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A comparative assessment of the LC-MS profiles and cluster analysis of four *Centaurea* species from Turkey

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ABSTRACT

The present study was designed to delineate the chemical characterization of four *Centaurea* species sampled from Turkey. The chemical profiles were determined by UHPLC-ESI/HRMS and multiple correspondence analysis were carried out to observe variabilities of the tested extracts. To study the variability of the four samples, 16 qualitative variables represented by the 16 compounds (some acylquinic acids including 3-Caffeoylquinic, 5-Caffeoylquinic and 5-Feruloylquinic acids) have been used to perform the multiple correspondence analysis. There groups were recorded in the cluster analysis. The first group is represented by *C. urvielli* subsp. *hayekiana* and *C. kotschi* var. *persica* species while the second and third group is composed of *C. drabifolia* subsp. *detonsa* and *C. patula*, respectively. Finding presented herein has established baseline data that could spark further studies on the pharmacological potential of these *Centaurea* species.

1. Introduction

Centaurea L. (tribe: Cynareae) is one of the largest genus of the family Asteraceae. The genus is represented by more than 700 species, predominately distributed around the Mediterranean area and in West Asia (Taşar et al., 2018). Turkey is one of the main centres of origin of this genus, particularly in central, southwest and east of the Anatolia (Kilic and Bagci, 2016) and regarded as the third largest genus after *Astragalus* and *Verbascum* in Turkey (Biyikoglu et al., 2018). In Turkey, *Centaurea* is represented by 194 taxa, of which 118 are endemic and it is the richest genera in terms of endemic species with the rate of 64% (Taşar et al., 2018). The members of the genus are annual, biennial, or perennial plants and they are rarely evergreen large shrubs (Janaćković et al., 2008). The species are commonly known as star-thistle, cornflower and knapweed (Albayrak et al., 2017).

During the past decade, several studies have systematically analyzed the consumption and gathering of medicinal plants in World including Turkey (Arık, 2018; Islam et al., 2019; Nadiroğlu and Behçet, 2018). Interestingly, in the Turkish traditional medicine, the plants of *Centaurea* genus are used as natural medications for treating various ailments such as stomach ache, abscesses, asthma, headache, hemorrhoids, diarrhea, hyperthermia, stypsis, cardiac disorder, embolism and rheumatoid arthritis (Korga et al., 2017; Polat, 2018; Zater et al., 2016; Zengin et al., 2016b). Several reports have highlighted on important biological activities from *Centaurea* species. For instance, the methanolic extract obtained from *Centaurea iberica* has been reported to exhibit remarkable wound healing properties and a significant, dose-dependent anti-inflammatory activity *in vivo* (Koca et al., 2009). Chloroform extracts of *C. cuneifolia*, *C. kilaea* and *C. salicifolia* have exhibited pronounced *in-vitro* antioxidant, anti-inflammatory and anti-cancer activity against human hepatocellular cancer HepG2 cell line (Sekerler et al., 2018). Tatlı et al. (2009) have been reported on the anti-inflammatory and anti-nociceptive effects *C. drabifolia* subsp. *drabifolia*. A series of phytochemical studies on *Centaurea* species have revealed that the plant contains a diverse number of compounds including sesquiterpenes (Marco et al., 2005; Saroglou et al., 2005), flavonoids (Ahmed and Kamel, 2014; Mishio et al., 2015; Uddin et al., 2017), alkaloids (Hodaj et al., 2017), lignans (Hodaj et al., 2017), steroids, triterpenes, hydrocarbons, polyacetylenes, anthocyanins (Kilic, 2013; Mishio et al., 2015; Sen et al., 2017).

Previously, Zengin et al. (2010) have reported the *C. patula* possess remarkable antioxidant property and significant amount of essential

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fatty acids, with α-linolenic acid as the major fatty acid. Another report has revealed the presence of spathulenol, *n*-hexadecanoic acid, 1-pentadecene and phytol as the major components of the essential oils from *C. patula* (Zengin et al., 2016a). While *Centaurea urvillei* subsp. *hayekiana* has been documented to possess moderate antioxidant capacity and high content of linoleic acid and oleic acid (Zengin et al., 2011). The inhibitory activity against key physiological enzymes involved in common pathologies such as neurodegenerative diseases (cholinesterases – AChE and BChE), hyper-pigmentation (tyrosinase) and diabetes (α-amylase and α-glucosidase) of the ethyl-acetate and chloroform extracts of *Centaurea patula*, Centaurea *urvielli* subsp. *hayekiana*, *Centaurea kotschi* var. *persica*, *Centaurea drabifolia* subsp. *dentosa* have been reported (Zengin et al., 2016b).

In our continuous attempt to search for potential therapeutic compounds from plants from this genus for the management of chronic diseases, the present work was designed to establish the chemical profile of four Turkish *Centaurea* species (*Centaurea patula*, Centaurea *urvielli* subsp. *hayekiana*, *Centaurea kotschi* var. *persica*, *Centaurea drabifolia* subsp. *dentosa*). The main aim was to evaluate and compare the chemical profile of these four species that could spark the development sustainable phytomedicines. The chemical profiles were determined by ultra-high-performance liquid chromatography–electrospray/high resolution mass spectrometry (UHPLC-ESI/HRMS) and multiple correspondence analysis were carried out to observe variabilities of the tested extracts.

2. Materials and methods

2.1. Plant material and extractions

Centaurea species (*Centaurea patula*, Centaurea *urvielli* subsp. *hayekiana*, *Centaurea kotschi* var. *persica*, *Centaurea drabifolia* subsp. *dentosa*.) were collected in May and June 2009 from Konya, Turkey. The plants have been identified by Dr. Tuna Uysal and Dr. Evren Yıldıztugay, Faculty of Science, Selcuk University. The voucher specimens have been deposited in KNYA herbarium at Department of Biology, Selcuk University.

The aerial parts (as mixed) were divided and dried for 10 days at the room temperature. Then, these samples were powdered with a laboratory mill. The dried plants (10 g) samples were macerated with methanol (200 ml) until the solvent become colorless. Methanol was preferred as one of the most used solvent to extraction of phenolic (Belwal et al., 2018; Boeing et al., 2014). Then, the extracts were filtered. After filtration, the extracts were concentrated using a rotary evaporator under vacuum at 40 °C. The extracts were stored at +4 °C until further analysis.

2.2. UHPLC-ESI/HRMS

Protocatechuic (**1**), neochlorogenic acid (**2**), chlorogenic acid (**3**), caffeic acid (**4**), orientin (**6**), vitexin (**7**), quercetin-3-O-glucoside (**8**), luteolin-7-O-rutinoside (**10**), luteolin-7-O-glucoside (**11**), isovitexin (**12**), quercetin (**13**), apigenin (**14**) and luteolin (16) were obtained from Extrasynthese (Genay, France).

UHPLC-ESI/HRMS were acquired on LC/HRMS system consisting of an Q Exactive Plus (ThermoFisher Scientific, Inc., Bremen, Germany) mass spectrometer, equipped with a heated HESI-II source coupled to a UHPLC system Dionex Ultimate 3000RSLC (ThermoFisher Scientific, Inc.). Chromatographic separation was achieved on a AkzoNobel Kromasil ExternityXT-1.8-C18 (Bohus, Sweden) narrow-bore column (2.1 × 100 mm, 1.8 µm), equipped with Phenomenex Security Guard ULTRA UHPLC EVO C18 (Torrance, USA) and maintained at 40 °C. The instrument parameters for negative mode were as follows: spray voltage was 2.5 kV, sheath gas 38 psi and auxiliary gas 12 a.u., while all other parameters were the same as in positive mode. Mass resolution in full scan mode in mass range m/z 100–1500 was set to 70000 FWHM (at m/z 200), while in data dependent MS/MS was 17500 FWHM (at m/z 200) and 1.0 amu isolation window of precursor ions was used for structural elucidation studies. All solvents were of LC-MS grade and were purchased from Ficher Scientific (Waltham, USA). The proposed structures were theoretically studied by Mass Frontier 5.1 Software (ThermoScientific Co, USA).

Table 1
Peak assessment of phenolic compounds in methanol extracts of *Centaurea* species.

Peak №	[M-H]⁻ m/z Molecular formula	Proposed compound
1	153.0181 $C_7H_5O_4$	Protocatechuic acid[a,b,c,d]
2	353.0887 $C_{16}H_{17}O_9$	3-Caffeoylquinic acid[b,c]
3	353.0894 $C_{16}H_{17}O_9$	5-Caffeoylquinic acid[b,c,d]
4	179.0340 $C_9H_7O_4$	Caffeic acid[a,b,c]
5	367.1031 $C_{17}H_{19}O_9$	5-Feruloylquinic acid[a,b,c,d]
6	447.0926 $C_{21}H_{19}O_{11}$	Orientin[a,b,c,d]
7	431.0982 $C_{21}H_{19}O_{10}$	Vitexin [a,b,c,d]
8	463.0884 $C_{21}H_{19}O_{12}$	Quercetin-3-O-glucoside[b,c,d]
9	493.1923 $C_{21}H_{33}O_{13}$	Patuletin-O-hexoside[a,d]
10	593.1511 $C_{27}H_{29}O_{15}$	Luteolin-7-O-rutinoside[b,c,d]
11	447.0927 $C_{21}H_{19}O_{11}$	Luteolin-7-O-glucoside[a,b,c,d]
12	431.0979 $C_{21}H_{19}O_{10}$	Isovitexin [a,b,c,d]
13	301.0354 $C_{15}H_9O_7$	Quercetin[a,b,c,d]
14	269.0454 $C_{15}H_9O_6$	Apigenin[a,b,c,d]
15	299.0560 $C_{16}H_{11}O_6$	Hispidulin[a,b,c,d]
16	285.0402 $C_{15}H_9O_6$	Luteolin[a,c,d]

*Tentative identification.
[a] Centaurea patula.
[b] *Centaurea urvielli* subsp. *hayekiana*.
[c] *Centaurea kotschi* var. *persica*.
[d] *Centaurea drabifolia* subsp. *detonsa*.

2.3. Data evaluation

Multiple correspondence, cluster and biplot analysis were carried out to observe variabilities of the tested extracts. The statistical procedures were performed by R software v. 3.5.1.

3. Results and discussion

3.1. Chemical composition

In the present study, IUPAC numbering system was used for the acylquinic acids and they summarized in Table 1. Their assessment was carried out according to the hierarchical key for identification of phenolic acids of (Clifford et al., 2003). Total ion chromatograms of studied *Centaurea* species are presented in Fig. S1. Also, Table S1 shows identified compounds with analytical parameters. The collision-induced dissociation (hcd 25) corresponded to MS^3 spectra of Cliford's hierarchical key. Two isobaric compounds (**2** and **3**) shared the same deprotonated molecule [M-H]⁻ at *m/z* 353.089 (Table 1). In (−) ESI/MS/MS both precursor ions produced a base peak (100%) at *m/z* 191.055

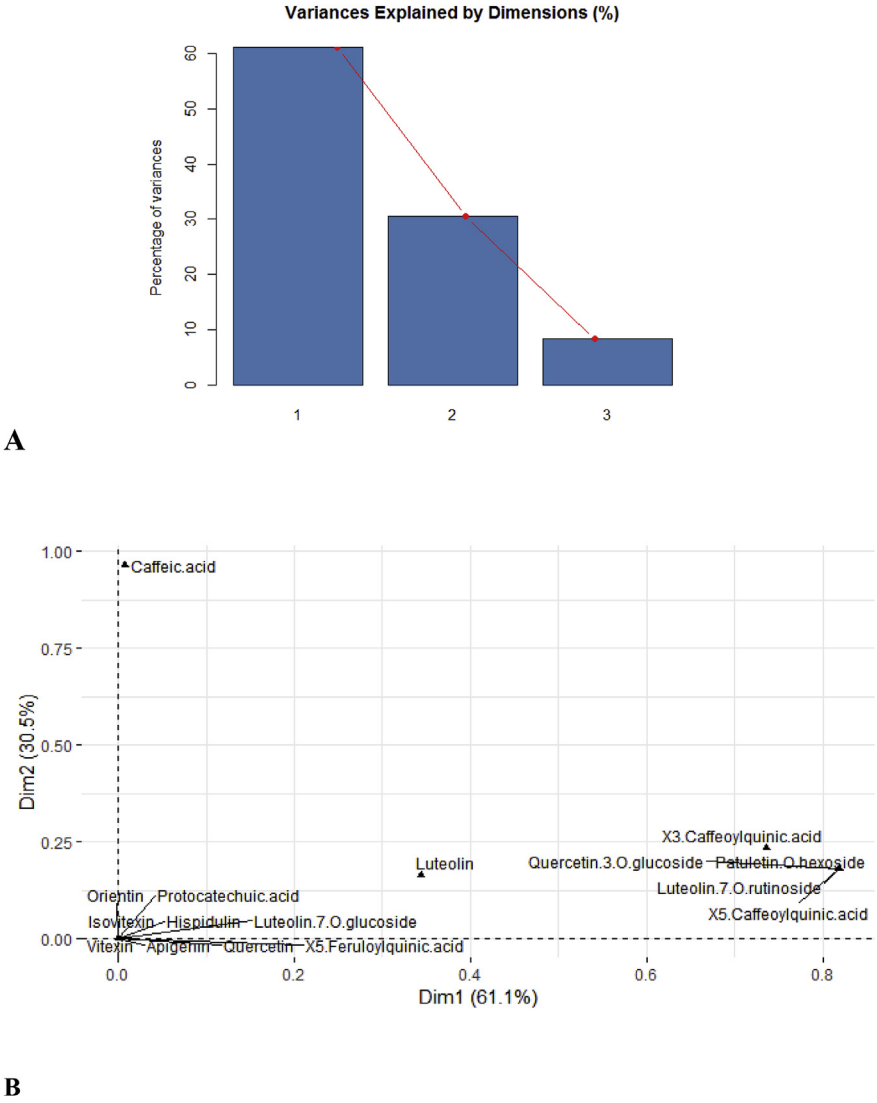

Fig. 1. Percentage variability explained by dimensions and relation between the 16 initial descriptors and first two identified factors. A. Percentage variability explained by dimensions B. and relation between the 16 initial descriptors and first two identified factors.

[quinic acid-H]$^-$ corresponding to the loss of caffeoyl moiety. In addition, **2** gave abundant fragment ion at *m/z* 179.034 [M-H-174]$^-$ (79.2%) indicating a caffeoyl residue supported by the fragment ions at *m/z* 135.044 [caffeoyl-H-CO_2)]$^-$ (7.6%) and 161.023 [caffeoyl-H-H_2O)]$^-$. Concerning **3**, fragment ions at 179 and 135 showed lower abundance (below 2%). By comparison with reference standards, **2** and **3** were identified as neochlorogenic (3-caffeoylquinic) and chlorogenic (5-caffeoylquinic) acid, respectively. In the same way, 5 ([M-H]$^-$at *m/z* 367.103) was assigned as 5-feruloylquinic acid (Table S1).

Compounds **1** ([M-H]$^-$at *m/z* 153.018) and **4** ([M-H]$^-$at *m/z* 179.034) afforded base peaks at *m/z* 109.028 and 135.044 [M-H-CO_2]$^-$, respectively, indicating a loss of CO_2. They were identified as protocatechuic and caffeic acid, respectively, confirmed by comparison with reference standards.

MS/MS spectra of the compounds **6** and **11** with [M-H]$^-$ at *m/z* 447.093 were acquired. The prominent ions at *m/z* 327.051 [M-H-120]$^-$ (100%) and 357.062 [M-H-90]$^-$ (33.5%), indicated *C*-linked hexosyl unit in compound **6**. The aforementioned fragments resulted from the cross-ring cleavages of the hexose $^{0,3}X^-$ (−90) and ($^{0,2}X^-$) (−120), as was observed for 8-*C*-glucosyl-luteolin (orientin) (Zheleva-Dimitrova et al., 2018). Concerning **11**, the loss of a hexose moiety afforded a base peak at *m/z* 285.040 indicating *O*-glycosidic bond. In both **6** and **11**, the aglycone was assigned to luteolin witnessed by the fragment ion at *m/z* 257.046 [Lu-H-CO]$^-$ together with RDA cleavages $^{1,3}B^-$ at *m/z* 133.029 and $^{1,3}A^-$ at *m/z* 151.003 in (−) ESI/MS (Table 1). Thus, **11** was identified as luteolin-*O*-glucoside. Consistent with the *C*-8 flavon glycosides fragmentation fingerprint, **7** (vitexin) afforded a base peak at *m/z* 311.056 [M-H-120]$^-$ supported by *m/z* 341.066 [M-H-90]$^-$. In line with our earlier study, *C*-6 isomer **12** (isovitexin) displayed a base peak at *m/z* 431.098 [M-H]$^-$ and a low abundant ion at *m/z* 311.056 (Zheleva-Dimitrova et al., 2018). In both **7** and **12**, the aglycone apigenin was deduced from the low abundant fragment ion at *m/z* 269.040 (Table S1). The MS/MS spectrum of **10** exhibited prominent fragment ions at *m/z* 431.049 [M-H-162]$^-$ (0.8%), 285.040 [M-H-162-146]$^-$ (100%) indicating a loss of inner hexose unit and concomitant loss of hexose and deoxyhexose, respectively. The loss of the internal sugar residue was in agreement with 7-*O*-substituted flavon (de Rijke et al., 2006). Accordingly, **10** was ascribed to luteolin-7-*O*-rhamnosyl-(1 → 6)-glucoside (luteolin-7-*O*-rutinoside) -, evidenced by comparison with reference standard.

The MS/MS spectrum of **8** showed a loss of a hexose unit yielding aglycone at *m/z* 301.035. supported by the radical aglycone at *m/z* 300.027 as was seen previously for the quercetin-3-*O*-glycosides (Cuyckens and Claeys, 2004). RDA cleavages generated $^{1,3}A^-$ at *m/z* 151.002 and $^{1,2}B^-$ at *m/z* 121.028 (Table 1). Thus, **8** was assessed as quercetin-3-*O*-glucoside (isoquercitrin). Retention times, fragmentation

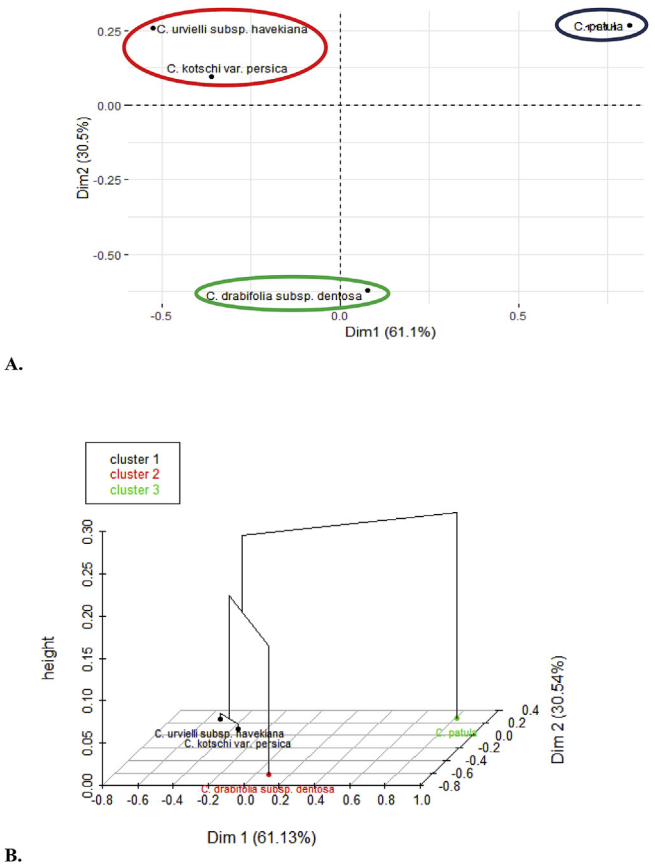

Fig. 2. Structuring of the variability observed based on the factorial map of MCA and the dendrogram AHC. A. factorial map of MCA B. dendogram of AHC partitioning the samples into three groups.

patterns and monoisotopic profiles of **6**, **7**, **8**, **10**, **11** and **12** were in good agreement with those of reference standards.

Peak **9** exhibited a loss of a hexosyl moiety $[M\text{-}H\text{-}162]^-$ (100%) at *m/z* 331.046 $[C_{16}H_{11}O_8]^-$ indicating *O*-glycoside. The fragmentation pattern involved the fragment ion $[Agl\text{-}H\text{-}15]^-$ at *m/z* 316.022 suggesting a methoxy group on the aglycone. Moreover, a prominent fragment ion at *m/z* 287.301 indicated concomitant losses of CH_3 (15 Da) and HCO (29 Da), while the low abundant ion at *m/z* 243.123 resulted from the loss of $(CH_3 + HCO + CO_2)$. No A- and B-ring fragments were observed. These data was consistent with 6-methoxylated flavonoid patuletin according to the Justesen's key for differentiation between methoxylated flavonoids (Justesen, 2001). Thus, **9** was identified as patuletin-*O*-hexoside. The fragmentation fingerprint of **15** ($[M\text{-}H]^-$ at *m/z* 299.056) was also consistent with that of 6-methoxylated flavon, and was tentatively identified as hispidulin.

Regarding **13** (quercetin), the precursor ion at 301.035 afforded a series of neutral losses at *m/z* 273.040 $[M\text{-}H\text{-}CO]^-$, 243.123 $[M\text{-}H\text{–}CH_2O\text{–}CO]^-$, 229.051 $[M\text{-}H\text{–}CO\text{–}CO_2]^-$. RDA cleavages generated $^{1,3}A^-$ at *m/z* 151.002, $^{1,2}A^-$ at *m/z* 178.997, $^{1,2}B^-$ at *m/z* 121.028, $^{0,4}A^-$ at *m/z* 107.012 and $[M\text{-}B\ ring]^-$ at *m/z* 193.014 (Table). In the same way, the compounds **14** ($[M\text{-}H]^-$ at 269.045) and **16** ($[M\text{-}H]^-$ at 285.040) were assigned to apigenin and luteolin, respectively. Typical RDA cleavages gave $^{1,3}A^-$ at *m/z* 151.002 (**14** and **16**), $^{1,3}B^-$ at *m/z* 133.029 (**16**) and 117.033 (**14**). The identification of aforementioned flavonoid aglycones was confirmed by comparison with authentic standards.

3.2. Variability analysis of the samples

To study the variability of the 4 samples, 16 qualitative variables represented by the 16 compounds have been used to perform the multiple correspondence analysis. The percentage of variability represented by the first two factors (1–2) is 91.6% (Fig 1A). The first factor summarizing 61.1% of total inertia is defined by the compounds 3-Caffeoylquinic acid, patuletin-O-hexoside, luteolin 7-O-rutinoside, quercetin 3-0-glucoside and 5-Caffeoylquinic acid (Fig 1B). The second explaining 30.6% of the total variability is determinated by caffeic acid (Fig 1B).

The projection of the individuals represented by the samples in the factorial plane 1–2 is derived from the data based on the presence-absence of 16 compounds in the different samples (Fig 2A). The graph analysis completed by a hierarchical classification based on the coordinates of samples on the first two factor of the multiple correspondence analysis generate 3 distinct group (Fig. 2A and B). The first group is represented by *C. urvielli* subsp. *hayekiana* and *C. kotschi* var. *persica* species while the second and third group is composed of *C. drabifolia* subsp. *detonsa* and *C. patula* respectively.

Biplot analysis of individuals (samples) and descriptors (compounds) shows that all of 4 species together contain 9 molecules which are, Protocatechuic acid, 5-Feruloylquinic acid, Orientin, Vitexin, Luteolin-7-O-glucoside, Isovitexin, Quercetin, Apigenin and Hispidulin

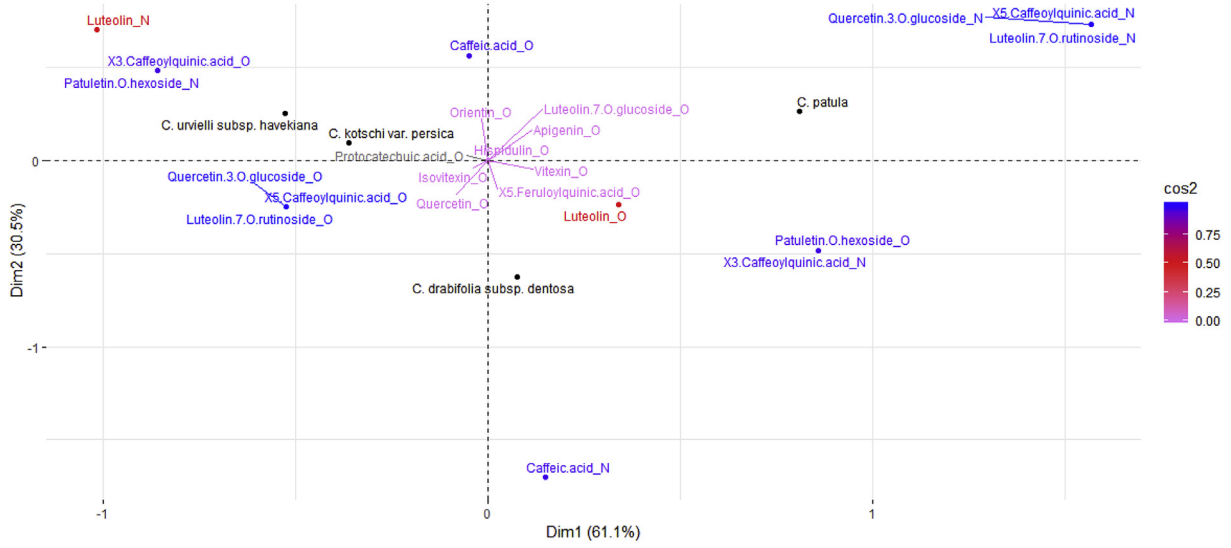

Fig. 3. Biplot analysis of samples and compounds.

(Fig 3). 3-Caffeoylquinic acid particularly characterizes the species of the first group while five compounds namely protocatechuic acid, quercetin-3-O-glucoside, 5-caffeoylquinic acid, patuletin-O-hexoside and caffeic acid, are isolated from species belonging to different groups. The three first compounds are present at the species of the group 1 and 2, the following at the group 2 and 3 and the latest compound at the group 1 and 3.

4. Conclusion

In conclusion, the study is the first attempt to compare the chemical profiles of four *Centaurea* species. Several bioactive compounds have been identified from these plant species that warrants further evaluation. Finding presented herein has established key baseline data that could spark further studies on the pharmacological potential of these *Centaurea* species and initiate future drug development programmes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101189.

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