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Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Metabolomic profiling and antioxidant activity of some *Acacia* species



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Received 14 November 2013; revised 19 March 2014; accepted 23 March 2014

Available online 31 March 2014

KEYWORDS

Acacia seyal;
Antioxidant capacity;
DPPH radical scavenging activity;
Flavonoids;
Metabolome;
Multivariate data analysis;
Saponins

Abstract Metabolomic profiling of different parts (leaves, flowers and pods) of *Acacia* species (*Acacia nilotica*, *Acacia seyal* and *Acacia laeta*) was evaluated. The multivariate data analyses such as principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) were used to differentiate the distribution of plant metabolites among different species or different organs of the same species. *A. nilotica* was characterized with a high content of saponins and *A. seyal* was characterized with high contents of proteins, phenolics, flavonoids and anthocyanins. *A. laeta* had a higher content of carbohydrates than *A. nilotica* and *A. seyal*. On the basis of these results, total antioxidant capacity, DPPH free radical scavenging activity and reducing power of the methanolic extracts of studied parts were evaluated. *A. nilotica* and *A. seyal* extracts showed less inhibitory concentration 50 (IC₅₀) compared to *A. laeta* extracts which means that these two species have the strongest radical scavenging activity whereas *A. laeta* extracts have the lowest radical scavenging activity. A positive correlation between saponins and flavonoids with total antioxidant capacity and DPPH radical scavenging activity was observed. Based on these results, the potentiality of these plants as antioxidants was discussed.

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1. Introduction

Acacia has a wide range of ecological amplitudes and is distributed in many regions all over the world. The genus includes more than 1350 species (Seigler, 2003). In spite of the huge

number of *Acacia* species, there are very few researches regarding the phytochemistry of these plants. *Acacia nilotica* is described as a multipurpose medicinal and pharmaceutical plant (Ali et al., 2012). In traditional medicine, *A. nilotica* is used for the treatment of many diseases including tuberculosis, pneumonia, gonorrhoea and small pox. *A. nilotica* showed a strong antimicrobial activity against both bacteria and fungi (Saini et al., 2008). Methanolic extract of *A. nilotica* leaves and ethanolic extract of stem bark were investigated against Gram positive and Gram negative bacteria. The results indicated that the extracts revealed antimicrobial activity against both types of bacteria (Mahesh and Satish, 2008; Banso, 2009). The ethanolic extract of *A. nilotica* leaves showed antimicrobial activity against *Campylobacter coli* isolated from

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goats (Solomon-Wisdom and Shittu, 2010). Saini et al. (2008) studied the antimicrobial activity of five species of *Acacia* and the results indicated that *A. nilotica* had the highest antifungal activity against *Aspergillus niger* and *Candida albicans*. Methanolic leaf extract of *A. nilotica* revealed a high antifungal activity against *Aspergillus flavus*, *Drechslera turcica* and *Fusarium verticillioides* (Mahesh and Satish, 2008). *A. nilotica* bark extract prevents hepatic malondialdehyde formation and reduces liver injury (Singh et al., 2009). *A. nilotica* pods have been evaluated for the antihypertensive and antispasmodic activity. Methanolic extract of *A. nilotica* inhibited the spontaneous contraction of rabbit jejunum (Gilani et al., 1999).

Regarding *Acacia laeta*, there are a few studies relating to this species. Many species of *Acacia* are distributed in both Nile valley and desert regions in Egypt. The most common species in Egypt are: *A. nilotica*, *A. laeta*, *Acacia seyal*, *Acacia*

raddiana, *Acacia ehrenbergiana* and *Acacia tortilis*. Although there are many studies that have been published regarding the phytochemical composition of *A. nilotica*, there are very few studies dealing with the phytochemistry and antioxidant activity of other species of *Acacia*.

In recent years, metabolomics which is defined as monitoring of metabolite concentration in a cell, tissue, organ or a whole plant (Ott et al., 2003) has become prominent as a part of systems biology. Moreover, metabolomics is of interest in chemical classification of plants for chemotaxonomy. Differentiation between different species of *Acacia* based on their metabolomic profiling has not been carried out yet.

In this study, a spectrophotometric method coupled with different multivariate data analyses such as PCA and PLS-DA was applied to *Acacia* metabolome aiming to investigate the metabolomic variation among different species of

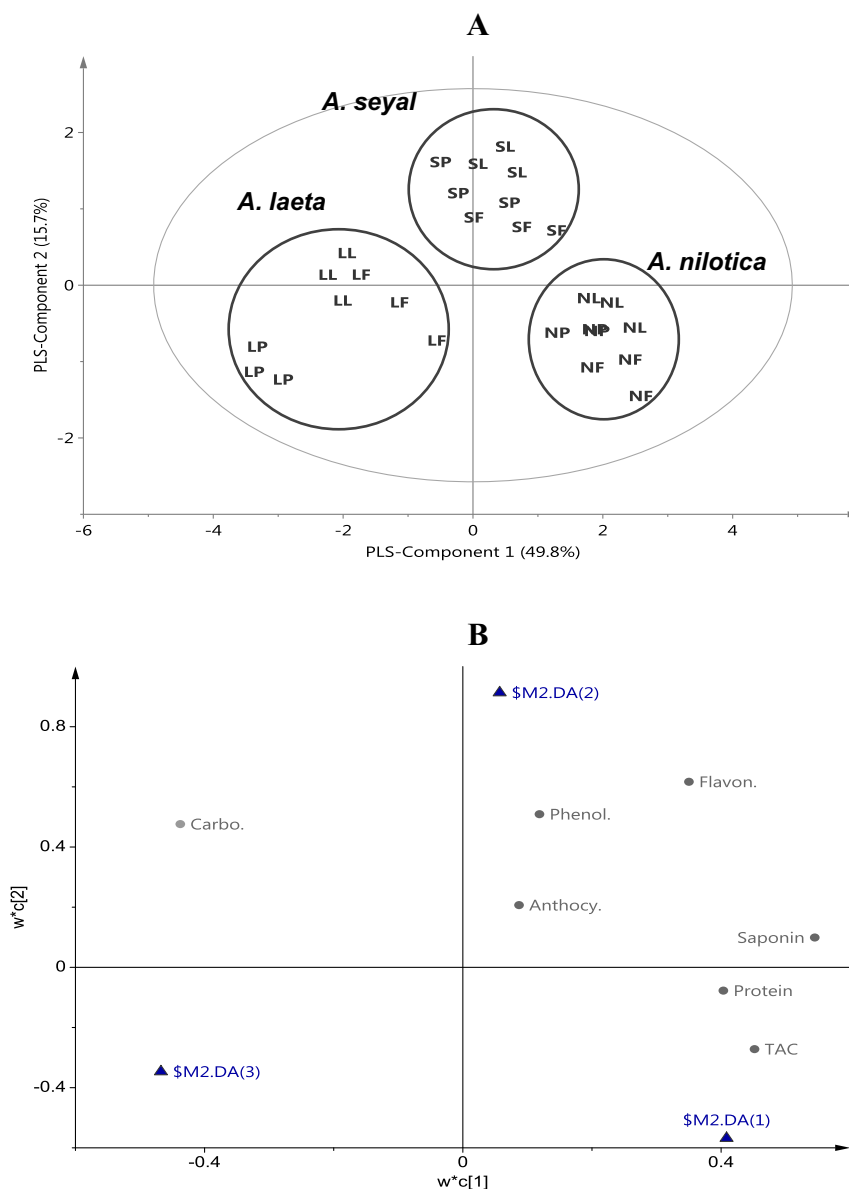


Figure 1 Score scatter plot (A) and score loading plot (B) of PLS-DA of three groups of *Acacia* species. NL = *A. nilotica* leaves, NF = *A. nilotica* flowers, NP = *A. nilotica* pods, SL = *A. seyal* leaves, SF = *A. seyal* flowers, SP = *A. seyal* pods, LL = *A. laeta* leaves, LF = *A. laeta* flowers, LP = *A. laeta* pods.

Acacia and among different organs of the same species and to evaluate these species as antioxidant potentialities.

2. Materials and methods

2.1. Collection of plant materials

Leaves, flowers and pods of three *Acacia* species (*A. nilotica*, *A. seyal* and *A. laeta*) were collected from the desert garden, Aswan University, 15 km south west of Aswan city in May 2012. Plant parts were dried in the shade at room temperature. The dried materials were powdered using an electrical grinder.

2.2. Spectrophotometrical analysis

2.2.1. Proteins, carbohydrates and anthocyanins determination

Powdered plant materials were used directly for the determination of carbohydrates, proteins and anthocyanins.

Carbohydrates were determined using anthrone reagent (Morris, 1948) and protein was determined using Folin Ciocalteu reagent. The anthocyanin content of the plant was determined according to the modified method of Padmavati et al. (1997). The anthocyanin content in the supernatant was measured spectrophotometrically at 530 and 657 nm. The absorbance values were indicated as A530 and A657. The concentration was calculated using the following equation:

$$\text{anthocyanin concentration } (\mu\text{mol/g}) = ([A_{530} - 0.33 \times A_{657}] / 31.6) \times (\text{volume [ml]} / \text{weight [g]}).$$

2.2.2. Phenolics, flavonoids, saponins, total antioxidant capacity (TAC), reducing power and DPPH free radical scavenging activity determination

Powdered plant materials (100 mg) were dissolved in 4 ml 80% methanol and the mixtures were vortexed for 1 min before

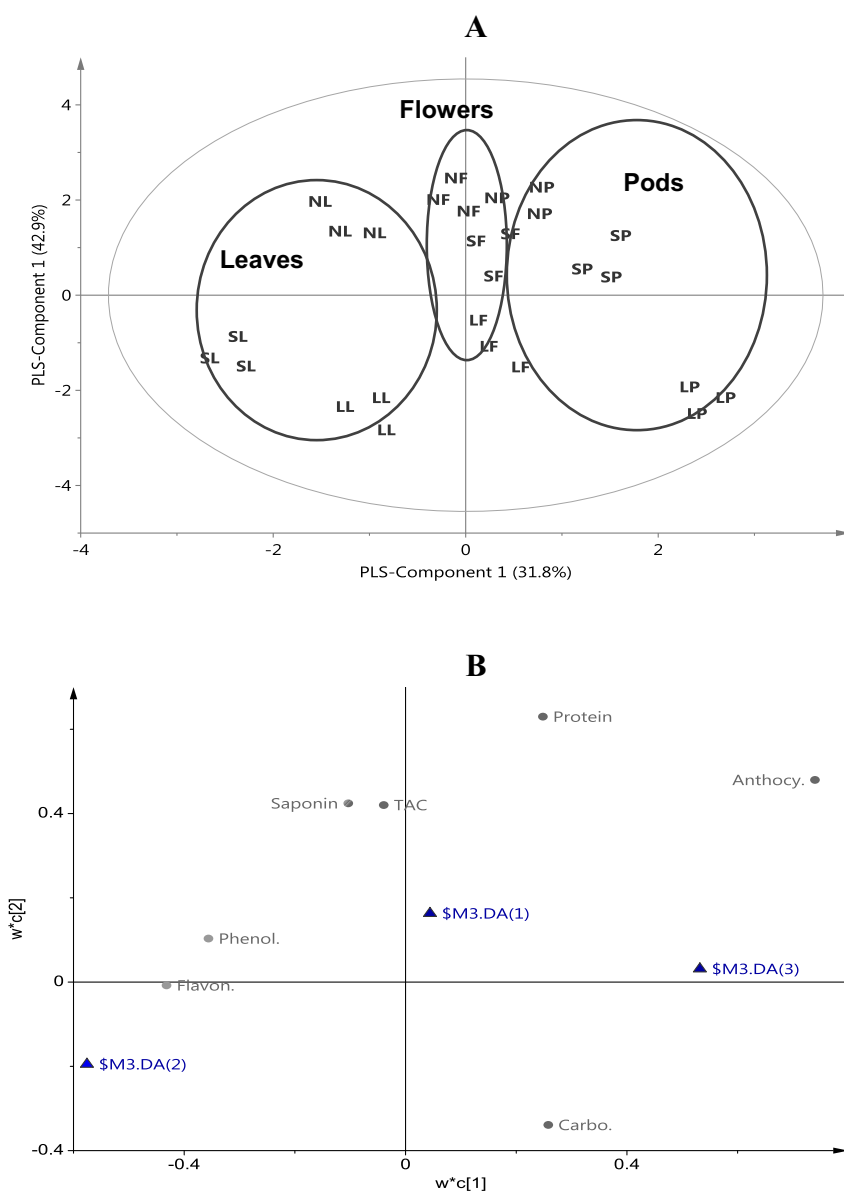


Figure 2 Score scatter plot (A) and score loading plot (B) of PLS-DA of three groups of organs (leaves, flowers and pods) of *Acacia* species. Labeling of each group is the same as Fig. 1.

placing in a water bath for 1 h at 60 °C. The mixtures were centrifuged at 800 rpm for 10 min and the supernatants were used for the determination of saponins, flavonoids, phenolics and total antioxidant capacity. The total concentration of phenolics in the extracts was determined according to the Folin–Ciocalteu method (Singelton et al., 1999) with gallic acid as a standard and expressed (mg) as gallic acid equivalents per gram of extract. Total flavonoid content was determined using the aluminum chloride colorimetric method according to Zhi-shen et al. (1999). Quercetin was used to make the calibration curve and the results were expressed as mg quercetin equivalents per gram of extract. Saponin content was determined using vanillin solution according to Ebrahimzadeh and Niknam (1998). The absorbance of the samples was measured at 473 nm and saponin content in samples was calculated from a standard curve constructed with purified saponins. Total

antioxidant capacity (TAC) was determined using the phosphomolybdenum method according to Prieto and Pineda Aguilar (1999). The antioxidant capacity was expressed as ascorbic acid equivalent. Different concentrations of crude extracts (after evaporation of methanol) were used for evaluating the DPPH radical scavenging activity and reducing power. Free radical scavenging activity of the sample extracts was determined spectrophotometrically using the method of Blois (1958). The reaction mixture consisting of 0.5 M acetic acid buffer solution at pH 5.5, 0.2 mM DPPH in ethanol, and 50% (v/v) ethanol aqueous solution, was shaken vigorously with the extracts. After incubation at room temperature for 30 min in dark, the amount of DPPH remaining was determined by measuring the absorbance at 517 nm. Control without extract in the presence and absence of DPPH was used. The concentration of ascorbic acid was measured at the same

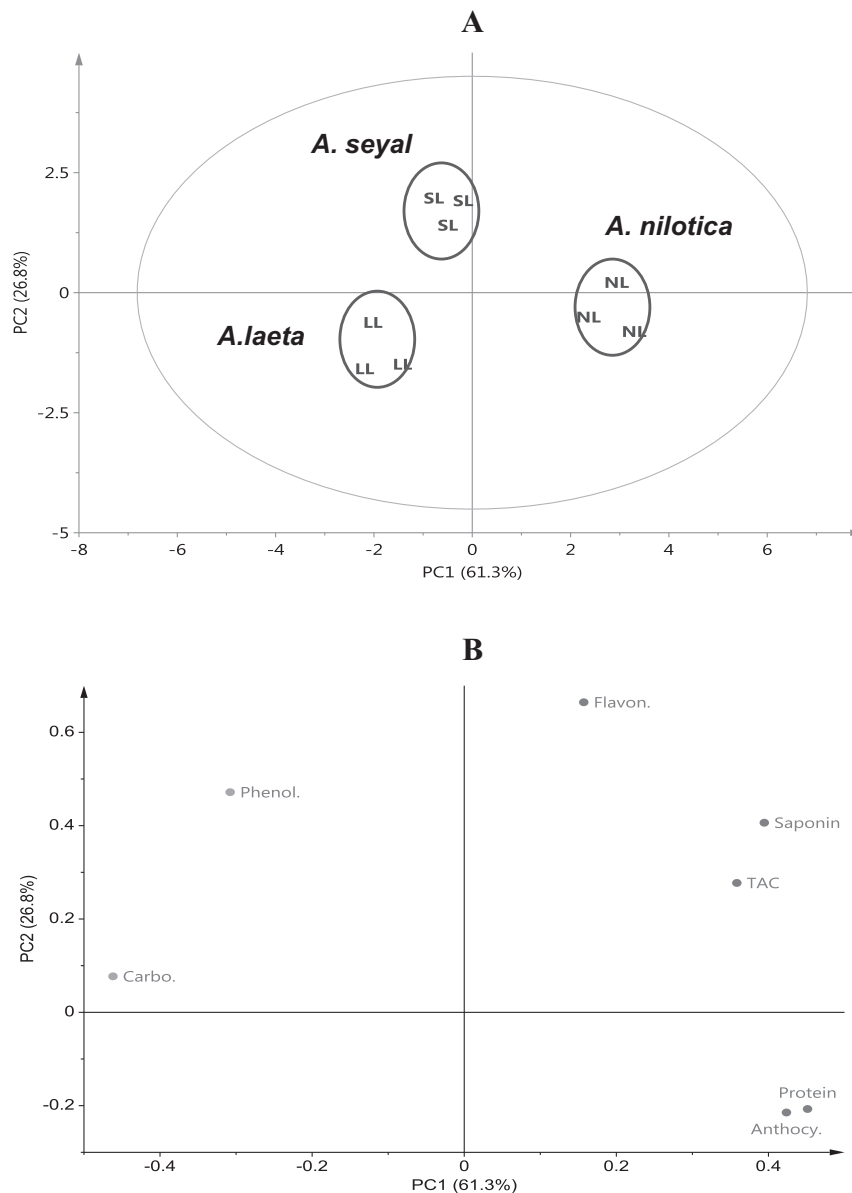


Figure 3 Score scatter plot (A) and score loading plot (B) of PCA of three groups of the leaves of *Acacia* species. NL = *A. nilotica* leaves, SL = *A. seyal* leaves, LL = *A. laeta* leaves.

wavelength. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation:

$$\% \text{ radical scavenging activity} = (Ac - As/Ac) \times 100$$

where Ac = Absorbance of negative control at 517 nm and As = Absorbance of sample at 517 nm (Wang and Mazza, 2002). The reducing power of the extracts was determined using the potassium ferricyanide reduction method (Oyaizu, 1986).

2.3. Data analysis

Principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) were performed with the SIMCA-P software (v. 11.0, Umetrics, Umeå, Sweden) with Unit variance scaling methods for spectrophotometrical data. An Analysis of variance (ANOVA) using Minitab (v. 12.21) was used to evaluate the significant difference of the metabolite content among different species and among the same organs of the studied species. Pearson's correlation was used to assess the correlation between the determined metabolites and both total antioxidant capacity and DPPH radical scavenging activity.

3. Results and discussion

3.1. Variation of metabolites among different *Acacia* species

The obtained data from spectrometric analysis of different *Acacia* species (*A. nilotica*, *A. seyal* and *A. laeta*) were subjected to PCA or PLS-DA. The most important information obtained from this analysis is the correlation between data sets which corresponds, in this study with the metabolome of the three species of *Acacia*. The differences or similarity among the samples can be detected by visualization of the score scatter plot while the metabolites responsible for the differences or similarities can be identified by the score loading plot. The partial least square-discriminant analysis (PLS-DA) was used for the spectrophotometer data of three organs (leaves, flowers and pods) of three species of *Acacia* (*A. nilotica*, *A. seyal* and *A. laeta*). The PLS-components 1 and 2 explain about 65.5% of the variation among data. Score scatter plot of PLS-DA shows three discriminated groups: the first group is *A. nilotica*, the second is *A. seyal* and the third is *A. laeta* (Fig. 1A). The score loading plot shows the compounds responsible for this discrimination. *A. nilotica* shows high contents of saponins, proteins and TAC. *A. seyal* has high contents of flavonoids, phenolics and anthocyanins, whereas *A. laeta* shows a high content of carbohydrates. *A. nilotica* and *A. seyal* together are characterized with higher contents of phenolics, anthocyanins, flavonoids, saponins, proteins and TAC than *A. laeta* (Fig. 1B). There are many morphological differences among those *Acacia* species but the results of this study may confirm the difference among them from the point of chemotaxonomy. Multivariate data analysis combined with different spectroscopic techniques was used to differentiate between different species belonging to a particular genus (Choi et al., 2005) or varieties of a particular species (Abdel-Farid et al., 2007) indicating that the chemometrics

Table 1 Phytochemical analysis, total antioxidant capacity, DPPH radical scavenging activity and reducing power of the three organs (leaves, flowers and pods) of three species of *Acacia* (*A. nilotica*, *A. seyal* and *A. laeta*).

Plant species and organs →	Leaves			Flowers			Pods		
	<i>A. nilotica</i> (NL)	<i>A. seyal</i> (SL)	<i>A. laeta</i> (LL)	<i>A. nilotica</i> (NF)	<i>A. seyal</i> (SF)	<i>A. laeta</i> (LF)	<i>A. nilotica</i> (NP)	<i>A. seyal</i> (SP)	<i>A. laeta</i> (LP)
Carbohydrates (mg/g)	18.17 ± 0.328 ^a	30.90 ± 2.608 ^b	33.40 ± 0.771 ^b	18.82 ± 2.180 ^a	32.71 ± 0.323 ^b	29.83 ± 2.738 ^b	23.62 ± 1.573 ^a	35.20 ± 0.715 ^b	42.69 ± 0.708 ^c
Proteins (mg/g)	75.42 ± 2.409 ^a	14.57 ± 3.272 ^b	18.11 ± 1.739 ^b	77.41 ± 0.805 ^a	77.67 ± 1.555 ^a	37.05 ± 4.060 ^b	61.87 ± 3.909 ^a	62.80 ± 7.477 ^a	34.04 ± 3.785 ^b
Saponins (mg saponin equivalent/g extract)	60.78 ± 0.235 ^a	57.38 ± 0.489 ^b	43.14 ± 0.671 ^b	61.88 ± 0.136 ^a	57.18 ± 0.359 ^b	45.55 ± 0.151 ^c	61.49 ± 0.00 ^a	51.86 ± 2.475 ^b	40.02 ± 2.837 ^c
Phenolics (mg gallic acid equivalent/g extract)	9.51 ± 0.144 ^a	10.24 ± 0.104 ^b	9.92 ± 0.435 ^{ab}	8.40 ± 0.023 ^a	9.79 ± 0.011 ^b	9.78 ± 0.057 ^b	9.39 ± 0.013 ^a	10.11 ± 0.016 ^b	6.21 ± 0.306 ^c
Flavonoids (mg quercetin equivalent/g extract)	35.71 ± 0.427 ^a	48.60 ± 1.495 ^b	19.68 ± 0.796 ^{cb}	29.69 ± 1.719 ^a	32.62 ± 1.451 ^a	15.26 ± 0.057 ^b	23.01 ± 0.924 ^a	28.25 ± 0.398 ^b	7.36 ± 0.848 ^c
Anthocyanins (μmol/g extract)	1.60 ± 0.820 ^a	UD	UD	5.16 ± 0.238 ^a	6.42 ± 0.774 ^a	4.54 ± 0.792 ^{ac}	8.29 ± 0.654 ^a	11.63 ± 0.611 ^b	6.98 ± 0.827 ^{ac}
TAC (ascorbic acid equivalent/g extract)	1043 ± 294 ^a	827 ± 236 ^a	525 ± 25 ^{ab}	1257 ± 2 ^a	790 ± 283 ^b	599 ± 296 ^b	1251 ± 123 ^a	608 ± 238 ^b	387 ± 52 ^b
% DPPH (scavenging at 100 μg/ml)	65.86 ± 0.00 ^a	66.27 ± 0.00 ^a	39.16 ± 5.396 ^a	61.85 ± 43.54 ^a	ND	ND	63.86 ± 1.14 ^a	66.67 ± 2.27 ^b	42.17 ± 21.58 ^c
Reducing power (O.D.) at 100 μg	1.27 ± 0.06 ^a	0.93 ± 0.09 ^b	0.31 ± 0.15b ^c	1.17 ± 1.70 ^a	0.51 ± 0.01 ^b	0.08 ± 0.06 ^{ab}	2.01 ± 0.05 ^a	0.50 ± 0.02 ^b	0.07 ± 0.03 ^c

Different letters in each group (leaves, flowers and pods) show significant difference at $p < 0.05$, UD = under detectable level, ND = not detected and TAC = total antioxidant capacity.

(multivariate data analysis) combined with the spectroscopic data are valuable tools in chemotaxonomy of species, varieties and cultivars.

3.2. Variation of metabolites among different organs

PLS-components 1 and 2 interpret around 74.6% of the variance among the samples. The score scatter plot of PLS-component 1 versus 2 (Fig. 2A) shows three discriminated groups: leaves, flowers and pods. The score loading plot (Fig. 2B) reveals that leaves of the three species are characterized with high contents of flavonoids and phenolics. Flowers have high contents of saponins, proteins and TAC. Pods of the three species have high contents of anthocyanins and carbohydrates (Fig. 2B). It is expected for a plant to show variation of metabolites among different organs. High contents of flavonoids and phenolics in the leaves may reflect the contribution of leaves actively to plant fitness so they are expected to

contain a higher content of compounds associated to defense mechanisms. These findings are in accordance with previous reports with different plants and different secondary metabolites such as glucosinolates in *Arabidopsis* (Brown et al., 2003) and phenylpropanoids and glucosinolates in *Brassica rapa* (Abdel-Farid et al., 2007).

3.3. Variation of metabolites among the same organ of different species

3.3.1. Variation of metabolites among leaves of different species

PC1 and PC2 of principal component analysis (PCA) explain 88.1% of the variation among samples. Score scatter plot of PC1 versus PC2 reveals three distinct groups. The first group is *A. nilotica* leaves, the second *A. seyal* and the third *A. laeta* (Fig. 3A). The score loading plot shows that *A. nilotica* leaves have high contents of saponins, anthocyanins, proteins and TAC (Fig. 3B). *A. seyal* leaves have

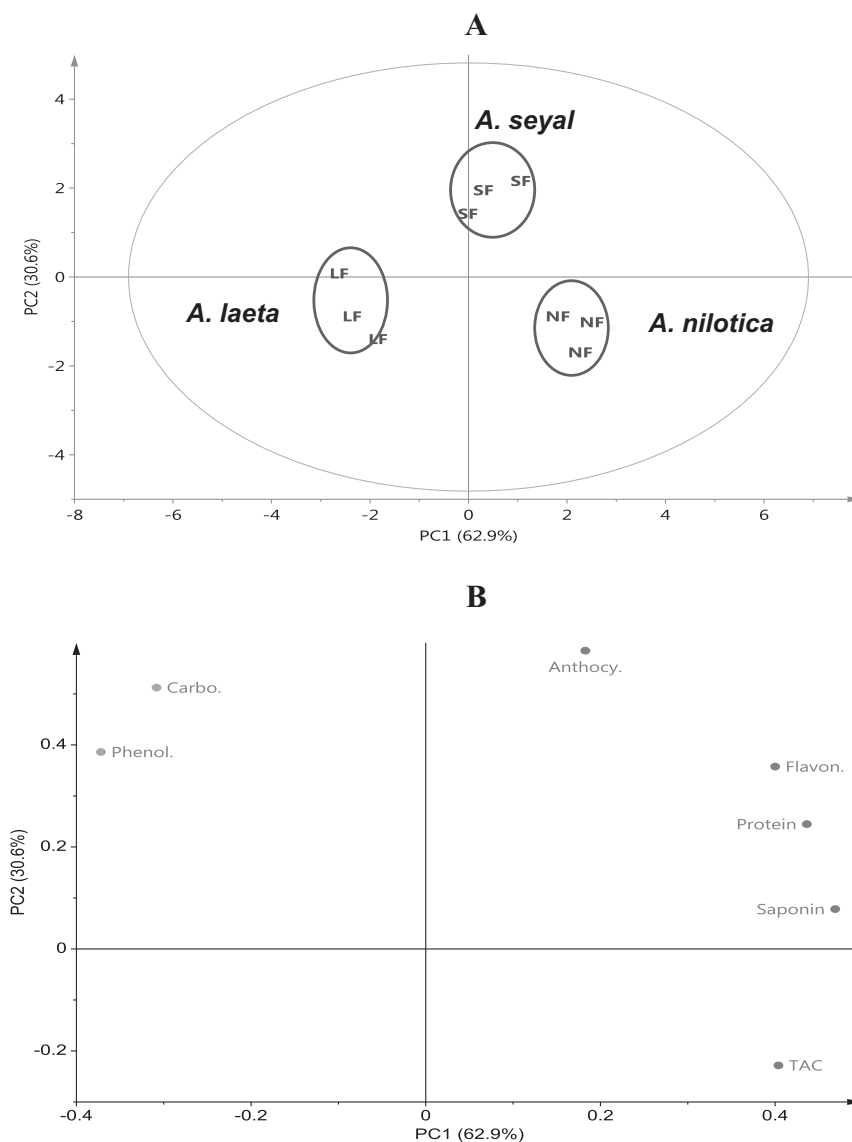


Figure 4 Score scatter plot (A) and score loading plot (B) of PCA of three groups of flowers of *Acacia* species. NF = *A. nilotica* flowers, SF = *A. seyal* flowers, LF = *A. laeta* flowers.

high contents of phenolics and flavonoids, whereas *A. laeta* leaves have a high content of carbohydrates (Fig. 3B). One way analysis of variance (ANOVA) was performed to evaluate the significant difference in metabolite content among the organs of different species (Table 1). Leaves of different species and even cultivars or varieties of the same species have different metabolomic profiling (Abdel-Farid et al., 2007).

3.3.2. Variation of metabolites among flowers of different species

Score scatter plot of PC1 versus PC2 explains 93.5% of variation among data and reveals three distinct groups. *A. nilotica*, *A. seyal* and *A. laeta* (Fig. 4A). The score loading plot shows that *A. nilotica* flowers have high contents of saponins and TAC, *A. seyal* flowers have high contents of carbohydrates, proteins, anthocyanins, phenolics and flavonoids, whereas *A. laeta* leaves have less contents of the previous detected metabolites (Fig. 4B).

Table 2 IC₅₀ values of methanol extracts of *Acacia* species potential as antioxidant (ND = not detected).

Species name	Organ's name	Inhibitory concentration (IC ₅₀) (µg/ml)
<i>A. nilotica</i>	Leaves	78.18
	Flowers	78.27
	Pods	76.14
<i>A. seyal</i>	Leaves	76.98
	Flowers	ND
	Pods	77.13
<i>A. laeta</i>	Leaves	130.59
	Flowers	ND
	Pods	145.18
	Ascorbic acid (AA)	5.99

3.3.3. Variation of metabolites among pods of different species

PLS-1 versus 2 of PLS-DA explains 96.4% of the variation among samples and reveals three groups: *A. nilotica*, *A. seyal*

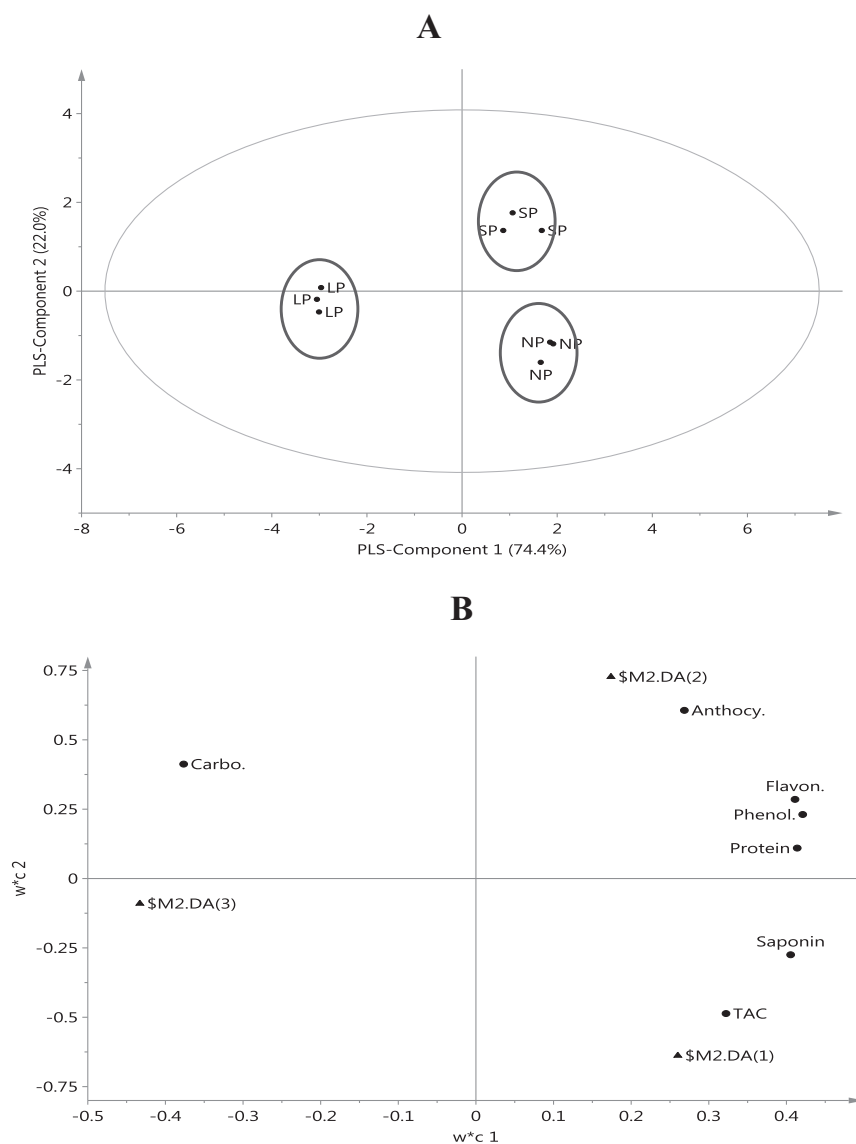


Figure 5 Score scatter plot (A) and score loading plot (B) of PLS-DA of three groups of pods of *Acacia* species. NP = *A. nilotica* pods, SP = *A. seyal* pods, LP = *A. laeta* pods.

Table 3 Correlation coefficient (r) and probability (p) between the detected metabolites and both total antioxidant capacity (TAC) and DPPH radical scavenging activity (ns = non significant).

Metabolites	Carbohydrates	Proteins	Saponins	Phenolics	Flavonoids	Anthocyanins
Total antioxidant capacity (TAC)	0.544 ($p = 0.002$)	0.451 ($p = 0.012$)	0.751 ($p = 0.00$)	ns	0.394 ($p = 0.031$)	ns
DPPH radical scavenging activity	ns	ns	0.828 ($p = 0.006$)	ns	0.716 ($p = 0.030$)	ns

and *A. laeta* (Fig. 5A). The score loading plot shows that *A. nilotica* pods have high contents of saponins and TAC, *A. seyal* pods have high contents of protein, flavonoids, phenolics, and anthocyanins whereas *A. laeta* pods have a high content of carbohydrates and a less content of other detected metabolites (Fig. 5B). In general *A. nilotica* is characterized with a higher saponin content and TAC, whereas *A. seyal* have a higher content of phenolics, flavonoids and anthocyanins and *A. laeta* has a higher content of carbohydrates.

3.4. TAC, DPPH free radical scavenging activity, IC_{50} and reducing power

Total antioxidant capacity, DPPH free radical scavenging activity and reducing power of methanol extracts of the three parts of the three species of *Acacia* are shown in Table 1. Total antioxidant capacity was high in *A. nilotica* and *A. seyal* extracts. At 100 $\mu\text{g/ml}$, *A. seyal* pods, leaves, *A. nilotica* leaf and pod extracts show the highest DPPH radical scavenging activity of 66.67%, 66.27%, 65.86% and 63.86%, respectively (Table 1). From the dose-dependent, *A. nilotica* pods, *A. seyal* leaves and pods and *A. nilotica* leaf extracts showed the lowest IC_{50} 76.14, 76.98, 77.13 and 78.18 $\mu\text{g/ml}$, respectively (Table 2) whereas *A. laeta* leaves and pods showed the highest IC_{50} 130.59 and 145.18 $\mu\text{g/ml}$, respectively (Table 2). *A. nilotica* pods, *A. seyal* leaves and pods and *A. nilotica* leaves have the lowest IC_{50} which means that of all extracts they have the strongest radical scavenging activity whereas *A. laeta* leaf and pod extracts have the lowest radical scavenging activity. The same extracts having the lowest IC_{50} have the lowest values of reducing power (Tables 1 and 2). *A. laeta* leaves and pods showed the lowest reducing power (Table 2). The highest content of secondary metabolites detected in both *A. nilotica* and *A. seyal* such as saponins, phenolics, anthocyanins and flavonoids may be the reason for increasing the potentialities of these extracts as antioxidants. The antioxidant activity of many plant extracts is attributed to the presence of saponins (Vu et al., 2013), flavonoids (Hamouz et al., 2011), anthocyanins (Sutharut and Sudarat, 2012) and phenolics (Basar et al., 2013).

3.4.1. Relation between antioxidant capacity and determined metabolites

Pearson's correlation was carried out to test the correlation between total antioxidant capacity and the determined metabolites in all samples (three species and three organs). Total antioxidant capacity was found to be positively correlated with total carbohydrates, proteins, saponins and flavonoids (Table 3). The correlation of TAC with flavonoids is in accordance with many reports (Hajimahmoodi et al., 2008; Hamouz et al., 2011). Saponin content of eleven extracts of traditional Chinese antidiabetic plants (Xi et al., 2008), Soy bean saponin content (Lee et al., 2011) and shallot saponin content (Vu et al., 2013) were evaluated for their antioxidant capacity

and a positive correlation between antioxidant activity and saponins was observed. Protein has a vital role in the prevention of lipid oxidation through inactivation reactive oxygen species and scavenging free radicals (Elias et al., 2008). In this study, carbohydrate was determined as total carbohydrates and this correlation may be attributed to the presence of glycosides in *Acacia* extracts. *Acacia* species is a rich source of some glycosides such as cyanogenic glycosides and these types of compounds may have a strong antioxidant activity. The results indicated that saponins and flavonoids showed a positive correlation with DPPH radical scavenging activity (Table 3) which confirm the importance of these compounds in antioxidant activity. Some species of *Acacia* such as *A. nilotica* have cytotoxic activity and reduce liver injury (Kaur et al., 2005; Singh et al., 2009). This potentiality may be attributed to its high contents from saponins, flavonoids and glycosides.

4. Conclusion

Metabolomic analysis combined with multivariate data analyses such as PCA and PLS-DA is an effective tool in the differentiation between different species of *Acacia* and also different organs. This study reveals that *A. seyal* and *A. nilotica* have higher contents of the detected metabolites than *A. laeta*. The metabolites contributing to differentiation between species and organs are saponins, flavonoids, proteins and carbohydrates. Also the antioxidant activity of *A. seyal* and *A. nilotica* extracts is higher than that of *A. laeta* and this may be attributed to the higher content of saponins and flavonoids in *A. seyal* and *A. nilotica* than *A. laeta*. Saponins and flavonoids were positively correlated with both total antioxidant capacity and DPPH radical scavenging activity. Some species of *Acacia* have been studied extensively, not only due to their wide range of ecological amplitude, but also due to their nutritional and beneficial effects. One of these species is *A. nilotica* which has been studied extensively from the point of its allelopathic potential against seeds germination and seedlings growth and also its antimicrobial activity. Having a high content of phenolics, flavonoids and anthocyanins in some parts of *A. seyal* may open the door for an extensive study of that species regarding its allelopathic and antimicrobial potentialities of its extracts comparing to *A. nilotica* particularly as this species is growing surrounding the crop fields.

Acknowledgement

The authors thank Dr. Magdi A. El-Sayed for his valuable discussions and providing valuable comments and suggestions.

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