*[a] $[M-H]^-$	Molecular formula	Error (ppm)	Score	Main fragments	UV (nm)	Proposed compound	1	2	3	4	5	6	7
7.65	331.0674	$C_{13}H_{16}O_{10}$	-0.1	90.77	313.0562, 169.0136, 168.0065, 125.0233	230, 256	Gallic acid hexoside I[b]	+	+	+	+	+	+	+
8.11	169.0142	$C_7H_6O_5$	0.7	93.82	125.0241	N.D.	Gallic acid*	+	+	+	+	-	+	+
8.20	329.0886	$C_{14}H_{18}O_9$	-2.0	82.2	167.0316, 122.0367	258	Vanillic acid-4-*O*-β-D-glucopyranoside	+	+	+	+	+	+	+
8.76	331.0673	$C_{13}H_{16}O_{10}$	-0.4	99.18	313.0569, 169.0138, 168.0058, 125.0242	254	Gallic acid hexoside II[b]	+	+	+	+	+	+	+
9.09	299.0777	$C_{13}H_{16}O_8$	-1.4	98.83	137.0245, 93.0346	N.D.	Hydroxybenzoic acid hexoside I[b]	+	+	+	+	+	+	+
9.20	299.0777	$C_{13}H_{16}O_8$	-1.4	98.53	137.0245, 93.0347	248	Hydroxybenzoic acid hexoside II[b]	+	+	+	+	+	+	+
9.34	461.1296	$C_{19}H_{26}O_{13}$	1.7	93.4	417.1397, 285.0992, 123.0459	N.D.	Vanillic acid hexoside pentoside I[c]	+	+	+	+	+	+	+
9.90	315.0733	$C_{13}H_{16}O_9$	-3.5	95.55	153.0195, 152.0117, 109.0299, 108.0217	254, 314	Dihydroxybenzoic acid hexoside I[b]	+	+	+	+	+	+	+
9.93	331.0670	$C_{13}H_{16}O_{10}$	0.3	99.65	313.0576, 169.0148, 168.0074, 125.0250	N.D.	Gallic acid hexoside III[b]	+	+	+	+	+	+	+
9.94	431.1205	$C_{18}H_{24}O_{12}$	-2.1	97.38	137.0244, 93.0349	N.D.	Hydroxybenzoic acid hexoside pentoside I[b]	+	+	+	+	+	+	+
10.09	315.0721	$C_{13}H_{16}O_9$	0.4	99.17	153.0183, 152.0119, 109.0119, 108.0219	236, 314	Dihydroxybenzoic acid hexoside II[b]	+	+	+	+	+	+	+
10.19	461.1298	$C_{19}H_{26}O_{13}$	1.1	97.4	315.0782, 153.0227	N.D.	**Dihydroxybenzoic acid hexoside deoxyhexoside**	+	+	+	+	+	+	+
10.39	315.0726	$C_{13}H_{16}O_9$	-1.2	98.35	153.0195, 152.0116, 109.0297, 108.0220	240, 314	Dihydroxybenzoic acid hexoside III[b]	+	+	+	+	+	+	+
10.53	431.1204	$C_{18}H_{24}O_{12}$	-1.8	97.95	299.0795, 137.0250, 93.0353	252	Hydroxybenzoic acid hexoside pentoside II[b]	+	+	+	+	+	+	+
11.01	431.1204	$C_{18}H_{24}O_{12}$	-2.0	97.08	299.0892, 137.0311, 93.0399	250	Hydroxybenzoic acid hexoside pentoside III[b]	+	+	+	+	+	+	+
11.49	461.1295	$C_{19}H_{26}O_{13}$	1.3	99.15	329.0879, 167.0347, 152.0111	254, 292	Vanillic acid hexoside pentoside II[c]	+	+	+	+	+	+	+
11.65	315.0728	$C_{13}H_{16}O_9$	-2.0	97.51	153.0200, 109.0299	238, 307	Dihydroxybenzoic acid hexoside IV[b]	+	+	+	+	+	+	+
12.13	447.1147	$C_{18}H_{24}O_{13}$	-0.5	99.64	315.0729, 153.0195, 152.0119, 109.0296, 108.0221	257, 305	**Dihydroxybenzoic acid hexoside pentoside I**	+	+	+	+	+	+	+
12.35	447.1143	$C_{18}H_{24}O_{13}$	0.4	99.15	315.0723, 153.0186, 152.0113, 109.0289, 108.0215	231, 316	**Dihydroxybenzoic acid hexoside pentoside II**	+	+	+	+	+	+	+
13.02	353.0876	$C_{16}H_{18}O_9$	0.9	99.06	191.0563, 179.0354, 135.0454	230, 288, 330sh	Caffeoylquinic acid I	-	+	-	+	-	+	+
13.07	325.0926	$C_{15}H_{18}O_8$	0.0	91	163.0401, 119.0501	N.D.	*p*-coumaric acid glucopyranoside	+	+	+	+	+	+	+
13.34	285.0616	$C_{12}H_{14}O_8$	0.0	98.84	153.0182, 152.0114, 109.0927, 108.0212	268	Dihydroxybenzoic acid pentoside[b]	+	+	+	+	+	+	+
13.64	609.1466	$C_{27}H_{30}O_{16}$	-0.6	97.84	447.0943, 285.0412, 284.0325, 151.0031	243, 314, 342	Kaempferol 3,7-*O*-β-D-diglucopyranoside	+	+	+	+	+	+	+
14.39	355.1043	$C_{16}H_{20}O_9$	-2.0	82.29	193.0509, 149.0607	232, 291, 314	Ferulic acid hexoside I[b]	+	+	+	+	+	+	+
15.03	353.0877	$C_{16}H_{18}O_9$	0.4	98.46	191.0562, 179.0344, 173.0453, 135.0449	N.D.	Caffeoylquinic acid II[b]*	+	+	+	+	+	+	+
15.14	385.1146	$C_{17}H_{22}O_{10}$	-1.5	90.56	223.0614, 208.0376, 191.0199, 179.0138,	258	Sinapic acid hexoside I	+	+	+	+	+	+	+
15.32	353.0876	$C_{16}H_{18}O_9$	0.6	99.11	191.0542, 173.0441, 179.0325, 161.0231, 135.0444	245, 290, 325	Caffeoylquinic acid III	+	+	+	+	+	+	+
15.42	153.0191	$C_7H_6O_4$	1.2	99.51	109.0297	248, 322	Dihydroxybenzoic acid I	+	+	+	+	+	+	+
15.72	137.0247	$C_7H_6O_3$	-1.6	99.44		256	*p*-hydroxybenzoic acid*	+	+	+	+	+	+	+

15.79	401.0721	$C_{16}H_{18}O_{12}$	1.4	98.33	357.0842, 315.0723, 153.0194, 152.0115, 109.0297, 108.0217	230, 279	**Dihydroxybenzoic acid malonyl hexoside I**	+	+	+	+	+	+	+
15.90	401.0740	$C_{16}H_{18}O_{12}$	-0.3	97.31	357.0827, 315.0730, 153.0204, 152.0125, 109.0309, 108.0230	230, 280	**Dihydroxybenzoic acid malonyl hexoside II**	+	+	+	+	+	+	+
16.03	289.0718	$C_{15}H_{14}O_6$	0.1	97.94	245.0820, 221.0819, 203.0713, 151.0401, 123.0453, 109.0297	230, 279	(+)-Catechin*	+	+	+	+	+	-	+
16.05	353.0881	$C_{16}H_{18}O_9$	-0.8	99.19	191.0567, 179.0353, 135.0456	250, 292, 326	Caffeoylquinic acid IV	-	+	+	+	+	-	+
16.40	827.1894	$C_{35}H_{40}O_{23}$	-0.4	99.03	783.2006, 621.1472, 447.0898, 285.0389, 284.0333, 151.0035	265, 353	**Kaempferol malonyl dihexoside pentoside I**	+	+	+	+	+	+	+
16.60	609.1461	$C_{27}H_{30}O_{16}$	0.6	95.74	447.0924, 285.0410, 283.0252, 151.0035	259, 324, 342	Kaempferol 3-*O*-β-D-diglucopyranoside	+	+	+	+	+	+	+
16.84	355.1038	$C_{16}H_{20}O_9$	-1.8	90.93	193.0517	N.D.	Ferulic acid hexoside II[b]	+	+	+	+	+	+	+
17.20	167.0353	$C_8H_8O_4$	-2.0	98.88	152.0115, 122.0373, 108.0217	230, 260, 296	Vanillic acid*	+	+	+	+	+	+	+
17.28	445.1360	$C_{19}H_{26}O_{12}$	-1.5	98.04	151.0407, 137.0218, 136.0173	255	Methoxy hydroxybenzoic acid hexoside pentoside[c]	+	+	+	+	+	+	+
17.43	385.1138	$C_{17}H_{22}O_{10}$	0.7	98.4	223.0616, 208.0375, 191.0198, 179.0139	256	Sinapic acid hexoside II	+	+	+	+	+	+	+
17.46	353.0878	$C_{16}H_{18}O_9$	-0.4	97.55	191.0567, 179.0355, 135.0454	252, 294, 325	Caffeoylquinic acid V	+	+	+	+	+	+	+
17.53	153.0191	$C_7H_6O_4$	1.4	99.54	109.0289	252	Dihydroxybenzoic acid II	+	+	+	+	+	+	+
17.57	827.1887	$C_{35}H_{40}O_{23}$	0.4	98.96	783.2003, 621.1499, 447.0977, 285.0419, 284.0337, 151.0025	348	**Kaempferol malonyl dihexoside pentoside II**	+	+	+	+	+	+	+
17.71	695.1478	$C_{30}H_{32}O_{19}$	-1.7	97.69	651.1556, 489.1038, 447.0923, 446.0851, 285.0409, 151.0023, 131.0714	266, 349	Kaempferol malonyl dihexoside I[c]	+	+	+	+	+	+	+
17.94	741.1878	$C_{32}H_{38}O_{20}$	1.1	98.66	579.1342, 447.0913, 285.0399, 284.0323, 179.0149	348	Kaempferol 3-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside-4'-*O*-β-D-glucopyranoside	+	+	+	+	+	+	+
18.06	289.0717	$C_{15}H_{14}O_6$	-1.3	90.86	245.0815, 221.0818, 203.0712, 151.0398, 123.0450, 109.0295	230, 278	(-)-Epicatechin*	+	+	+	-	+	-	-
18.17	727.2097	$C_{32}H_{40}O_{19}$	-0.8	99.23	565.1451, 445.1034, 433.1029, 271.0577, 151.0039, 145.0297	N.D.	Naringenin dihexoside pentoside	+	+	+	+	+	+	+
18.42	755.2038	$C_{33}H_{40}O_{20}$	0.5	96.63	609.1455, 301.0342, 300.0276, 151.0028	255, 358	Quercetin -3-*O*-rutinoside-7-*O*-α-L-rhamnopyranoside	-	+	+	+	+	+	+
18.54	449.1089	$C_{21}H_{22}O_{11}$	0.2	96.96	287.0563, 269.0450, 259.0609, 153.0183, 151.0030	N.D.	Aromadendrin-3-*O*-β-*D*-glucopyranoside	+	+	+	+	+	+	+
18.64	609.1466	$C_{27}H_{30}O_{16}$	-0.5	99.34	447.0942, 446.0869, 285.0416, 283.0260, 255.0309, 151.0047	264, 346	Kaempferol-3,4'-*O*-β-D-diglucopyranoside	+	+	+	+	+	+	+
18.85	755.2048	$C_{33}H_{40}O_{20}$	-0.8	98.27	593.1517, 431.1945, 285.0409, 284.0331, 151.0029	266, 347	Kaempferol-3-*O*-rutinoside-7-*O*-β-D-glucopyranoside	+	+	+	+	+	+	+
19.03	625.1413	$C_{27}H_{30}O_{17}$	0.0	98.42	463.0909, 445.0781, 301.0352, 300.0284, 151.0037	254, 368	Quercetin-3,7-*O*-di-glucopyranoside	+	+	+	+	+	-	-
19.08	741.1880	$C_{32}H_{38}O_{20}$	0.4	99.12	609.1450, 301.0304, 300.0279, 178.9986	256, 356	Quercetin-3-*O*-β-D-xylopyranosyl-(1→2)-rutinoside	+	+	+	+	+	-	+
19.40	771.1987	$C_{33}H_{40}O_{21}$	0.6	98.73	756.1761, 639.1565, 331.0458, 330.0375, 316.0224, 315.0149, 178.9981, 151.0033	248, 349sh, 366	**Myricetin-*O*-methyl ether hexoside deoxyhexoside pentoside**	+	+	+	+	+	+	+
19.69	695.1475	$C_{30}H_{32}O_{19}$	-1.5	95.34	651.1729, 489.1049, 447.0937, 285.0411, 221.0250, 151.0031	266, 343	Kaempferol malonyl dihexoside II[c]	+	+	+	+	+	+	+
20.09	725.1935	$C_{32}H_{38}O_{19}$	-0.1	98.84	593.1526, 431.1001, 285.0415, 284.0337, 178.9994,	262, 347	Kaempferol 3-*O*-lathyroside-7-*O*-α-L-	+	+	+	+	+	+	+

					151.0042		rhamnopyranoside									
20.30	595.1664	$C_{27}H_{32}O_{15}$	-0.6	99.36	433.0999, 287.0559, 151.0037, 135.0451	N.D.	Aromadendrin 7-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranoside	+	+	-	-	-	-	-
20.38	609.1466	$C_{27}H_{30}O_{16}$	-0.7	98.89	301.0355, 300.0287, 151.0054	256, 356	Rutin* [quercetin 3-*O*-rutinoside]	+	+	+	+	+	+	+
20.43	755.2045	$C_{33}H_{40}O_{20}$	-0.8	98.56	623.1618, 461.1259, 315.0510, 300.0275, 178.9991, 151.0086	256, 368	Isorhamnetin 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside	+	+	+	+	+	+	+
20.51	639.1567	$C_{28}H_{32}O_{17}$	-0.1	95.92	331.0459, 316.0216	256, 368	Myricetin-*O*-methyl ether hexoside deoxyhexoside	+	+	+	+	+	+	+
20.70	593.1516	$C_{27}H_{30}O_{15}$	-0.9	98.84	447.0922, 285.0387, 284.0318, 255.0288, 151.0030	264, 348	Kaempferol-3-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranside	+	+	+	+	+	+	+
20.74	163.0403	$C_9H_8O_3$	-1.1	99.4	119.0505, 101.0384	310	*p*-coumaric acid*	+	+	+	+	+	+	+
20.76	695.1460	$C_{30}H_{32}O_{19}$	0.8	99.16	651.1576, 609.1458, 301.0347, 300.0248, 151.0024	N.D.	Quercetin 3-*O*-(6"-malonylneohesperidoside)	-	+	+	+	-	-	-
21.14	533.1665	$C_{26}H_{30}O_{12}$	-0.6	92.89	431.1873, 389.2187, 371.2075	N.D.	Dalpanin I	+	+	+	+	+	+	+
21.24	463.0880	$C_{21}H_{20}O_{12}$	0.3	99	301.0361, 300.0275, 271.0246, 255.0295, 151.0032	256, 357	Quercetin 3-*O*-β-D-glucopyranoside*	+	+	+	+	+	+	+
21.24	579.1352	$C_{26}H_{28}O_{15}$	0.6	99.43	447.0949, 285.0402, 284.0329, 255.0301, 151.0193	264, 348	Kaempferol 3-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside	+	+	+	+	+	+	+
21.37	477.0675	$C_{21}H_{18}O_{13}$	-0.4	98.3	301.0359, 151.0037	N.D.	Quercetin-3-*O*-β-D-glucopyranuronic acid	+	+	+	+	+	-	-
21.40	223.0615	$C_{11}H_{12}O_5$	-1.3	98.56	208.0378, 193.0141, 179.0146, 164.0483	254, 318	Sinapic acid*	+	+	+	+	+	+	+
21.69	593.1516	$C_{27}H_{30}O_{15}$	-0.8	99.22	447.0920, 285.0405, 255.0295, 151.0036	266, 345	Kaempferol 3-*O*-rutinose*	+	+	+	+	+	+	+
21.71	193.0508	$C_{10}H_{10}O_4$	1.0	96.7	134.0385	230, 286sh, 316	Ferulic acid*	+	+	+	+	+	+	+
21.79	533.1670	$C_{26}H_{30}O_{12}$	-2.1	92.35	473.1567, 431.1873, 389.1829, 371.1144, 353.1062, 341.1048, 326.0762, 206.0459, 121.0300	N.D.	Dalpanin II	+	+	+	+	+	+	+
22.27	563.1410	$C_{26}H_{28}O_{14}$	-0.2	97.97	431.0978, 269.0453, 175.0756	254, 323	Genistein 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside	+	+	+	+	+	+	+
22.31	665.1351	$C_{29}H_{30}O_{18}$	1.6	98.09	621.1460, 489.1050, 327.0509, 285.0416, 284.0342, 255.0298, 151.0059	266, 348	Kaempferol-3-*O*-[6"-malonyl-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside]	+	+	+	+	+	+	+
22.52	121.0297	$C_7H_6O_2$	-1.7	99.61	77.0394	232, 284	Benzoic acid	+	+	+	+	+	+	+
22.55	565.1562	$C_{26}H_{30}O_{14}$	-0.1	98.07	445.1031, 433.1148, 271.0618, 151.0033, 145.0291	N.D.	Naringenin hexoside pentoside I[b]	+	+	+	+	+	+	+
22.59	447.0935	$C_{21}H_{20}O_{11}$	-0.3	99.81	327.0521, 285.0406, 284.0331, 255.0299, 227.0352, 151.0033	264, 348	Kaempferol 3-*O*-β-D-glucopyarnoside*	+	+	+	+	+	+	+
22.70	477.1038	$C_{22}H_{22}O_{12}$	-0.2	97.23	315.0503, 314.0432, 300.0263, 299.0199, 285.0401, 271.0240, 179.0473, 151.0025	262, 356	Isorhamentin 3-*O*-β-D-glucopyranoside	+	+	+	+	+	+	+
22.80	431.0984	$C_{21}H_{20}O_{10}$	0.0	98.76	269.045, 268.0378, 239.0345, 224.0475, 135.0215, 132.0215	257, 327	Genistin [genistein-7-*O*-β-D-glucopyranoside]	+	+	+	+	+	+	+
22.89	565.1573	$C_{26}H_{30}O_{14}$	-1.6	96.69	433.1106, 271.0612, 151.0041	N.D.	Naringenin hexoside pentoside II[b]	+	+	+	+	+	+	+
23.45	433.1144	$C_{21}H_{22}O_{10}$	-1.2	98.69	271.0641, 151.0035, 119.0491	N.D.	Prunin [naringenin 7-*O*-β-D-glucopyranoside]	+	+	+	+	+	+	+
23.62	533.0940	$C_{24}H_{22}O_{14}$	-0.4	99.35	489.1054, 447.1205, 285.0407, 284.0326, 255.0285, 151.0026	260, 348	Kaempferol-3-*O*-(6"-malonyl-)-β-D-glucopyranoside	+	+	+	+	+	+	+

24.14	461.1087	$C_{22}H_{22}O_{11}$	-0.2	96.64	313.0566, 299.0558, 284.0321, 169.0144, 151.0031, 147.0457, 107.0139, 103.0555	244, 310	Pratensein 7-*O*-β-D-glucopyranoside	+	+	+	+	+	+	+
24.52	269.0457	$C_{15}H_{10}O_5$	-1.2	98.01	251.0437, 241.0481	N.D.	Apigenin*	+	+	+	+	+	+	+
24.75	287.0566	$C_{15}H_{12}O_6$	-2.2	93.56	259.0617, 177.0557, 151.0031, 125.0241	290, 310sh	Dihydrokaempferol [Aromadendrin]	+	+	+	+	+	+	+
25.00	273.0767	$C_{15}H_{14}O_5$	0.1	99.09	167.0353, 151.0395, 137.0241, 123.0445, 121.0297, 109.0298	244, 277	(Epi)afzelechin	+	+	+	-	-	-	-
25.68	253.0506	$C_{15}H_{10}O_4$	-0.1	92.1	242.0477, 225.0575, 209.0591, 197.0600, 135.0088, 133.0295	250, 310	Daidzein	+	+	+	-	+	+	+
26.02	269.0459	$C_{15}H_{10}O_5$	-0.9	96.13	159.0452, 133.0294, 119.0501, 107.0139	N.D.	Genistein*	+	-	-	-	-	-	-
26.31	285.0406	$C_{15}H_{10}O_6$	-0.3	99.88	249.1047, 217.0515, 151.0034, 133.0259, 107.0137	278, 313	Orobol	+	+	+	+	+	+	+
26.39	445.1151	$C_{22}H_{22}O_{10}$	-3.2	93.35	283.0618, 268.0385, 248.9730, 217.0036, 132.0202	256, 325	Biochanin A 7-*O*-β-D-glucopyranoside	+	+	+	+	+	+	+
26.67	283.0615	$C_{16}H_{12}O_5$	-1.4	82.07	268.0381, 250.0318, 239.0341, 164.2169, 151.0032, 132.0221, 115.0774, 107.0128	250, 311	Methyl isoflavone isomer I[d]	+	+	+	+	+	+	+
27.31	283.0610	$C_{16}H_{12}O_5$	1.1	98.76	268.0342, 250.0224, 239.0324, 151.0032, 132.0215, 117.0325	258, 303	Methyl isoflavone isomer II[d]	+	+	+	+	+	+	+
27.74	271.0614	$C_{15}H_{12}O_5$	-0.9	95.1	151.0045, 119.0496, 107.0147, 93.0343	N.D.	Naringenin*	+	+	+	-	+	-	+
27.90	285.0405	$C_{15}H_{10}O_6$	0.3	99.09	257.0461, 239.0356, 229.0514, 185.0614, 151.0039, 107.0142, 93.0350	N.D.	Kaempferol*	+	+	-	+	+	+	+
28.02	299.0567	$C_{16}H_{12}O_6$	-2.1	98.52	284.0328, 255.0294, 211.0394, 151.0038, 135.0095	264, 296	Pratensein	+	+	+	+	+	+	+
28.55	267.0651	$C_{16}H_{12}O_4$	-1.1	98.91	252.0430, 251.0341, 223.0403, 132.0281	252, 301	Biochanin B	+	+	+	+	+	+	+
29.60	299.0563	$C_{16}H_{12}O_6$	-1.7	95.17	285.0381, 284.0332, 151.0034, 107.0148	N.D.	Kaempferide*	+	+	+	+	+	+	+
29.66	283.0616	$C_{16}H_{12}O_5$	-1.3	98.47	268.0385, 250.0246, 239.0349, 151.0028, 132.0217, 107.0131	260, 329	Biochanin A	+	+	+	+	+	+	+

RT, retention time; Exp., experimental. N.D., below 5 mAU or masked by compound with higher signal. Compounds in bold letter indicate new proposed structures. Rutinoside, rhamnopyranosyl-(1→6)-β-D-glucopyranoside; lathyroside, xylopyranosyl-(1→2)-galactopyranoside; 6"-malonylneohesperidoside, (2"-*O*-α-L-rhamnopyranosyl-6"-*O*-malonyl)-β-D-glucopyranoside.

*Identification confirmed by comparison with standards.

[a]All detected ions were $[M-H]^-$.

[b]Only the isomer corresponding to chlorogenic acid and 1-*O*-galloyl-β-D-glucopyranoside, *p*-hydroxybenzoic acid 4-*O*-β-D-glucopyranoside, salicylic acid primeveroside, protocatechuic acid hexoside, gentisic acid 5-*O*-β-D-xylopyranoside, ferulic acid-4-*O*-β-D-glucopyranoside and naringenin-7-*O*-(β-D-xylopyranosyl-(1→2))-β-D-glucopyranoside have previously been described in Fabaceae.

[c]Vanillic acid 1-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside] ester, methyl salicylate β-primeveroside and kaempferol 3-*O*-β-D-(6"-malonyl-)glucopyranoside-7-*O*-β-D-glucopyranoside have previously been identified in Apiaceae, Clethraceae and Equisetaceae, respectively.

[d]Glycitein has previously been reported in chickpeas according to[22] while kakkatin, prunetin, isoprunetin, 6-hydroxyformononetin and 8-hydroxyformononetin in Fabaceae according to Reaxys database. The UV data agrees with[12,27,28,41].

Table 2. Non phenolic compounds characterized in seven Egyptian cultivars of chickpea: 'Giza 1' (1), 'Giza 2' (2), 'Giza 3' (3), 'Giza 4' (4), 'Giza 195' (5), 'Giza 531' (6) and 'Solala 104' (7).

RT (min)	Exp. *m/z*[a] $[M-H]^-$	Molecular formula	Error (ppm)	Score	Main fragments	UV (nm)	Proposed compound	1	2	3	4	5	6	7
2.67	195.0510	$C_6H_{12}O_7$	-0.1	99.2	135.0561	N.D.	Gluconic/ galactonic acid	+	+	+	+	+	+	-
2.98	133.0146	$C_4H_6O_5$	-2.8	86.6	115.0084	230, 264	Malic Acid	+	+	+	+	+	+	-
4.31	191.0197	$C_6H_8O_7$	0.4	99.6	173.0087 , 111.0084	230, 259	Isocitric acid	+	+	+	+	+	+	+
4.84	191.0206	$C_6H_8O_7$	-4.4	96.2	173.0095 , 111.0098	238	Citric acid*	+	+	+	+	+	+	+
4.86	167.0213	$C_5H_4N_4O_3$	-1.7	98.2	124.0193, 123.0136, 105.2227	284	Uric acid	+	+	+	+	+	+	+
5.67	130.0876	$C_6H_{13}NO_2$	-1.5	98.9	112.9856	230, 270sh	Leucine/Isoleucine	+	+	+	+	+	+	+
5.71	129.0195	$C_5H_6O_4$	-1.6	97.9	85.0297	230, 256sh	Itaconic acid	+	+	+	+	+	+	+
5.82	117.0193	$C_4H_6O_4$	-0.1	99.7	73.0277	230, 256sh	Succinic acid	+	+	+	+	+	+	+
6.23	180.0668	$C_9H_{11}NO_3$	-1.4	98.8	163.3854, 119.0506	224, 274	Tyrosine*	+	+	+	+	+	+	+
6.42	243.0626	$C_9H_{12}N_2O_6$	-1.4	99.2	200.0570, 152.0362, 140.0350, 110.0245	261	Uridine	+	+	+	+	+	+	+
8.27	266.0895	$C_{10}H_{13}N_5O_4$	0.5	91.9	134.0475	258	Adenosine	+	+	+	+	+	+	+
8.47	282.0852	$C_{10}H_{13}N_5O_5$	-2.0	93.3	150.042, 133.061	254	Guanosine	+	+	+	+	+	+	+
9.26	164.0718	$C_9H_{11}NO_2$	-0.9	99.7	147.0458, 103.0559	254	Phenylalanine*	+	+	+	+	+	+	+
10.64	309.1101	$C_{14}H_{18}N_2O_6$	-2.7	95.87	180.0671, 163.0405, 128.0356, 119.0507	230, 252, 270	Gamma-glutamyl-tyrosine I	+	+	+	+	+	+	+
10.71	218.1033	$C_9H_{17}NO_5$	0.1	99.7	146.0818	N.D.	Pantothenic acid (Vit B5)	+	+	+	+	+	+	+
10.77	309.1101	$C_{14}H_{18}N_2O_6$	-2.7	95.86	180.0656, 163.0392, 128.0350, 119.0501	230, 250, 271	Gamma-glutamyl-tyrosine II	+	+	+	+	+	+	+
11.41	380.1564	$C_{15}H_{27}NO_{10}$	-0.1	98.5	218.1027, 146.0817	255, 293	Pantothenic acid hexoside	+	+	+	+	+	+	+
11.86	382.1002	$C_{14}H_{17}N_5O_8$	0.7	98.8	266.0896, 250.546, 206.0682, 134.0473, 115.0043	256	Succinyladenosine	+	+	+	+	+	+	+
12.74	326.1244	$C_{15}H_{21}NO_7$	0.8	97.9	164.0718, 147.0301	260	Phenylalanine hexoside	+	+	+	+	+	+	+
12.88	203.0834	$C_{11}H_{12}N_2O_2$	-3.6	96.2	159.0932, 142.0672, 116.0507	278	Tryptophan*	+	+	+	+	+	+	+
13.89	443.1925	$C_{21}H_{32}O_{10}$	-0.2	99.5	281.1399, 237.1508, 219.1339, 161.0443	260	Dihydrophaseic acid 4'-*O*-β-D-glucopyranoside	+	+	+	+	+	+	+
14.81	175.0613	$C_7H_{12}O_5$	-0.2	98.3	115.0394,113.0611	N.D.	Isopropylmalic acid	+	+	+	+	+	+	+
15.13	293.1157	$C_{14}H_{18}N_2O_5$	-4.5	93.3	164.0722, 147.0497, 128.0359	258, 285	Gamma-glutamyl-phenylalanine	+	+	+	+	+	+	+
16.08	431.1205	$C_{18}H_{24}O_{12}$	-2.1	97.58	125.0245	256	Licoagroside B[b]	+	+	+	+	+	+	+
16.13	387.1668	$C_{18}H_{28}O_9$	-2.5	91.7	369.1579, 225.1101, 207.1026, 163.1129	N.D.	Tuberonic acid hexoside (hydroxyjasmonic acid hexose) I	+	+	+	+	+	+	+

16.60	387.1667	$C_{18}H_{28}O_9$	-2.0	95.9	369.1581, 225.1114, 207.1019, 163.1152	N.D.	Tuberonic acid hexoside II	+	+	+	+	+	+	+
17.01	519.2085	$C_{23}H_{36}O_{13}$	-0.3	99.0	387.1650, 225.1128, 207.1022, 163.1126	N.D.	**Tuberonic acid hexoside pentoside**	+	+	+	+	+	+	+
17.18	387.1667	$C_{18}H_{28}O_9$	-1.4	82.4	369.1551, 225.1122, 207.1031, 163.1134	N.D.	Tuberonic acid hexoside III	+	+	+	+	+	+	+
17.66	281.1396	$C_{15}H_{22}O_5$	-0.4	99.9	237.1537, 219.1424, 207.1420, 189.1317, 171.1210, 153.0955, 151.0759, 139.0788	N.D.	Dihydrophaseic acid	+	+	+	+	+	+	+
18.97	403.161	$C_{18}H_{28}O_{10}$	-1.1	95.7	241.1083, 225.1134, 179.0146	N.D.	Dihydroxyjasmononic hexoside	+	+	+	+	+	+	+
22.14	245.0931	$C_{13}H_{14}N_2O_3$	0.3	99.0	203.0833, 159.0927, 142.0664	N.D.	Acetyltryptophan	+	+	+	+	+	+	+
23.77	187.0985	$C_9H_{16}O_4$	-1.5	99.4	169.0879, 125.0976	N.D.	Azelaic acid	+	+	+	+	+	+	+
27.04	941.5123	$C_{48}H_{78}O_{18}$	-0.3	98.6	795.4499, 615.3946, 457.3681	196, 202	Soyasaponin I I	+	+	+	+	+	+	+
27.57	1081.5227	$C_{54}H_{82}O_{22}$	-0.4	97.6	935.4680, 917.4524, 755.4041,710.4044, 579.9793	294	Lablab saponin I	+	+	+	+	+	+	+
27.67	1083.5393	$C_{54}H_{84}O_{22}$	-1.3	95.9	1043.5481,983.5138, 895.5151, 595.2939, 571.2937, 447.2656, 279.2351	291	Soyasaponin αg I	+	+	+	+	+	+	+
27.92	941.5123	$C_{48}H_{78}O_{18}$	-0.7	99.0	795.4589, 615.3942, 457.3744	196, 202	Soyasaponin I II	+	+	+	+	+	+	+
28.03	911.5022	$C_{47}H_{76}O_{17}$	-1.2	98.7	893.4905, 615.3876, 457.3690	196, 202	Soyasaponin II [Astargaloside VIII]	+	+	+	+	+	+	+
28.04	925.5162	$C_{48}H_{78}O_{17}$	1.1	97.3	779.4625, 617.4048, 599.3931, 441.3698	196, 198	Kaikasaponin III[c]	+	+	+	+	+	+	+
28.16	925.5161	$C_{48}H_{78}O_{17}$	0.7	98.5	779.4499, 599.3927, 441.3687	196, 198	Kaikasaponin II[c]	+	+	+	+	+	+	+
28.36	939.4966	$C_{48}H_{76}O_{18}$	-0.8	99.2	793.4408, 613.3761, 455.3524	196, 198	Dehydrosoyasaponin I	+	+	+	+	+	+	+
28.19	1083.5388	$C_{54}H_{84}O_{22}$	0.3	99.6	1043.5426, 983.5120, 921.1329, 895.5089, 595.2888, 571.2888, 447.2521, 279.2332	N.D.	Soyasaponin αg II	+	+	+	+	+	+	+
28.27	1067.5427	$C_{54}H_{84}O_{21}$	1.0	97.6	1049.5325, 879.5080, 733.0341, 205.0719, 143.0358, 125.0259	296	Soyasaponin βg I	+	+	+	+	+	+	+
28.64	1083.5366	$C_{54}H_{84}O_{22}$	1.5	97.9	897.5132, 895.4976, 595.2823, 571.2828, 447.2482, 279.2322	290	Soyasaponin αg III	+	+	+	+	+	+	+
28.84	1067.5436	$C_{54}H_{84}O_{21}$	0.2	97.4	1049.5301, 879.5108, 733.4540, 205.0741, 143.0376, 125.0270	295	Soyasaponin βg II	+	+	+	+	+	+	+

RT, retention time; Exp., experimental. N.D., below 5 mAU or masked by compound with higher signal. Compounds in bold letter indicate new proposed structures.

*Identification confirmed by comparison with standards.

[a]All detected ions were $[M-H]^-$.

[b]Maltol 3-*O*-[6-*O*-(3-hydroxy-3-methyl-glutaroyl)]-β-D-glucopyranoside

[c]The characterization is based on the elution pattern in similar conditions.[64]

The UV data agrees with[42,57,58,63].

Figures captions

Fig. 1. Total phenol content (TPC) (mg of gallic acid/100 g of chickpea seeds) and yield (%) of extracts of chickpea seeds from cultivar 'Giza 1' obtained by three different solid-liquid extraction procedures (M1, M2 and M3, according to experimental section). Data are given as mean ± standard deviation.

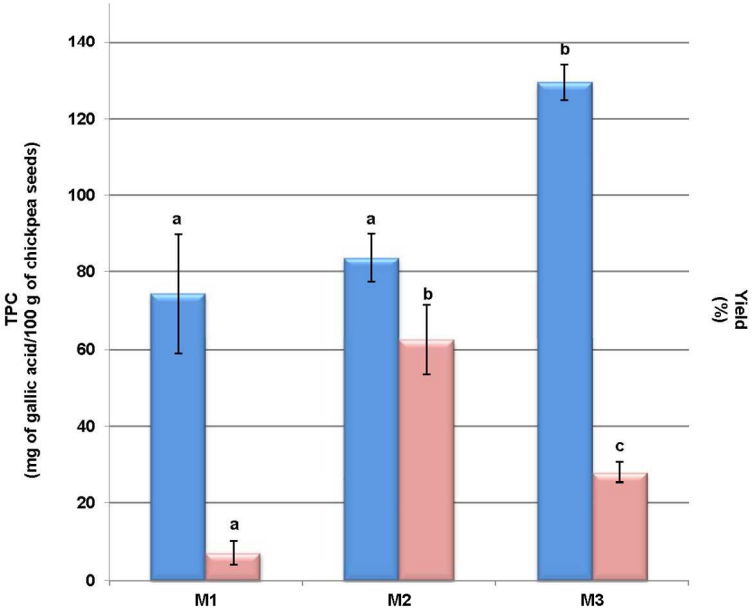
Fig. 2. Base peak chromatogram (BPC) and UV chromatogram at 280 nm of chickpea extracts from 'Giza 1' cultivar obtained by three different extraction methods (M1, M2 and M3, according to experimental section). Bar chart represents the total area from the characteristic chromatograms obtained at UV 280, 240, 320, 330 and 350 nm and BPC. The most critical areas are highlighted.

Fig. 3. Base peak chromatograms of the seven studied Egyptian chickpea cultivars.

Fig. 4. Fragmentation pattern of a) dihydroxybenzoic acid malonyl hexoside (isomer II) and b) kaempferol malonyl dihexoside pentoside (isomer I).

Fig. 5. Total phenol content (TPC) (mg of gallic acid/100 g of chickpea seeds) and antioxidant activity determined by the trolox equivalent antioxidant capacity (TEAC) assay (μmol of trolox/100 g of chickpea seeds) of the seven studied Egyptian chickpea cultivars. Data are given as mean ± standard deviation.

Fig. 6. Relative amounts expressed as total area of each phenolic subclass and other compound classes in Egyptian chickpea cultivars. The bar for other compounds includes nucleosides, dihydrophaseic acid derivatives and a maltol.



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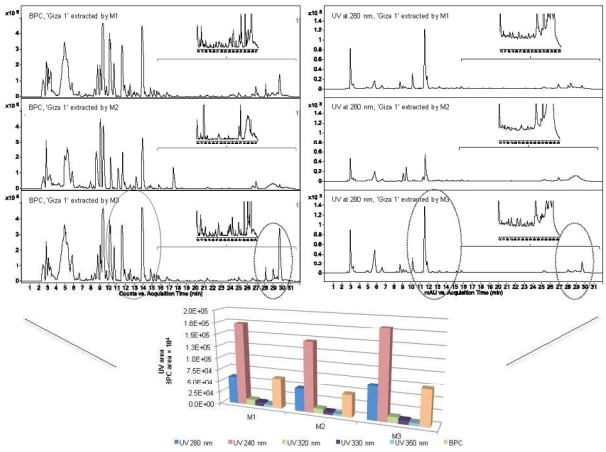

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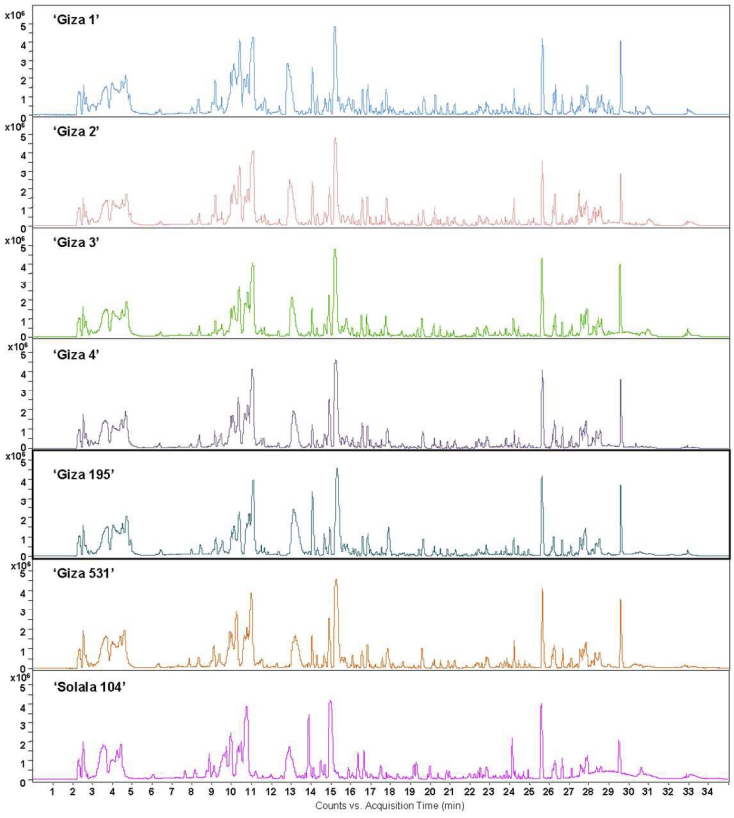

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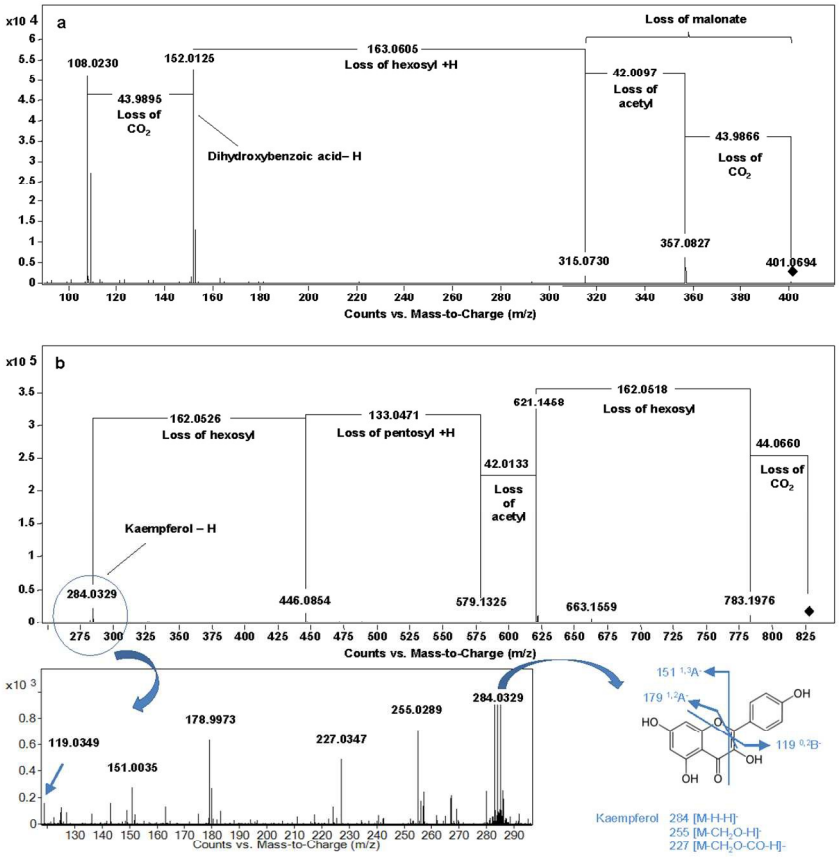

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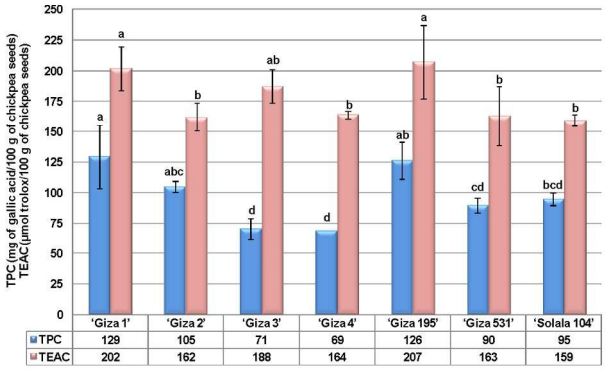

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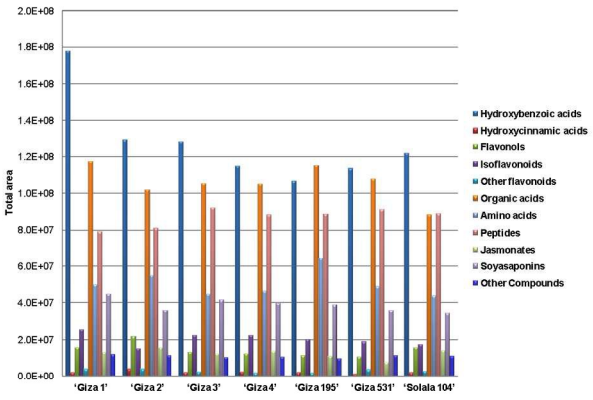

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