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Metabolomic Profiling and Antioxidant Activity of *Trigonella foenum-graecum* and *Trigonella hamosa*

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THE METABOLOMIC profiling of *Trigonella foenum-graecum* and *Trigonella hamosa* seeds was performed using NMR spectroscopy combined with multivariate data analysis (MVDA). The database of the chemical shifts and the *J*-resolved two-dimensional NMR technique were used to identify the metabolites. Additionally, the identified metabolites were confirmed by comparison with previously identified compounds as well. Among the elucidated metabolites, choline, sterols, leucine, valine, flavonoids derivatives and trigonelline were found to contribute to distinguish between the two species of *Trigonella* in score scatter and loading plots of multivariate data analysis. Phytochemical analysis of plant seeds extracts for total carbohydrates, proteins, phenolics, flavonoids, saponins and anthocyanins was performed by spectrophotometer. The antioxidant potential of both species was evaluated by phosphomolybdenum method and DPPH radical scavenging activity. *T. foenum-graecum* was characterized by higher concentrations of trigonelline, sterols, protein, flavonoids derivatives, saponins and anthocyanins, and lower IC_{50} in DPPH radical scavenging activity assay than *T. hamosa*. It was observed that total antioxidant capacity was positively correlated with glucose, trigonelline, protein, flavonoids, saponins and anthocyanins. *T. foenum-graecum* revealed an interesting metabolomic pattern with antioxidant activity that assigns this plant as a promising candidate to explore its targeted and non-targeted metabolomics profile along with potential bioactivities.

Keywords: Antioxidant activity, Flavonoids, NMR spectroscopy, PLS-DA, Trigonelline.

Introduction

Metabolites are defined as compounds produced by plants to perform principal functions, such as growth and development (primary metabolites), and specialized functions, such as defense against most of the attackers herbivores and pathogens (secondary metabolites) (Hartmann, 2007; Fernie & Pichersky, 2015; Pagare et al., 2015; Erb & Kliebenstein, 2020). It is impossible for any analysis to reflect the whole metabolome of a specific tissue, due to its great chemical diversity (Weckwerth, 2003; Kopka et al., 2004; Lu et al., 2017).

The genus *Trigonella* belongs to Fabaceae family and includes eleven annual herbaceous species in the Egyptian flora (Täckholm, 1974; Boulos, 1999). Among these is *Trigonella hamosa* an annual herb that widely distributed in Egypt as a weed (Boulos, 1999). A few researches have been carried out on the phytochemistry of *T. hamosa*. Steroidal saponins were isolated from the Egyptian *T. hamosa* seeds (Hamed, 2007) and showed promising biological activities against diabetes, glucose homeostasis and lipid profiles (Salah et al., 2007). Moreover, saponins from *T. hamosa* seeds alleviated the detrimental effects of chronically diabetes (Salah et al., 2007; Kumar et al., 2011).

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In several countries including Egypt, India, and Pakistan, *Trigonella foenum-graecum* leaves are consumed as a vegetable meal (Sharma, 1986; Rajagopalan, 2001). Furthermore, the *T. foenum-graecum* seeds are also used to supplement maize and wheat flour for bread baking (Al-Habori & Raman, 2002).

Trigonella seeds are rich with valuable medicinally active compounds such as fatty acids, volatile oils, alkaloids, flavonoids, saponins, and polysaccharides (Nandagopal et al., 2012). More than one hundred and eighty metabolites were identified from *Trigonella* seeds (*T. caerulea, T. corniculata* and *T. foenum-graecum*) using UPLC-MS and GC/MS (Farag et al., 2016). Most of these metabolites belong to the groups of peptides, phenolic acids, flavonoids, saponins, sugars, free fatty acids, fatty acyl esters, organic acids and amino acids (Farag et al., 2016). Using different extraction methods, many polyphenols were identified from *T. foenum-graecum* seeds growing in Algeria by using HPLC. The identified polyphenols included kaempferol, genistein, myrecitin, rutin and vanillin (Benziane et al., 2019).

T. foenum-graecum seeds contain approximately 4-8% saponins and about 1% alkaloids (Gupta et al., 1986). Phytochemical analysis of *T. foenum-graecum* seeds revealed the presence of different steroidal saponins, two of them were identified as furostanol saponins (Gupta et al., 1986). Another new six furostanol known as trigoneosides were identified in the Egyptian *T. foenum-graecum* seeds (Murakami et al., 2000). Many major flavonoids glycosides dominated by apigenin, luteolin, chrysoeriol and tricin were identified in *T. coerulescens* and *T. foenum-graecum* and *Medicago* species (Saleh et al., 1982). Triterpenoid saponins and steroids moiety were also detected in *T. esculenta* by NMR spectroscopy (Graziani et al., 2018).

The term metabolome has been used to monitor the metabolites content in a particular organelle, a cell, a tissue, an organ or an organism (Oliver et al., 1998; Ott et al., 2003). Metabolomic is very important in chemical classification of plants. Metabolome of a given plant is changeable and several factors may influence the metabolome (Sampaio et al., 2016). Among these factors cultivar, species (Choi et al., 2005; Abdel-Farid et al., 2007; Ali et al., 2011; Abdel-Farid et al., 2014) and many biotic or abiotic factors may affect plant metabolome (Sampaio et al., 2016; Jahangir et al., 2008a,b; Abdel-Farid et al., 2009). Studying the effect of the most effective factors such as species variability on the metabolome of plants is urgently needed. In this study, metabolomic profiling of *T. foenum-graecum* and *T. hamosa* using NMR spectroscopy coupled with MVDA and spectrophotometer analysis was evaluated. The aim of the study is to differentiate between these two species based on both metabolomic profiling and antioxidant potentiality.

Materials and Methods

Sample collection

T. foenum-graecum seeds were obtained from a traditional local market in Aswan city, while *T. hamosa* seeds were collected from plants growing naturally in Aswan University campus at Aswan city.

Extraction and NMR analysis

The extraction of samples was performed using methanol-d_4 in D_2O (KH_2PO_4 buffer, pH 6.0) containing 0.05% TSP (trimethyl silyl propionic acid sodium salt, w/v) and analysis through a 500MHz Bruker DMX-500 spectrometer (Bruker, Germany) operating at a proton NMR frequency of 500.13MHz (Abdel-Farid et al., 2007; Jahangir et al., 2008a, Kim et al., 2010). Compounds were identified by 1D NMR and 2D-*J*-resolved spectral analysis and the metabolites were confirmed by comparison with the previously reported data as well.

Spectrophotometer analysis

Lowery method was used to determine the content of proteins. A series of different concentrations of proteins was prepared. At 700nm, the absorbance of standard and samples was measured. Concentration of proteins in plant seed materials was determined form the standard curve (Lowery et al., 1951). Carbohydrates were determined according to Morris (1948) using anthrone reagent. A series of different concentrations of glucose was prepared. The developed blue-green color in samples and standard were read at the wavelength of 620nm against a blank containing only water and anthrone reagent. The concentration of carbohydrates in plant seed materials was determined from the constructed standard curve.

Furthermore, the content of anthocyanin was estimated by dissolving plant materials in acidified methanol in dark bottles which were placed in refrigerators for 24hrs. After centrifugation, the absorbance of the supernatant was read at 530 and 657nm (Padmavati et al., 1997).

Determination of phenolics, flavonoids and saponins

Methanol-water extract was used for estimation of total secondary metabolites in seed extracts. Folin-Ciocalteau was used to estimate the content of phenolics (Singleton et al., 1999). The content of total flavonoid was evaluated by using aluminum chloride in presence of quercetin as a standard, as previously reported (Zhishen et al., 1999). Furthermore, the saponins were also determined according to Ebrahimzadeh & Niknam (1998) in presence of acidified vanillin and a purified saponin compound was used as standard.

Determination of total antioxidant activity and DPPH free radical scavenging activity

Total antioxidant activity of aqueous methanol extract was estimated by phosphomolybdenum method using ascorbic acid as a standard based on Prieto et al. (1999). The powder of *T. foenum-graecum* and *T. hamosa* seeds were immersed in 80% methanol and the mixture was filtrated through Whatman filter paper. The filtrate was evaporated under low pressure by rotary evaporator and the resulted crude extract was used for evaluating the DPPH radical scavenging activity. DPPH free radical scavenging activity of the crude extracts of the samples was determined at different concentrations in a spectrophotometer at 517nm based on Blois (1958) using 2,2diphenyl picryl hydrazyl. The inhibition percentage (%) was calculated and finally the IC_{50} of plant extracts was calculated.

Data analysis

AMIX software (v. 3.7, Bruker Biospin) was used to automatically bin the ^{1}H-NMR spectra. Total intensity scaling method was used to scale the spectral intensities. The reduction of spectral intensities was done for integrated regions to have equal width (0.04ppm) of spectral buckets, corresponding to the regions from δ 0.4ppm till δ 10.0 ppm. The omission of signals of residual water and methanol (δ 4.8 – δ 4.9 and δ 3.28 – δ 3.34, respectively) was assured. SIMCA-P software (v. 11.0, Umetrics, Umeå, Sweden) as a Chemometrics tool for principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) of NMR data bucket was used. Additionally, one way analysis of variance (ANOVA) for spectrophotometer data and quantitative intensities of ^{1}H-NMR signals was performed by using Minitab (ver. 12.21). The significant difference between metabolites contents either those identified by NMR or spectrophotometer is evaluated and considered significant at $P< 0.05$. Correlation between the detected metabolites and total antioxidant capacity was performed using Pearson's correlation from Minitab software.

Results

Visualization of 2D J-resolved spectra

Different arrays of primary metabolites including amino and organic acids, carbohydrates, unsaturated fatty acids (USF) and secondary metabolites including flavonoids derivatives, trigonelline and sterols were identified from the seeds of *T. foenum-graecum* and *T. hamosa* using ^{1}H-NMR with the assist of 2D *J*-resolved spectra (Table 1 and Fig. 1A-C). The region of amino and organic acids (δ 0.8 - δ 4.5) is characterized with the signals of aspartic acid, leucine, valine, alanine, and malic acid (Table 1 and Fig. 1A). Signals of aspartic acids were identified as double doublets at δ 2.62 (dd, *J*= 16.0 and 7.5Hz) and δ 2.82 (dd, *J*= 16.0 and 4.6Hz). Signals of leucine were identified as doublets at δ 0.96 (d, *J*= 6.7) and δ 0.98 (d, *J*= 6.7). Signals of valine were identified as doublets at δ 1.00 (d, *J*= 7.0Hz), δ 1.05 (d, *J*= 7.0Hz) and as a multiplet at δ 2.3 (m). Signals of alanine were identified as doublet at δ 1.47 (d, *J*= 7.0Hz) and as a quartet at δ 3.73 (q). Signals of malic acid were identified as double doublets at δ 2.6 (dd, *J*= 16.0 and 7.5Hz), δ 2.80 (dd, *J*= 16.0 and 4.6Hz) and δ 4.36 (dd, *J*= 7.2 and 4.1Hz) (Fig. 1A-B).

The region of carbohydrates (δ 4.1 – δ 5.4) showed 2D *J*-resolved of the anomeric proton of fructose of sucrose, β-glucose, α-glucose and sucrose at δ 4.17 (d, *J*= 7.7Hz), δ 4.58 (d, *J*= 7.7Hz), δ 5.13 (d, *J*= 3.7Hz) and δ 5.4 (d, *J*= 3.7Hz), respectively (Table 1 and Fig. 1B). Unsaturated fatty acids were identified at δ 0.95 as triplet, δ 1.3 and δ 5.35 as broad singlet (Table 1 and Fig. 1A - B).

TABLE 1. ^{1}H chemical shifts (δ in ppm) and coupling constants (*J* in Hz) of some metabolites detected on ^{1}H-NMR and *J*-resolved spectra of *Trigonella foenum- graecum* and *T. hamosa*

Compound	Chemical shifts and coupling constants
Amino/ organic acids	
Aspartic acid	δ 2.62 (dd, *J* = 16.0, 7.5 Hz), δ 2.82 (dd, *J* = 16.0, 4.6 Hz)
Leucine	δ 0.96 (d, *J* = 6.7), δ 0.98 (d, *J* = 6.7)
Valine	δ 1.00 (d, *J* = 7.0 Hz), δ 1.05 (d, *J* = 7.0 Hz), δ 2.3 (m)
Alanine	δ 1.47 (d, *J* = 7.0Hz), δ 3.73 (q)
Malic acid	δ 2.6 (dd, *J* = 16.0, 7.5 Hz), δ 2.8 (dd, *J* = 16.0, 4.6 Hz), 4.32 (dd, 7.2, 4.1 Hz)
Sugar	
Fructose of sucrose	δ 4.17 (d, *J* = 7.7 Hz)
Sucrose	δ 5.4 (d, *J* = 3.7 Hz)
β-glucose	δ 4.57 (d, *J* = 7.7 Hz)
α-glucose	δ 5.13 (d, *J* = 3.7 Hz)
Other compounds	
Trigonelline	δ 4.46 (s), δ 8.1 (dd, *J* = 8.0, 1.6 Hz), δ 8.85 (d, *J* = 6.2), δ 9.12 (s)
Sterols	δ 0.81 (s), δ 0.84 (s)
USF	δ 0.95 (t, *J* = 7.5), δ 1.3 (s), δ 5.35 (s)
Choline	δ 3.22 (s)

s= Singlet, d= Doublet, dd= Double of doublet, t= Triplet, q= Quartet, m= Multiplet, USF= Unsaturated fatty acids.

Despite the low intensity of the signals of secondary metabolites and overlapping of many signals from different compounds, 2D *J*-resolved spectrum was efficient to solve this problem. Using 2D *J*-resolved spectrum with the assistance of previous reports, it was possible to identify very few secondary metabolites such as trigonelline and flavonoids derivatives (mainly flavonols) at the aromatic region (δ 6.0 - δ 9.0) as well as sterols and choline at the aliphatic region (Table 1 and Fig. 1 A-C).

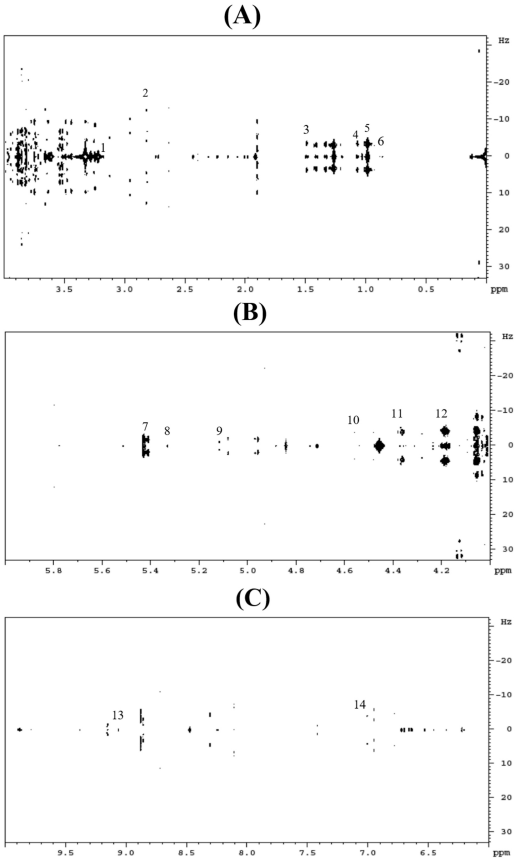

Fig. 1 Two dimensional *J*-resolved spectrum of *T. foenum-graecum* seeds in the range of δ 0 - δ 4 (A), δ 4 - δ 6 (B) and in the range of δ 6 - δ 10 (C) [1: Choline, 2: Aspartic acid, 3: Alanine, 4: Valine, 5: Leucine, 6: Sterols, 7: Sucrose, 8: Unsaturated fatty acids, 9: α-glucose, 10: β-glucose, 11: Malic acid, 12: Fructose, 13: Trigonelline, 14: Flavonoids derivative]

Multivariate data analysis

The graph of score plot of PCA resulted from MVDA shows any grouping among the *Trigonella* species based on their metabolomic profiling. The metabolites responsible for any grouping (spectrophotometer data and NMR chemical shifts) were shown in the corresponding loading plot. The peak areas of ^{1}H-NMR data of *T. foenum-graecum* and *T. hamosa* were subjected to PCA analysis, but as no separation between *T. foenum-graecum* and *T. hamosa* was noticed, further PLS-DA evaluation of this data was conducted (Fig. 2).

The application of supervised analyses like PLS-DA is the next step in multivariate data analysis (MVDA) if no separation has been gotten using PCA. The score plot for component

1 *versus* component 2 of PLS from the NMR data showed that several metabolites contributed to the differentiation between the two species. *T. foenum-graecum* showed higher concentrations of alanine, malic, aspartic acids, α-glucose, sterols and trigonelline than *T. hamosa*, whereas *T. hamosa* showed higher concentrations of valine, leucine, β-glucose, fructose, choline and flavonoids derivatives than *T. foenum-graecum* (Fig. 2). For the confirmation of multivariate data analysis, the ANOVA was applied on NMR dataset. Choline, sterols and trigonelline are among the metabolites those showed significant differences between the two species ($P< 0.05$).

Relative quantification of metabolites

The metabolites of the two species were relatively quantified from the peak areas of the NMR signals. Through ANOVA, it is confirmed that several metabolites are contributing factors to discriminate the two species, with statistically significant difference ($P< 0.05$). The relative quantities of the identified metabolites in both species were shown in Fig. 3. The relative quantity was calculated based on the mean peak intensities of the characterized and identified metabolites signals. *T. foenum-graecum* is dominated by sterols and trigonelline which significantly differ in amounts from those of *T. hamosa* ($P= 0.05$ and 0.006, respectively) whereas *T. hamosa* is dominated by choline which significantly differed from that of *T. foenum-graecum* ($P= 0.038$) (Fig. 3).

Phytochemical analysis of T. foenum-graecum and T. hamosa using spectrophotometer

Total carbohydrates, total proteins, total phenolics, total flavonoids, anthocyanins, saponins and total antioxidant capacity were estimated in *T. foenum- graecum* and *T. hamosa*. All detected metabolites showed higher concentrations in *T. foenum-graecum* than *T. hamosa*. These metabolites showed significant difference between the two species after data subjection to ANOVA (Table 2). The data of the metabolites detected in *T. foenum-graecum* and *T. hamosa* by spectrophotometer were subjected to multivariate data analysis which showed discrimination between the two species (Fig. 4A). PCA revealed that all contents of detected metabolites in *T. foenum graecum* were higher than those of *T. hamosa* (Fig. 4B).

DPPH radical scavenging activity

DPPH free radical scavenging activities of the hydro-methanol extracts of the two species of *Trigonella* were shown in Table 2. The inhibition percentages of DPPH free radical scavenging activity at 50µg/mL were 34.5 and 28.5% in *T. foenum- graecum* and *T. hamosa*, respectively.

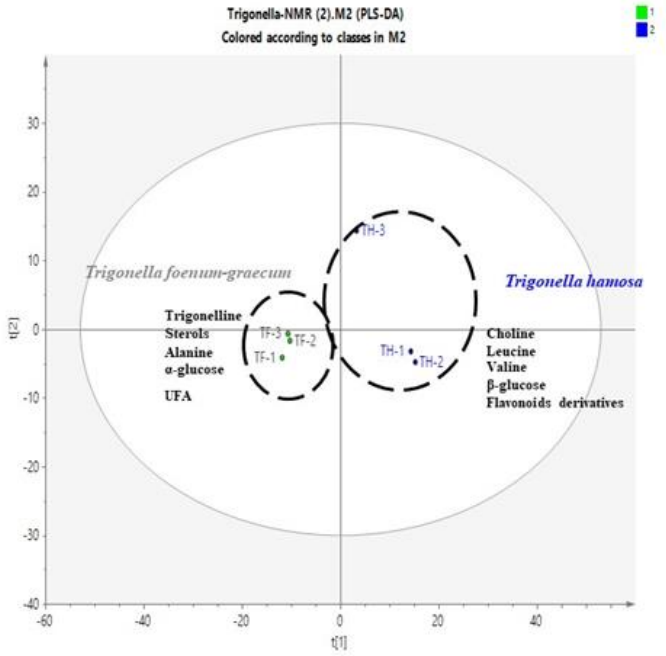

Fig. 2. Score scatter and score loading plot of PLS-DA of NMR spectra of two groups of *Trigonella* species. TF= *Trigonella foenum-graecum*, TH= *T. hamosa*

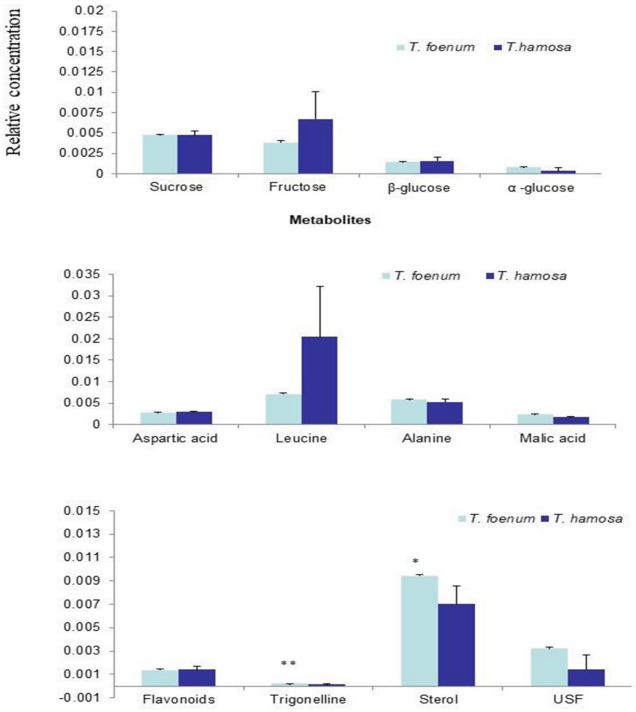

Fig. 3 Relative quantification of some metabolites in *T. foenum-graecum* and *T. hamosa* seeds based on the mean peak area of the associated NMR signals

TABLE 2. Phytochemical analysis, total antioxidant capacity (TAC) and DPPH radical scavenging activities of *T. foenum- graecum* and *T. hamosa*

	Carbohy. mg/g	Proteins mg/g	Phenolics mg gallic acid equivalent / g extract	Flavonoids mg quercetin equivalent /g extract	Saponin mg saponin equivalent /g extract	Anthocyanins µmole/ g extract	TAC mg ascorbic acid equivalent /g extract	% DPPH scavenging at 50µg/ mL
T. foenum-graecum	47.66± 0.28	18.92± 0.94***	2.44± 0.12	12.03± 2.19*	61.9± 0.8***	2.8± 0.16***	0.570± 0.164*	34.5± 3.4*
T. hamosa	47.59± 0.34	7.94± 2.8	2.21± 0.09	8.96± 1.12	53.9± 0.10	UD	0.216± 0.006	28.5± 0.00

* = Significant at P< 0.05, *** = Very highly significant at P< 0.001, UD= Under detectable level.

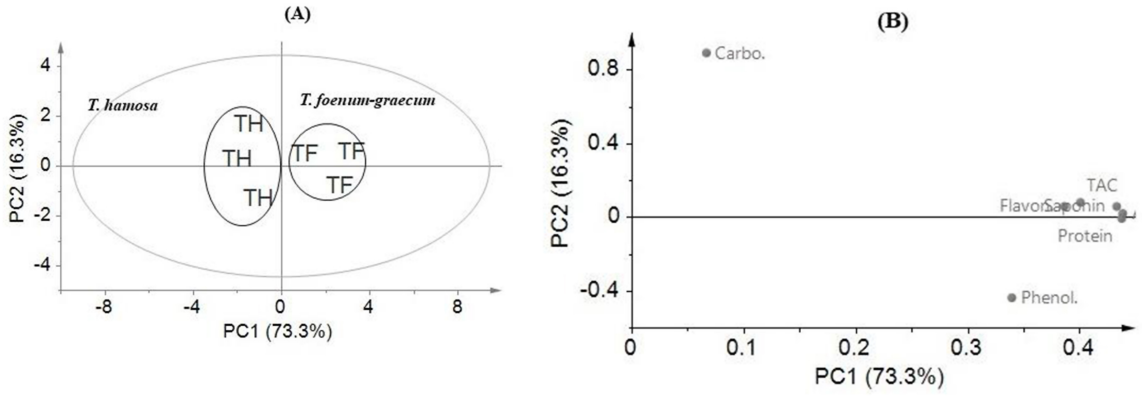

Fig. 4 Score scatter (A) and loading (B) plots of PCA for spectrophotometer data of two groups of *Trigonella* species. TF= *Trigonella foenum-graecum*, TH= *T. hamosa*

The inhibitory concentration (IC_{50}) values for DPPH radical scavenging for *T. foenum-graecum* and *T. hamosa* were 76.5 and 86.5µg/mL, respectively. *T. foenum-graecum* has lower IC_{50} than *T. hamosa* which means that *T. foenum-graecum* has more strong DPPH radical scavenging activity than *T. hamosa*.

Pearson's correlation showed a positive correlation between total antioxidant capacity (TAC) and total flavonoids, total saponin, anthocyanins, total protein, trigonelline and α-glucose and negative correlation with choline (Table 3).

TABLE 3. Correlation coefficient (r) and probability (p) between total antioxidant capacity (TAC) and the determined metabolites by spectrophotometer and NMR spectroscopy

Metabolites	r	P
Flavonoids	0.89	0.017
Saponin	0.82	0.044
Anthocyanin	0.87	0.023
Trigonelline	0.85	0.031
Protein	0.88	0.019
α-glucose	0.85	0.029
Choline	-0.90	0.013

Discussion

The low intensity of secondary metabolites signals as well as the overlapping of many signals from different metabolites is the most common problems which hinder the identification of metabolites in NMR analysis. Despite of these problems, the 2D *J*-resolved spectrum was successfully applied to overcome the signal overlapping (Choi et al., 2006). The use of ^{1}H-NMR and 2D *J*-resolved has significantly demonstrated the potentiality to identify several metabolites of *Trigonella*. Numerous primary and secondary metabolites were identified which included great chemo-diversity, either from primary metabolites such as amino, organic acids and sugars or secondary metabolites such as flavonoids derivatives, alkaloids (trigonelline) and sterols. As compared to other analytical techniques, the NMR spectroscopy has strong ability to detect a diverse range of metabolites in a single run (Choi et al., 2006; Verpoorte et al., 2008; Graziani et al., 2018).

The importance of PCA as a technique of multivariate data analysis is attributed to its role in the reduction of the dimensionality of multivariate datasets. Any groupings among the biological samples could be shown by the score scatter plots. Moreover, the metabolites responsible for grouping could be detected in the score loading plots (Sumner et al., 2003; Ali et al., 2011).

NMR coupled with MVDA has been extensively used as a profiling and fingerprinting technique in metabolomic studies in plants. It has been used effectively for the metabolic fingerprinting and profiling of not only cultivars but also species (Choi et al., 2005; Abdel-Farid et al., 2007; Ali et al., 2011; Abdel-Farid et al., 2014).

Metabolomic characterization using NMR spectroscopy coupled with MVDA of two *Trigonella* species has been performed. Using this technical application, *T. foenum-graecum* not only discriminated from *T. hamosa* but also the differences between the two species regarding their metabolome and determination of the biomarkers compounds in both species were revealed. *T. foenum-graecum* is characterized by high concentrations of some secondary metabolites that have nutritional and medicinal importance. Among these metabolites, sterols which have especially important role in reduction of cholesterol level in humans (Ostlund et al., 2003). Additionally, trigonelline is used as a potential therapeutic compound for diabetics and the diseases related to the central nervous system (Zhou et al., 2012). Trigonelline has beneficial effect for diabetes through up-regulating antioxidant enzyme activity and decreasing lipid peroxidation (Zhou et al., 2013).

Polyphenols including flavonoids, phenolics and anthocyanins are important bioactive compounds that promote human health activities. These groups of compounds were reported as antioxidants, antiproliferative, anti-hyperglycemic and antidiabetic (Alim et al., 2019; Al-Ishaq et al., 2019; Muniyandi et al., 2019). Saponins also are important secondary metabolites that have important biological activity such as antimicrobial, anti-tumor, anti-insect, hepatoprotective, hemolytic and anti-inflammatory activities (Salah et al., 2007; Barbosa, 2014). Profiling of *T. foenum-graecum* and *T. hamosa* for these classes of compounds revealed higher concentrations in *T. foenum-graecum* than in *T. hamosa*. Flavonoids, saponins, anthocyanins and

proteins showed significant differences between the two *Trigonella* species. Generally, there is a correlation between total antioxidant capacity and total phenolics, flavonoids and anthocyanins in different plants (Reyes et al., 2005; Hu¨lya Orak, 2007; Sathishkumar et al., 2008; Hamouz et al., 2011; Basar et al., 2013; Abdel-Farid et al., 2014; Atito et al., 2019; Taha et al., 2020). In our study, *Trigonella species* have high concentrations of proteins, saponins, flavonoids and anthocyanins. Pearson's correlation revealed significant correlation between the content of these metabolites and total antioxidant capacity. *Trigonella species* particularly *T. foenum-graecum* are used as antidiabetic (Kumar et al., 2011) through improving insulin synthesis and removing the oxidative stress (Salah et al., 2007). This activity might be due to the high concentrations of saponins, flavonoids and anthocyanins in *Trigonella* seeds. Generally, TAC and DPPH radical scavenging are highly correlated with the content of total phenolics (Hassas-Roudsari et al., 2009; Dragović-Uzelac et al., 2010; Basar et al., 2013; Atito et al., 2019; Korat Avni et al., 2019; Taha et al., 2020). TAC in *Trigonella species* in this study has no correlation with the content of total phenolics. This may be attributed to the low content of total phenolics in these plants comparing to other secondary metabolites such as anthocyanins, flavonoids and saponins. Anthocyanins showed a positive correlation with TAC which is supported by previous reports in *Solanum tuberosum* genotypes (Reyes et al., 2005) and rice (Sutharut & Sudarat, 2012). Furthermore, TAC was highly correlated with flavonoids content which is in line with many previous reports (Abdel-Farid et al., 2014). Trigonelline exhibited positive correlation with TAC. Votavova et al. (2009) reported a positive correlation between the content of trigonelline and TAC in Robusta coffee. Saponins content was highly correlated with TAC in our study which is in line with some other previous studies (Xi et al., 2008; Hoa et al., 2013).

Conclusions

NMR spectroscopy coupled with MVDA and chemometrics is a promising tool contributing to metabolomic assessment of the studied species. NMR spectroscopy combined with MVDA has a potentiality to discriminate *T. foenum-graecum* from *T. hamosa* based on their metabolomic composition. Different arrays of primary and secondary metabolites were detected and elucidated by NMR spectroscopy and based on their concentrations, MVDA differentiated between the two species. Due to its high contents of flavonoids, saponins and anthocyanins, *T. foenum-graecum* had lower IC_{50} of DPPH radical scavenging activity than *T. hamosa*. TAC was positively correlated with trigonelline, glucose, protein, flavonoids, saponins and anthocyanins reflecting the roles of these classes as antioxidant potentialities in these species particularly in *T. foenum-graecum*. Seeds of *T. foenum-graecum* should be consumed regularly because they have sufficient content of some important secondary metabolites such as anthocyanins, flavonoids, trigonelline and some compounds in glycoside form.

Conflict of interest: The authors declare that there is no conflict of interest.

Authors contribution: Ibrahim Abdel-Farid: Conceptualization, formal analysis, methodology, software and visualization and writing the original draft of the manuscript. Usama Mahalel: Conceptualization, methodology, investigation and revising the manuscript. Magdi El-Sayed: Conceptualization, formal analysis, methodology, writing and revising the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethical approval: Not Applicable.

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التحليل الأيضي والنشاط المضاد للأكسدة لنباتي الحلبة (التريجونيلا فوينم جرايكم) وعشبة الملك (التريجونيلا هاموزا)

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تم إجراء التحليل الأيضي لبذور كل من نباتي الحلبة (التريجونيلافوينم جرايكم) وعشبة الملك (التريجونيلا هاموزا) وذلك باستخدام تقنية الرنين المغناطيسي النووي والمرتبط بالتحليل متعدد العوامل للبيانات. تم استخدام قاعدة بيانات الازاحة الكيميائية للمركبات الأيضية وتقنية الرنين المغناطيسي النووي ثنائي الابعاد للتعرف على المركبات الايضية الموجودة في بذور النباتين. وبالإضافة إلى ذلك، تم تأكيد التعرف على المركبات الايضية عن طريق مقارنتها مع المركبات المعرفة سابقاً. من بين المركبات الايضية التي تم التعرف عليها، وجدنا أن الكولين، والستيرولات، والليوسين، والفالين، ومشتقات الفلافونويدات والتريجونيلين قد ساهمت ايضياً في التفرقة بين بذور النباتين كما ظهر ذلك في التحليل متعدد العوامل للبيانات. كما تم تقدير محتويات المواد الكربوهيدراتية والبروتينات والفينولات والفلافونويدات والمواد الصابونينية وأصباغ الانثوسيانين الكلية في بذور النباتين باستخدام المطياف الضوئي (الاسبكتروفوتوميتر). ولقد تم تقييم القدرة المؤكسدة الكلية والنشاط المضاد للأكسدة لبذور النباتين باستخدام طريقة موليبدات الفوسفات والنشاط الكاسح للشوارد الحرة. وقد تميزت بذور نبات الحلبة بمحتويات أعلى من التريجونيللين، والستيرولات، والبروتينات، ومشتقات الفلافونويدات، والمواد الصابونينية وأصباغ الأنثوسيانينوبقيمة أقل للتثبيط النصفي في طريقة النشاط الكاسح للشوارد الحرة بثنائي فينيل البيكريل هيدرازيل مقارنة بعشبة الملك. وقد لوحظ أن القدرة الكلية المضادة للأكسدة تتناسب طرديا مع محتويات كل من الجلوكوز، والتريجونيلين، والبروتينات، والفلافونويدات، والمواد الصابونينية، وأصباغ الانثوسيانين. وقد أظهر نبات الحلبة نمطاً عالي النشاط المضاد للأكسدة مما يرشحه ليكون نبات واعد ليتم استكشاف مركباته الايضية من خلال تقنيات التحليلات الايضية المختلفة بالإضافة إلى انشطته البيولوجية المحتملة.