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Mineral and phenolic content of leaves of Tunisian caprifig (*Ficus carica* L.) accessions

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Abstract: Leaves of twenty accessions of Tunisian caprifig (*Ficus carica* L.) accessions were investigated for their mineral and phenolic content. Results showed significant differences among accessions for all traits studied. Furthermore, leaves of caprifig are an important source of phenolic and mineral content. Potassium (379.05-1412.84mg/100g) is the major mineral content, followed by calcium (499.20mg/100g), magnesium (320.06mg/100g), phosphorus (175.54mg/100g), sodium (160.36mg/100g), iron (39.08mg/100g), manganese (2.54mg/100g) and zinc (1.41mg/100g). Total phenolic, flavonoid and flavonol contents ranged between 11.88-36.13mg.g-^{1}GAE DW; 5.88-20 mg.g-^{1}QE DW; 3-11.08 mg.g-^{1}QE DW respectively. Cluster analysis and PCA grouped accessions studied in four groups.

Keys worlds: Caprifig, *Ficus carica* L., leaves, mineral content, phenolic compound Tunisia.

1. Introduction

Ficus carica is one the oldest fruit species in the world (Ahmad et al . 2013) and among the first plants cultivated by humans (Mawa et al. 2013). Its seems to be originated from the Southern Arabia and the eastern part of the Mediterranean areas (Ikegami et. 2009). In Tunisia, fig has been traditionally cultivated in diverse edaphoclimatic conditions (Mars 1995) resulting in a high number of local varieties (Chatti et al., 2004, Mars et al. 2008). In fact, variation is an essential criterion for any programs of survival and conservation of species (Osman 2013) and is a key component of any agricultural production system (Frankel et al. 1995). Furthermore, to facilitate breeding program, it is necessary to analyses typical varieties in terms of minerals and micronutrients content (Nicolle et al. 2004).

Minerals are an inorganic substance that plays an important role in health and disease states of humans and domestic animals (Soetan et al. 2010) and necessary for almost metabolic processes (Ndukwe et al.2013). They are classified as macroelements (K, P, Mg, Na and Ca) and microelements (Mn, Fe and Zn) (Sulaiman et al.2011).

Phenolics compounds are the secondary metabolites ubiquitous in plants (Marinova et al. 2005) that have multiple physiological roles (Mawa et al. 2013) and pharmacological properties (Teixeira et al. 2005; Ghazi et al. 2012). Their synthesis can be influenced by diver's factors such as genotype (Saure 1990; Zadernoski, et al. 2005), nutrient availability, temperature, light (Saure 1990), divers biotic and abiotic stresses (Dixon and Paiva 1995), etc. Several work have been interested to mineral and phenolic content of fruit of female fig tree [Soloman et al. 2006; Oliveira et al. 2009; Aljane and Ferchichi 2009), rare in female fig leaves (Konyalioğlu, et . 2005; Teixeira et al. 2006; Oliveira et al. 2009; Ghazi et al. 2012) and absent in caprifig leaves.

Traditionally, *Ficus carica* has been used for several purposes (Kalaskar, et al. 2010). Their leaves can constitute an excellent source of phenolics compounds (Oliveira et al. 2009). It have anti-inflammatory activities (Patil and Patil 2011; Tchombé and A.Louajri 2015) and used for several diseases such as hemorrhoids, insect stings and bites (Tchombé and A.Louajri 2015), antioxidant, antimicrobial activity (Ahmad et. 2013). Also, leaf mineral analysis is an important guide for sustainable plant nutrition (Lewko et al. 2004).
Therefore, this study is interested to evaluate mineral and phenolic content of leaves of twenty Tunisian caprifig accessions.

2. Materials and Methods

2.1. Plant material

Leaves of twenty accessions of Tunisian caprifig (Table 1) were collected from the fig germplasm collection of Arid Land Institute of Médenine established in 'El Gordhab', Tataouine in the South-east of Tunisia.

Table 1. Accessions names, code and geographic origin of 20 Tunisian caprifig accessions studied.

No.	Accessions names	Code	Geographic Origin
1	Magouli1	MAG1	Duiret (Tataouine)
2	Jrani	JRN	Ghadhabna (Mahdia)
3	Bithri1	BTH1	Kerkennah (Sfax)
4	Assafri	ASF	Kerkennah (Sfax)
5	Bouharrag1	BHG1	Bir Amir (Tataouine)
6	Bithri2	BTH2	Bir Amir (Tataouine)
7	Dhokkar1	DHK1	Djebba (Béja)
8	Limi	LIM	Kébéli
9	Tebessi	TBS	Kébéli
10	Sawoudi	SWD	Kébéli
11	Magouli2	MAG2	Bir Amir (Tataouine)
12	Dhokkar2	DHK2	Tamaghza (Tozeur)
13	Dhokkar3	DHK3	Dégâche (Tozeur)
14	Dhokkar4	DHK4	Gafsa
15	Bouharrag2	BHG2	Toujen (Gabés)
16	Beldi	BLD	Zarzis (Médenine)
17	Dhokkar6	DKH6	Zarzis (Médenine
18	Dhokkar7	DHK7	Zammour (Médenine)
19	Bouharrag3	BHG3	Djerba (Médenine)
20	Khadhouri	KHD	Djerba (Médenine)

2.2. Mineral and phenolic analysis

2.2.1. Mineral compounds

For each sample about 1g of dried leaves was placed in porcelain capsule and heated at 550°C for 4h. After cooling, ashes were treated with 1ml of hydrochloric acid and 5ml of deoinised water, carried to boiling, filtered, adjusted to 100ml with deonised water and solutions were used for mineral analyses. Phosphorous contents were detected by a spectrophotometer (ANTHELIE ADVANCED, Microbeam, S.A.) while that of calcium, iron, potassium, magnesium, manganese, sodium and zinc were performed with flame atomic absorption method. For each accession the experiment was performed three times.

2.2.2. Phenolic compounds

The extraction of total phenolic was performed according to protocol previously described (Amin and Tan 2002) with minor modifications. About 0.5g of lyophilized leaves were homogenised with 25ml of boiled ultrapure water, agitated using an orbital shaker at 200 rpm for 2h at 50°C then submitted to centrifugation at 3000 rpm for 15 min at room temperature. The supernatant was conserved at -4°C for further analysis. For each accession extraction was performed three times.

Total phenolic, flavonoid and flavonol content were determined according to the method previously described (Kumaran and Karunakaran 2006). Total phenolic content was determined using the Folin–Ciocalteu reagent. Approximately, 100ul of extract were mixed with 500ul of FCR, 1.5ml of 20% sodium carbonate was added. The mixture was shaken thoroughly and completed to 10 ml with ultrapure water. The absorbance at 765 nm was determined after 2h. Results were expressed as mg gallic acid equivalents (GAE)/100g.

Flavonoid content was determined by aluminium chloride method. About 100ul extract was mixed with 100ul of 20% aluminium trichloride in methanol and a drop of acetic acid, and then diluted with ultrapure water to 5 ml. After 40 min the absorption at 415 nm was read. Results were expressed as mg quercetin equivalents (QE)/100g.

In order to determine a content of flavonols, about 1 ml of each extract was mixed with 1 ml aluminum trichloride (20 mg/ ml) and 3 ml sodium acetate (50 mg/ ml). The absorbance at 440 nm was read after 2.5 h. Results were expressed as mg quercetin equivalents (QE)/100g.

2.3. Statistical analysis

Analyses of Variance (ANOVA) were performed for data obtained from mineral and phenolic compound using the XLSTAT-Pro 7.5 program. The Fisher test (LSD) was used to compare the differences among accessions. Principal Component Analysis and cluster analysis of mineral and phenolic compounds were performed using the program NTSYS 2.11 (Exeter Software, Stauket, NY).

3. Results and Discussion

3.1. Minerals compounds

Our study showed that mineral composition varied depending on the accession (Table2). Potassium was the highest average content followed by calcium, magnesium, phosphorus, sodium, iron, manganese and zinc. Predominance of potassium content has been indicated in other species of Moraceae such as mulberry species [Ercisli and E.Orhan 2007; Gungor and Sengul. 2008), female figs (Aljane and Ferchichi 2009). The mineral content of caprifig leaves ranged between 379.05 to 1412.84mg/100g for potassium, 169.16 to779.05mg/100g for calcium, 121.14 to 416.27mg/100g for magnesium, 102.16 to 236.60 mg/100g for phosphorus, and 45.76 to 391.68 for sodium, 17.16 to 61.41 for iron, 0.84 to 6.09 for manganese and 0.76 to 2.30 for zinc. Results seem to be in the range to those found in female fig fruit (Soloman et al. 2006; Aljane and Ferchichi 2009).

3.2. Phenolic compounds

The concentration of total phenolic, flavonoid and flavonol contents are shown in Table 3. Average of total phenolic was 20.82±5.94mg.g-^{1}GAE DW. The highest value was recorded in TBS (31.38±0.83mg.g-^{1}GAE DW) while the lowest was in DKH3 (11.88±2.44 mg.g-^{1}GAE DW). Those values seem to be higher than those reported in leaves of female fig tree (Konyalioğlu et al. 2005). This higher value in phenolic compound can be attributed to genetic factor (Zadernoski, et al. 2005). But also, to conditions of water stress frequent in Southern Tunisia. In fact, insufficiency of the water in the plant tends to generate this situation of stress which induces the production of phenols (Par and Bolwell 2000).

Total flavonoid content varied from 5.88±0.45mg.g-^{1}QE DW (DKH3) to 17.21±2.43 mg.g-^{1}QE DW (DKH1) with an average of 10.43±2.61 mg.g-^{1}QE DW. Concerning the flavonol content, the values ranged from 3±1.03 mg.g-^{1}QE DW (DKH3) to 8.91±0.30 mg.g-^{1}QE DW (DKH1) with a mean of 5.88±1.77 mg.g-^{1}QE DW

Table 2. Mineral composition of leaves of Tunisian caprifig accessions (*Ficus carica* L.).

Accessions	Calcium	Iron	Potassium	Magnesium	Manganese	Sodium	Zinc	Phosphorus
MAG1	674.50±18.19^{BC}	48.22±6.53^{BCD}	1412.84±82.95^{A}	416.27±23.52^{A}	6.09±0.30^{A}	294.05±34.17^{B}	2.23±0.14^{ABC}	236.60±11.08^{B}
JRN	608.63±75.06^{CD}	37.01±1.22^{FG}	1107.29±38.52^{C}	378.20±32.75^{ABCDE}	4.27±0.34^{B}	209.05±2.83^{DEF}	1.73±0.30^{DEF}	176.30±10.98^{E}
BTH1	305.30±54.23^{GHI}	30.20±6.68^{G}	485.65±114.01^{G}	160.99±26.82^{G}	1.33±0.71^{GHI}	45.76±14.04^{K}	0.97±0.24^{IJ}	139.43±14.97^{F}
ASF	254.72±109.32^{IJ}	29.88±4.32^{G}	407.28±183.40^{G}	389.02±108.56^{ABCD}	1.98±1.62^{EFGH}	48.46±29.82^{K}	1.05±0.60^{HIJ}	162.49±25.94^{EF}
BHG1	169.16±29.08^{J}	17.16±2.15^{H}	379.05±74.57^{G}	121.14±10.76^{G}	0.84±0.60^{I}	95.57±18.60^{JK}	0.76±0.19^{J}	102.16±3.11^{G}
BTH2	375.84±39.47^{G}	30.17±6.03^{G}	819.55±208.73^{EF}	298.38±53.72^{EF}	2.92±0.65^{CDE}	100.77±8.77^{JK}	1.75±0.24^{DEF}	158.41±24.29^{EF}
DKH1	335.85±25.73^{GHI}	36.22±2.11^{FG}	781.47±128.67^{EF}	285.86±70.94^{F}	2.35±0.43^{DEF}	191.26±1^{EFG}	1.16±0.05^{HIJ}	197.21±16.09^{DE}
LIM	481.09±30.50^{EF}	43.91±6.37^{CDEF}	881.71±17.06^{E}	405.12± 70.09^{AB}	1.79±0.36^{FGH}	127.48±8.44^{IJ}	1.46±0.27^{DEFGH}	179.99±19.95^{DE}
TBS	467.81±16.77^{F}	51.07±3.08^{BCD}	1175.56±168.82^{BC}	326.25±49.76^{BCDEF}	1.23±0.32^{HI}	131.27±14.39^{HIJ}	1.37±0.37^{EFGHI}	189.57±23.9 6^{CDE}
SWD	773.65±18.22^{A}	42.63±0.90^{DEF}	1085.43±126.01^{CD}	398.27±55.60^{ABC}	2.80±0.23^{CDE}	187.93±15.05^{EFGH}	1.89±0.07^{BCD}	222.03±6.20^{BC}
MAG2	368.96±67.67^{GH}	50.21±10.01^{BCD}	825.09±67.19^{EF}	275.07±52.65^{F}	2.66±0.76^{CDEF}	175.51±41.94^{FGHI}	1.64±0.36^{DEFG}	212.40±32.87^{BCD}
DKH2	779.05±52.17^{A}	42.19±6.11^{DEF}	1166.07±102.79^{BC}	337.48±35.31^{ABCDEF}	2.87±0.22^{CDE}	234.13±38.34^{CDE}	1.33±0.38^{FGHI}	187.39±14.77^{DE}
DKH3	650.67±53.09^{BC}	37.68±0.86^{EFG}	795.96±120.89^{EF}	310.86±51.40^{DEF}	2.06±0.34^{EFGH}	134.63±21.39^{GHIJ}	1.33±0.06^{FGHI}	164.01±9.15^{EF}
DKH4	593.51±53.66^{CD}	46.23±9.09^{BCDE}	901.69±164.02^{DE}	395.61±66.72^{ABC}	2.39±0.32^{DEF}	252.33±72.79^{BCD}	1.38±0.3 1^{EFGHI}	184.85±23.77^{DE}
BHG2	649.33±22.35^{BC}	43.39±5.29^{CDEF}	673.31±70.50^{F}	302.40±45.43^{EF}	2.57±0.40^{DEF}	177.12±40.08^{FGHI}	1.13±0.23^{HIJ}	137.78±25.18^{F}
BLD	610.46±52.35^{CD}	46.19±3.83^{BCDE}	751.31±27.39^{EF}	340.51±9.34^{ABCDEF}	3.15±0.39^{CD}	260.18±60.20^{BCD}	1.26±0.23^{GHI}	189.56±32.28^{CDE}
DKH6	618.06±57.70^{CD}	44.53±2.48^{CDEF}	770.03±53.91^{EF}	406.77±49.66^{AB}	3.58±0.45^{BC}	274.01±34.85^{BC}	1.80±0.21^{CDE}	178.72±18.13^{E}
DKH7	286.11±46.73^{HI}	51.75±7.42^{BC}	1291.46±123.01^{AB}	315.37±25.56^{CDEF}	1.26±0.09^{HI}	297.68±39.51^{B}	2.38±0.26^{A}	323.80±19.08^{A}
BHG3	718.43±75^{AB}	54.26±2.82^{AB}	673.27±53.81^{F}	296.60±37.28^{EF}	2.26±0.55^{DEFG}	204.03±45.96^{DEF}	1.25±0.23^{GHI}	139.39±28.63^{F}
KHD	558.14±66.14^{DE}	61.41±7.02^{A}	1178.01±17.43^{BC}	302.95±23.74^{EF}	5.65±0.56^{A}	391.68±37.90^{A}	2.30±0.17^{AB}	186.47±10.43^{DE}
F test	35.12	10.67	19.96	7.08	17.06	19.95	8.03	14.94
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 3. Total phenolic, flavonoid and flavonol composition of leaves of Tunisian caprifig accessions (*Ficus carica* L.).

Accessions	Total phenolic	Flavonoid	Flavonol
MAG1	16.25 ± 0.54^{GH}	8.67 ± 1.31^{HI}	3.95 ± 0.96^{IJ}
JRN	16.81 ± 0.85^{GH}	10.08 ± 0.56^{FGHI}	5.41 ± 0.25^{GH}
BTH1	24.56 ± 6.94^{CD}	10.33 ± 1^{DEFGH}	7.35 ± 0.78^{C}
ASF	18.35 ± 3.70^{FG}	8.75 ± 1.31^{HI}	4.53 ± 0.75^{HI}
BHG1	26.85 ± 4.26^{BC}	11.88 ± 0.75^{DEF}	8.71 ± 0.46^{B}
BTH2	22.94 ± 3.74^{CDEF}	10 ± 1.09^{GHI}	5.44 ± 0.70^{FGH}
DKH1	26.25 ± 6.22^{BC}	17.21 ± 2.43^{B}	8.91 ± 0.30^{B}
LIM	16.71 ± 1.44^{GH}	11.04 ± 0.44^{DEFG}	6.11 ± 0.17^{EFG}
TBS	31.38 ± 0.83^{AB}	12 ± 0.66^{DE}	6.79 ± 0.38^{CDE}
SWD	17.15 ± 2.75^{GH}	12.08 ± 1.25^{D}	4.30 ± 0.75^{I}
MAG2	26.10 ± 1.90^{BC}	10.25 ± 0.38^{EFGHI}	7.15 ± 0.11^{CD}
DKH2	15.83 ± 2.01^{GH}	8.46 ± 0.26^{I}	4.21 ± 0.40^{I}
DKH3	11.88 ± 2.44^{H}	5.88 ± 0.45^{J}	3 ± 1.03^{J}
DKH4	19.96 ± 3.75^{DEFG}	8.50 ± 0.94^{I}	5.88 ± 0.95^{EFG}
BHG2	21.33 ± 0.57^{CDEFG}	11.25 ± 0.37^{DEFG}	6.41 ± 0.59^{CDEF}
BLD	18.54 ± 5.18^{EFG}	11.92 ± 1.59^{DE}	8.76 ± 0.22^{B}
DKH6	19.79 ± 0.63^{DEFG}	13.92 ± 1.65^{C}	8.39 ± 0.62^{B}
DKH7	36.13 ± 4.52^{A}	20 ± 1.62^{A}	11.08 ± 0.49^{A}
BHG3	24.17 ± 4.41^{CDE}	11.17 ± 0.29^{DEFG}	8.76 ± 0.30^{B}
KHD	17.65 ± 1.03^{FG}	9.46 ± 0.52^{GHI}	6.30 ± 0.25^{DEFG}
F test	8.64	24.22	37.13
Pr > F	< 0.0001	< 0.0001	< 0.0001

3.3. Cluster and Principal Compound Analysis

Cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) based on mineral and phenolic compound revealed that the 20 accessions studied could be divided into 4 groups (Figure 1). Group (A) included DKH7 accession where is content the most important values in zinc, phosphorus, total phenolic, flavonoid and flavonol. Group (B) contain two accessions (BHG1 and BTH1) were characterized by the low values in magnesium and zinc. Group (C) formed the almost of accessions and group (D) contain two accessions (KHD and MAG1) who are distinguished by the most important values in manganese. Group (C) can be subdivided in two subgroup. First subgroup (C.1) contains ASF accession and the second (C.2) is formed by the remaining of accessions.

Principal component analysis for mineral and phenolic compound indicates that the first three PCAs explained 39.90%, 30.03% and 11.72% of the total variation (Table 4). PCA results suggest that calcium, potassium, magnesium, manganese, sodium, zinc and phosphorus are the major mineral and phenolic compounds composing PCA1. Total Phenolic, flavonoid and flavonol are the important variables composing PCA2. Finally, iron is the trait that composing PCA3.

As in the UPGMA cluster analysis four groups have been found **(**Figure 1**)**. Both UPGMA and PCA showed that geographic origin is not the main critical trait to classify accessions studied.

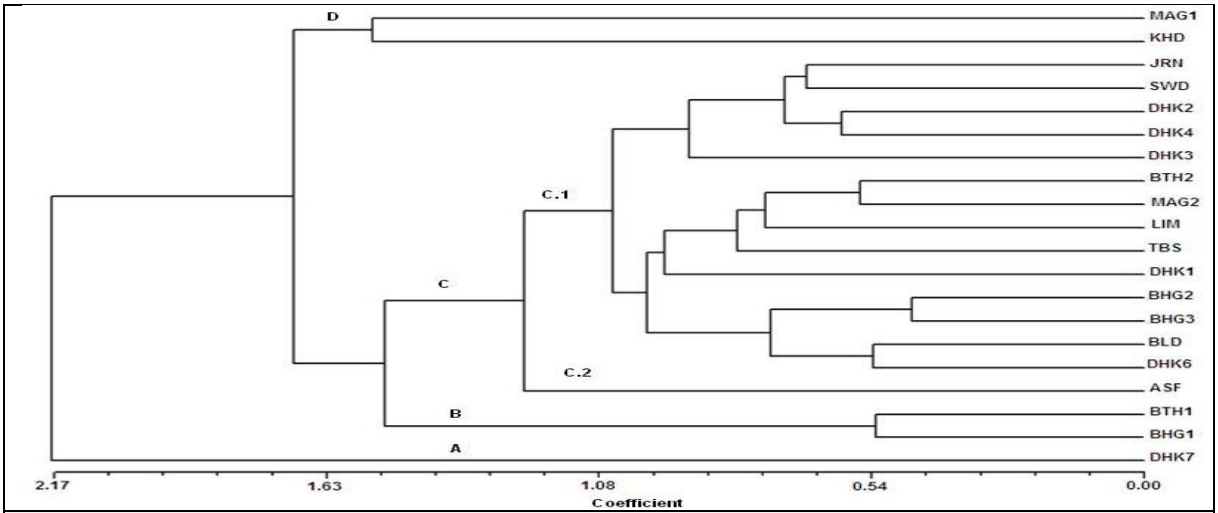

Figure 1. UPGMA cluster analysis of studied caprifig accessions based on mineral and phenolic content.

Table 4. Eigenvectors obtained from PCA analysis of studied caprifig accessions.

	PC1	PC2	PC3
Eigenvalue	4.39	3.30	1.29
Variance(%)	39.90	30.03	11.72
Accumulative variance(%)	39.90	69.93	81.66
Traits	**Eigenvalue**		
Calcium	0.68	0.38	0.32
Iron (Fer)	-0.27	0.45	-0.76
Potassium	0.84	-0.24	-0.30
Magnesium	0.74	0.18	-0.19
Manganese	0.79	0.22	0.12
Sodium	0.80	-0.38	0.37
Zinc	0.82	-0.38	-0.18
Phosphorus	0.61	-0.58	-0.43
Total phenolic	-0.42	-0.82	-0.17
Flavonoid	-0.11	-0.90	-0.07
Flavonol	-0.34	-0.85	0.28

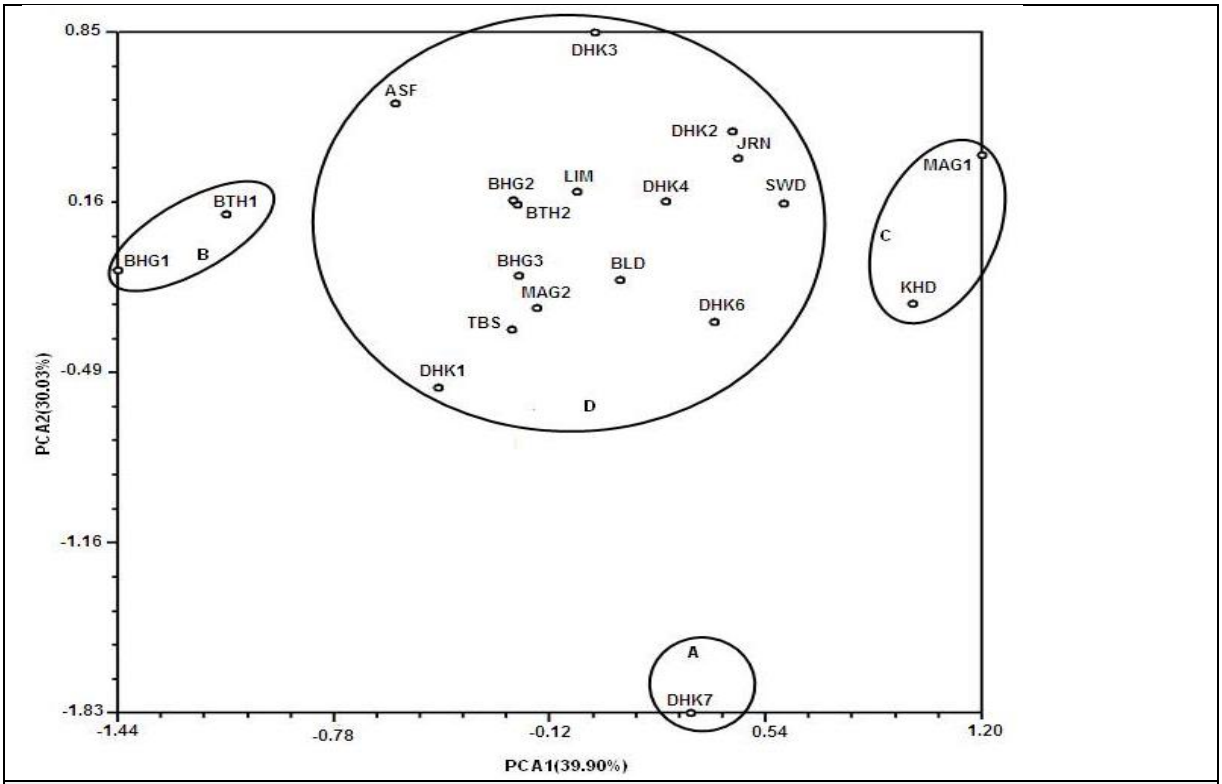

Figure 2. Distribution of caprifig accessions studied in the first two principal components (PCs) based on mineral and phenolic traits.

4. Conclusions

Caprifig leaves were analyzed for their content to mineral and phenolic content. Significant differences among accessions studied were observed indicating a great mineral and phenolic diversity. Potassium presents the highest amount in mineral content. Leaves of Tunisian caprifig accessions are good source of mineral and phenolic content. So, it is important to use this specie not only for genetic diversity but also for their therapeutic effect. Cluster analysis and PCA grouped accessions studied into four groups. Both PCA and UPGMA cluster analysis showed that geographic origin is not the main critical trait to discriminate between accessions of caprifig tree studied. DKH7 originated from Zammour (Médenine) diverged from other accessions by their most important values in zinc, phosphorus, total phenolic, flavonoid and flavonol.

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