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Comparative phenolic profiling and antioxidant activity of *Centaurea tougourensis* Boiss. & Reut. and *Centaurea dimorpha* Viv. from Algeria

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ABSTRACT

Centaurea L. plants are used in phytotherapy and as food. *C. tougourensis* (CT) and *C. dimorpha* (CD) are endemic of Algeria and have been scarcely studied up to now. Here, the phenolic content of their aerial parts was investigated using validated methods. Furthermore, the antioxidant properties of CT and CD were evaluated by performing DPPH, ABTS, FRAP, and phenanthroline assays. CD showed the highest total phenol (219.8 mg GAE/g) and total flavonoid (82.8 mg QE/g extract) contents. CT showed the highest flavonol content (46.3 mg QE/g). Chlorogenic acid, 4-OH-benzoic acid, and protocatechuic acid were the main compounds detected by LC-MS/MS. Several flavonoids were also detected in CD, while only hesperidin was detected in CT. Both the species showed antioxidant properties; however, radical scavenging activity and FRAP of CD were significantly higher than CT and were comparable with positive controls. Overall, this work affords a contribution to the characterisation and valorisation of the Algerian flora.

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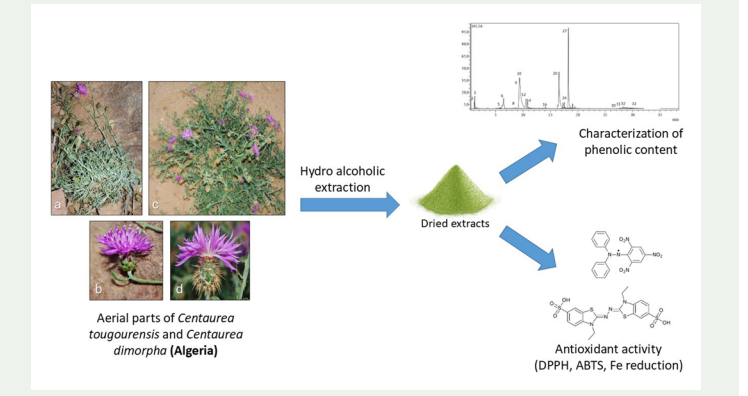

1. Introduction

The genus *Centaurea* L. (Asteraceae) comprises around 700 species that grow in Southern Europe, the Mediterranean Basin, Western Asia, and the American continent (Mabberley 2017; Nobarirezaeyeh et al. 2024). Of the 45 species naturally occurring in Algeria, seven are found in the Sahara Desert (Quezel and Santa 2015). *Centaurea* species are non-resinous or latex-free plants that grow in tufts or clumps, typically in spring, and are found in various habitats such as deserts and semi-deserts, steep slopes, high mountains, arable lands, periodically flooded areas, dry areas, and partially sun-exposed areas (Hellwig 2003). The common name of *Centaurea* in Algeria is 'Mers'gousse' (Bouafia et al. 2020). Many of the species belonging to the genus *Centaurea* are referenced for wide use in traditional medicine and have long been known in folk medicine for their wealth of natural healing substances used against several diseases. Several recent and older studies published in the literature have examined various species of *Centaurea* traditionally used to treat different ailments such as abscesses, haemorrhoids, gastric ulcers, microbial infections, and cancer. These species are also used as in food and pharmaceutical industries (Badalamenti et al. 2023; Bibi et al. 2024; Tüfekçi et al. 2024). For instance, the edible flowers of *Centaurea* plants are used as food supplements to ameliorate the symptoms of gastrointestinal atony, thanks to their stimulatory effects on the secretion of bile acids and motility of the gastrointestinal tract (Kubik et al. 2022).

Among the species growing spontaneously in Algeria are *C. tougourensis* Boiss. & Reut. (CT) and *C. dimoprha* Viv. (CD; Figure S1, Supplementary Material), which have been scarcely investigated up to now. Regarding CT, some studies have reported multiple bioactivities ranging from anti-inflammatory and neuroprotective, to antiulcer and antimicrobial (Bensaad, Dassamiour, Hambaba, Chawki, et al. 2021; Bensaad, Dassamiour, Hambaba, Kahoul, et al. 2022). Few phytochemical constituents putatively associated to these bioactivities have been described, mainly simple phenolic acids and few flavonoids (Nacer et al. 2006). To the best of our knowledge, a previous investigation on CD has only aimed to assess the chemical composition of essential oil (characterised by caryophyllene oxide, limonene, tetradecanoic acid, and

spathulenol mainly) and its antioxidant properties, measured by DPPH assay (Belkassam et al. 2019). To fill the knowledge gaps, in this study we focused on the phytochemical characterisation of the aerial parts of CP and CD and tested their antioxidant properties using different assays *in vitro*. Phytochemical characterisation was performed by spectrophotometric assays to determine the total polyphenol content. Individual compounds were then screened by LC-MS/MS. The method was validated for 37 bioactive natural compounds, comprising common polyphenols like phenolic acids and flavonoids (Table S1 of the Supplementary Material).

2. Results and discussion

2.1. Quantification of total phenolic, flavonoid and flavonol contents

Results of TPC, TFC, and TFL quantification in methanolic extracts of CP and CD are shown in Table S2 (Supplementary Material). Conferring to the data attained, the highest TPC (219.8 mg GAE/g) and TFC (82.8 mg QE/g extract) were detected in CD, and differences between the two plants were significant ($p < 0.05$). Conversely, TFL of CT (46.3 mg QE/g) was significantly greater than CD (29.01 mg QE/g). These amounts are higher compared to those already reported in other *Centaurea* species. For instance, (Albayrak et al. 2017) examined TPC and TFC of extracts of *C. aksoyi* and *C. amaena* obtained through maceration, Soxhlet and ultrasonication extractions and reported 12.92 mg GAE/g DW and 7.69 mg QE/g DW as highest TPC and TFC, respectively (*C. aksoyi*). However, other Authors reported TPC, TFC and TF amounts in other *Centaurea* species that were comparable to CP and CD, such as *C. castriferrei* (Kubik et al. 2022), *C. cyanus* (Escher et al. 2018), *C. kroumirensis* and *C. sicula* (Dhouibi et al. 2020). Overall, variations in phenolic content are expected among plant species of the same genus as this is influenced by genetic factors and growing conditions such as temperature, soil composition and altitude (Giorgi et al. 2010). Different extraction solvents and methods can also contribute to chemical variations (Escher et al. 2018).

2.2. LC-MS/MS method validation

Validation of the LC-MS/MS method was performed by evaluating linearity, precision, recovery, sensitivity (limits of detection: LODs), limits of quantification (LOQs), and specificity for each compound. Results are reported in Table S3 (Supplementary Material). The method showed good linearity ($r \geq 0.989$), precision (RSDs %: 0.058–3.209 for intra-day, 0.076–2.605 for inter-day), and reproducibility (the percentage of recovery ranged from 98.47 to 104.09%) in the ranges of concentration tested. The LODs and LOQs were 0.003–0.821 and 0.004–0.859 mg/L, respectively, indicating good performance limits. The relative standard uncertainties were within ±2.82% for all the analysed compounds.

2.3. LC-MS/MS characterisation of C. tougourensis and C. dimorpha extracts

Among the 37 phytocompounds monitored (Figure S2, Supplementary Material), 16 were determined in CD and 10 in CT (Table S4 and Figures S3 and S4, Supplementary

Material). Specifically, 8 phenolic acids were detected in CD, while 7 were found in CT. Phenolic acids determined in both extracts included p-coumaric acid, gallic acid, caffeic acid, salicylic acid, 4-OH-benzoic acid, chlorogenic acid, and protocatechic acid, albeit at varying concentrations. Chlorogenic acid was the most prevalent, with concentrations of 2689.66 µg/g and 3147.35 µg/g in CD and CT, respectively. In addition, 4-OH-benzoic acid (168.11 and 397.12 µg/g for CD and CT, respectively) and protocatechuic acid (82.2 and 137.49 µg/g for CD and CT, respectively) were similarly noticed. Sinapinic acid was exclusively found in CD, although in a low quantity.

Besides, 7 flavonoids were determined in CD (hesperidin, rutin, isoquercitrin, apigetrin, apigenin, rhoifolin, and nicotiflorin), with nicotiflorin being the most representative (5556.34 µg/g), followed by rutin (3319.17 µg/g). Conversely, only one flavonoid, namely hesperidin, was detected in CT, with a relatively low concentration of 79.35 µg/g. Additionally, two non-phenolic organic acids were identified, with quinic acid being the most abundant compound at 20533.79 µg/g and 571641.3 µg/g in CD and CT, respectively, followed by malic acid (2452.56 µg/g and 8402.23 µg/g for CD and CT, respectively). Fumaric acid was not detected in any of the studied extracts.

Previous research has reported sesquiterpene lactones, terpenes, flavonoids and acetylenes as major phytochemical components of *Centaurea* plants (Gürbüz and Yesilada 2007). Among flavonoids, quercetin, luteolin, kaempferol, salvigenin, apigenin, hispidulin, cirsimaritin, apigenin 7-O-glucoside and isokaempferide have been reported (Melikoglu et al. 2018). However, only a couple of articles on phytochemical characterisation of CT have been published up to now. In these, a total of ten compounds from ethyl acetate and dichloromethane extracts were reported, six of them being flavonoid aglycones (3′-O-methyleupatorin, jaceosidine, nepetine, eupatiline, apigenin, kaempferol), while the other were sesquiterpene lactones [8α-(3,4-dihydroxy-2-methylene-butanoyloxy)-dehydromelitensin, cnicin, (6R, 7R, 8S, 3′R) 8α-(3, 4-dihydroxy-2-methylene-butanoyloxy)-15-acetoxy-helianga 1(10), 4(5), 11(13) trien-6-olide, and (6R, 7R, 8S, 3′R) 8α-(3, 4-dihydroxy-2-methylene-butanoyloxy)-15-oxo-helianga 1(10), 4(5), 11(13) trien-6-olide] (Nacer et al. 2006; 2012). Regarding CD, the results presented here are the first concerning its composition in polyphenols since previous works have focused merely on its volatile constituents (Belkassam et al. 2019). Overall, our results represent a further contribution to the phytochemical characterisation of these plant species and to the identification of bioactive compounds potentially linked to their therapeutic effects.

According to Sokovic et al. (Sokovic et al. 2017) flavonoids and sesquiterpene lactones are mainly responsible for the biological activities of *Centaurea* spp. Among the main compounds found in our study, nicotiflorin may represent the most important flavonoid for CD, due to its antioxidant (Vergallo et al. 2019), hepatoprotective (Zhao et al. 2017), neuroprotective (Li et al. 2006) and anti-inflammatory effects (Wang et al. 2018). Conversely, CT was characterised by a low content of flavonoids but presented a higher content of quinic acid and chlorogenic acid than CD. Both compounds represent potential bioactive constituents of CD but also CT, considering that they have been previously associated to several bioactivities like antioxidant, anti-inflammatory and antimicrobial (Jang et al. 2017; Bai et al. 2022; Feng et al. 2023; La Rosa et al. 2023).

2.4. Antioxidant capacity in vitro

Despite numerous studies evaluating the antioxidant capacity of plant extracts, a single method capable of fully measuring this capacity has not yet been established. Consequently, it is required to evaluate antioxidant activity through a series of chemical analyses. In this context, our study employed various antioxidant assays, including DPPH, FRAP, ABTS, and phenanthroline assay. The results, shown in Table S5 (Supplementary Material), demonstrate a significant difference in antioxidant potential between the two plants, with CD being more active than CT in the different assays. Notably, CD showed a comparable or even higher activity than positive standards in almost all the assays, except for the phenanthroline assay. Overall, these results can be associated to the amounts of polyphenols in the two *Centaurea* species, considering that the species presenting the highest TPC, TFC, and TF were those with the highest antioxidant capacity. This association is corroborated by existing literature, which provides extensive evidence of the antioxidant properties of polyphenols. However, it must be highlighted that other compounds not included in our analyses may be co-responsible of the antioxidant properties of the two *Centaurea* extracts. This aspect deserves further investigation.

In a work by Bensaad et al. (Bensaad, Dassamiour, Hambaba, Chawki, et al. 2021), organic extracts of *C. tougourensis* aerial parts revealed a significant antioxidant capacity in different assays, being the n-butanol extract the most active. Regarding *C. dimorpha*, Belkassam et al. reported a percentage of inhibition of the DPPH radical of 77.01 at a concentration of 1 M (Belkassam et al. 2019). The antioxidant activity of various *Centaurea* spp. has been extensively documented. For instance, Marian et al. reported that the DPPH scavenging activity of *C. cyanus* from Romania reached an inhibition percentage of 83.42% (Marian et al. 2017). Surveys into *C. calcitrapa* subsp. *calcitrapa*, *C. spicata*, and *C. ptosimopappa* proved antioxidant potential across all three species. Notably, the highest activity on DPPH was observed for the aqueous extracts of *C. calcitrapa* and *C. spicata*, whereas it was more pronounced in the *C. ptosimopappa* methanol extract. This scavenging activity exhibited a skyward trend with increasing extract concentrations across all species (Erol et al. 2011). More recently, antiradical properties against DPPH and ABTS were reported also for *C. antitauri* (dichloromethane extract) and *C. amanicola* (ethyl acetate extract) (Nobarirezaeyeh et al. 2024).

Our results on CT and CD agree with all these data: they show that both the species can act as antioxidants by scavenging radicals such as DPPH and ABTS or exerting reductive effects on transition metals (Fe). However, it must be highlighted that all these data on the bioactivity of *Centaurea* spp. have to be considered as preliminary, since their antioxidant properties should be reproduced in more complex models *in vitro* (i.e. cells or isolated tissues and organs) and *in vivo*.

3. Experimental

Materials and methods are exhaustively reported in the Supplementary Material.

4. Conclusions

In conclusion, the objective of this work was to analyse the phenolic profile of hydroalcoholic extracts derived from the aerial parts of *C. dimorpha* and *C. tougourensis*, including total phenolic, flavonoid, and flavonol contents and targeted phenolic compounds of particular interest, along with their potential antioxidant effects. Our findings revealed that both the species represent valuable sources of polyphenols such as chlorogenic acid and nicotiflorin, and other bioactive secondary metabolites like quinic acid. Both extracts demonstrated promising antioxidant potential across various assays, with *C. dimorpha* displaying higher activity, comparable to that of known antioxidants such as tocopherol and ascorbic acid. These results suggest that *C. dimorpha* and *C. tougourensis* could serve as original and alternative sources of natural antioxidants for food applications or in phytotherapy. However, further experiments are required to confirm these potential biological activities in more complex *in vitro* and *in vivo* models. Furthermore, future studies should focus on elucidating the specific compounds responsible for the observed antioxidant activities.

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Abstract

Centaurea L. plants are used in phytotherapy and as food. *C. tougourensis* (CT) and *C. dimorpha* (CD) are endemic of Algeria and have been scarcely studied up to now. Here, the phenolic content of their aerial parts was investigated using validated methods. Furthermore, the antioxidant properties of CT and CD were evaluated by performing DPPH, ABTS, FRAP, and phenanthroline assays. CD showed the highest total phenol (219.8 mg GAE/g) and total flavonoid (82.8 mg QE/g extract) contents. CT showed the highest flavonol content (46.3 mg QE/g). Chlorogenic acid, 4-OH-benzoic acid, and protocatechuic acid were the main compounds detected by LC-MS/MS. Several flavonoids were also detected in CD, while only hesperidin was detected in CT. Both the species showed antioxidant properties; however, radical scavenging activity and FRAP of CD were significantly higher than CT and were comparable with positive controls.

Overall, this work affords a contribution to the characterization and valorisation of the Algerian flora.

Supplementary material

Experimental

Chemicals and reagents

Analytical standards, acetonitrile, LC-grade ammonium formate and formic acid were purchased from Sigma–Aldrich (Milano, Italy). Quercetin, Folin–Ciocalteu's reagent were obtained from Merck (Darmstadt, Germany). All other reagents were purchased from Sigma (St. Louis, MO, USA), unless indicated. Analytical grade reagents and solvents were consumed throughout the work.

Plant material

Whole aerial parts of CP and CD (Figure S1) were collected in a full bloom. CP was collected from the park national of Taza in the province of Batna and CD was collected in the province of M'sila. The species were identified by Dr. N. Souilah from Skikda University, Algeria. A voucher specimen was deposited in the Herbarium of the University of Constantine 1 for both the species (ID codes: CTlabo025 and CDlabo026, respectively).

Extraction of plant material

The aerial parts of the two species were dried at room temperature in shade for one week and powdered. Afterwards, 200 g of powder for each plant were exhaustively extracted by maceration in a mixture of methanol/water (80/20, v/v) at a ratio of 1:10 (w/v) for 24 h with constant stirring (speed of 200 rpm) and at room temperature. The solvents were evaporated at 40 °C using a Rotavapor (Büchi R-200, Germany) to afford 4.67 mg extract for *C. tougourensis* and 5.26 mg for *C. dimorpha*. Crude extracts were kept at 4 °C until further analysis.

Determination of phenolic content by chemical assays

Quantification of total phenolic content

The concentration of total phenolic compounds (TPC) was determined spectrophotometrically using the colorimetric assay with Folin-Ciocalteu's phenol reagent according to (Djeridane et al. 2006). Gallic acid was used as reference standard. The absorbance of all samples was measured at 765 nm and the results were expressed in mg gallic acid equivalents (GAE)/dry weight of the plant (g). Ultrapure water (18.2 MΩ·cm) was produced using a Milli-Q water purification system (MQ) from Millipore (Milford, MA, USA).

Quantification of total flavonoid content

The flavonoid content in extracts (TFC) was determined spectrophotometrically according to a previously published method (Quettier-Deleu et al. 2000). 0.5 mL of 2% aluminum chloride ($AlCl_3$) in methanol and 0.5 mL of extracts were mixed to form a complex flavonoid–aluminium. Absorbance was measured at 430 nm and quantification of compounds was performed using a calibration curve. Rutin was used as external standard for this purpose.

Quantification of flavonol content

Total flavonols (TFL) were determined according to the procedure described by (Adedapo et al. 2008). It consists in adding 500 µL of a 2% $AlCl_3$ solution and 750 µl of a sodium acetate solution to 250 µL of extract. After an incubation of 2.5 h in the dark, the absorbance is measured at 440 nm. The flavonol concentration is determined with reference to a calibration curve carried out with quercetin, and the results are expressed in mg equivalent of quercetin (QE) per gram of extract.

LC-MS/MS analysis of extracts

Samples were prepared by dissolving dried extracts in methanol at a concentration of 1 mg/L. Solutions were purified via a 0.2 µm syringe filter before being subjected to LC-MS/MS analysis. The quantitative profile of phytochemicals in CP and CD was determined by using a Shimadzu Nexera X2 LC-30AD UHPLC system coupled to an 8040 tandem mass spectrometer (MS) equipped

with an ESI source. An RP-C18 Insertil ODS-4 analytical column (100 mm x 2.1 mm, 2 µm; GL Sciences) was used for chromatographic separation. The column temperature was set to 35 °C. Eluent A (MQ water, 10 mM ammonium formate, and 0.1 % formic acid) and eluent B (acetonitrile) were used in the elution gradient. The gradient program was as follows: 5-20% B (0-10 min), 20% B (10-22 min), 20-50% B (22-36 min), 95% B (36-40 min), 5% B (40-50 min). The injection volume was 4 µL and the solvent flow rate was kept at 0.25 mL/min. The ESI source was operating in negative ion mode (ESI-). The MS working conditions were as follows: interface temperature, 350°C; DL temperature of 250°C; thermal block temperature of 400°C; nebulization gas flow of 3 L/min; and drying gas stream of 15 L/min. LabSolutions software (Shimadzu) was used for data collection and processing.

The quantitative LC-MS/MS method allowed to analyse 37 phytocompounds in CP and CD extracts in a targeted mode. Common phenolic compounds such as chlorogenic acid, caffeic acid, gallic acid, vanillic acid, and p-coumaric acid were included, as well as flavonoids such as hesperidin, quercetin, quercitrin, kaempferol and apigenin (Table S1). Compounds were chosen considering different criteria: 1) wide diffusion in the plant kingdom; 2) evidence of multiple beneficial effects on human health, according to the scientific literature [e.g., (Kiokias and Oreopoulou 2021; Al-Khayri et al. 2022; Sun and Shahrajabian 2023)]; 3) availability and affordability of reference standards. To achieve the highest sensitivity, specific fragmentations of the different targeted compounds were monitored through multiple reaction monitoring (MRM), as shown in Table S1. A representative chromatogram of standards is reported in Figure S2.

Determination of antioxidant activity of extracts

Free radical scavenging activity (DPPH assay)

Free radical scavenging activity was determined spectrophotometrically using the 2,2-diphenyl-1-trinitrophenylhydrazine (DPPH) assay (M.S. Blois 1958). 40 µl aliquots of different concentrations of samples (extracts and standards) were added to 160 µl of DPPH in methanol (0.1 mM) in a 96-

well microliter plate. In addition to DPPH reagent, a blank sample was prepared using methanol. After incubation at 37°C for 30 minutes, the absorbance of each solution at 515 nm was measured with a microplate reader. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), alpha tocopherol, and ascorbic acid were used as standards for activity comparison.

ABTS cation radical decolourisation assay

ABTS scavenger activity was determined by the method published in (Re et al. 1999) with slight modifications. $ABTS^{\cdot+}$ was prepared from ABTS (7 mM) and potassium persulfate (2.45 mM). The aqueous solutions of the two products were mixed and stored at room temperature in the dark for 12-16 hours. A 96-well microplate was added with 40 µL of each dilution of the different plant extracts, then 160 µL of $ABTS^{\cdot+}$ solution was added to the samples. After 10 min, the percent inhibition of each concentration relative to the absorbance of the blank at 734 nm was calculated. The scavenging ability of $ABTS^{\cdot+}$ was calculated using the following formula, and the results were expressed as IC_{50} values (µg/mL).

$$ABTS^{\bullet+} \text{ scavenging activity (\%)} = [(A_{Control} - A_{Sample})/ A_{Control}] \times 100$$

Reducing power (FRAP assay)

The method described by (Oyaizu 1986) was employed to assess the reducing capacity of each extract, with slight modifications. In brief, 10 µL of extracts (or standard antioxidants) at various concentrations (12.5-800 µg/mL) were combined with 40 µL of phosphate buffer (pH 6.6) and 50 µL of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 minutes. Afterwards, 10% chloroacetic acid (50 µL) was added to the reaction, and the mixture was centrifuged at 3000 rpm for 10 minutes. Subsequently, 40 µl of distilled water and 10 µl of $FeCl_3$ solution (0.1%) were added, and the absorbance was measured at 700 nm.

Phenanthroline test

The method outlined by Szydlowska-Czerniak et al. (Szydłowska-Czerniak et al. 2008) was followed with minor modifications to fix the activity of phenanthroline. BHT was used as a standard in this assay, which depends on the reduction of Fe^{3+} to Fe^{2+} ions by an antioxidant. The Fe^{2+} ions react with ortho-phenanthroline to form an orange-red complex. In this procedure, 10 µl of various dilutions of the plant extracts were combined with 50 µl of $FeCl_3$ solution and 30 µl of 1,10-phenanthroline solution in a volumetric flask, and the volume was adjusted to 110 µl with methanol. The resulting solution was mixed and left at room temperature in the dark. After 20 minutes, the absorbance of the orange-red solution was measured at 510 nm. A blank sample was prepared in the same manner, substituting the extract with the solvent used (methanol).

Statistical analysis

All the analyses were performed in triplicate and results are presented as mean ± standard error (SE). Statistical analyses and data manipulation were conducted using GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA). Treatment comparisons between groups were assessed via one-way ANOVA followed by post-hoc Tukey's test. Significance levels were denoted as * for $P<0.05$, ** for $P<0.01$, *** for $P<0.001$, **** for $P<0.0001$, and "ns" indicating no significance ($P\geq0.05$). Additionally, the IC_{50} values were determined using the GraphPad Prism software.

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FIGURES

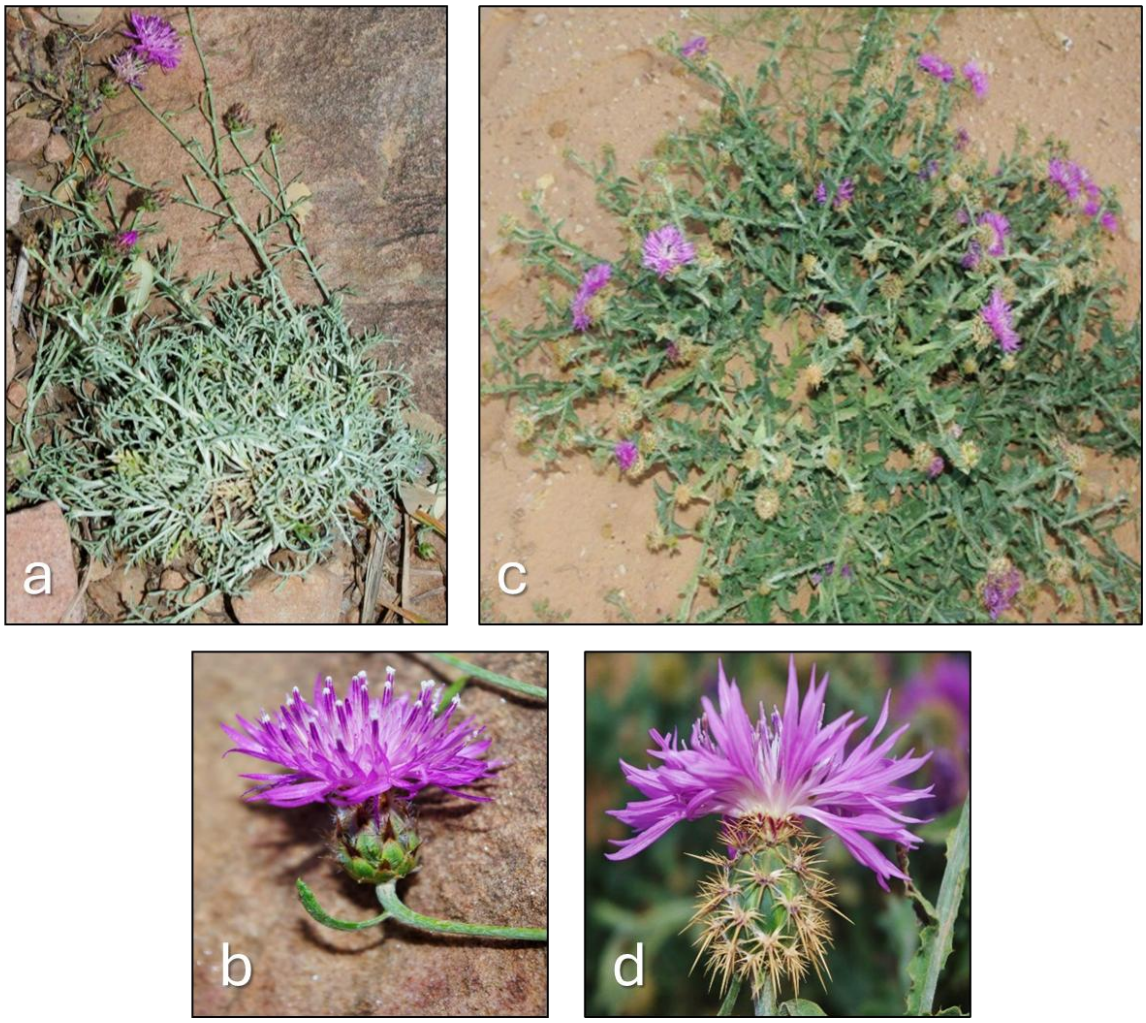

Figure S1. Aerial parts of *C. tougourensis* (a) and *C. dimoprha* (c) collected from Algeria. Panels b and d show details of *C. tougourensis* and *C. dimoprha*, respectively.

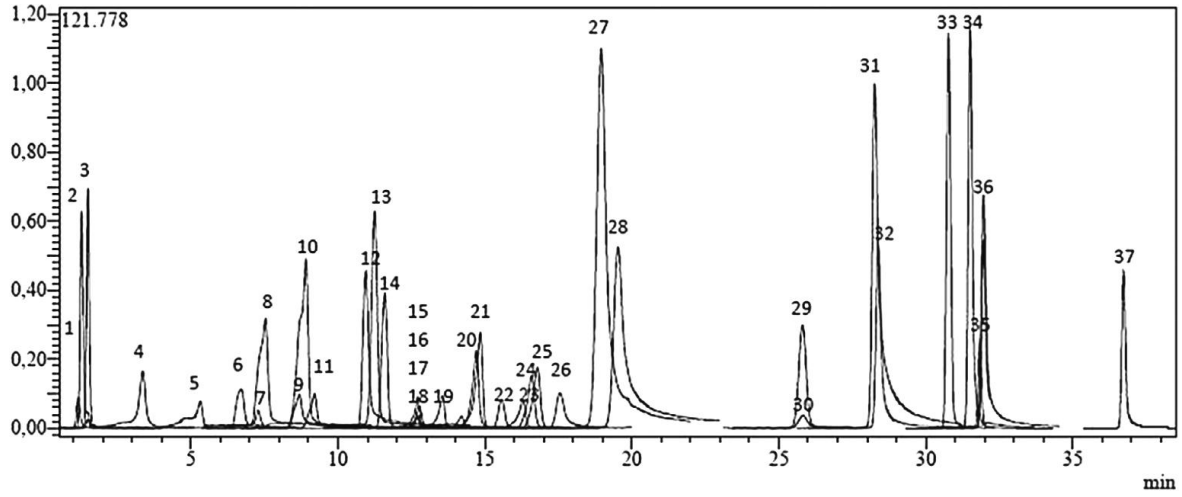

Figure S2. LC-MS/MS chromatograms of the standards mixture (1 µg/ml). Legend**:** (1) quinic acid, (2) malic acid, (3) fumaric acid, (4) gallic acid,(5) protocatechic acid, (6) pyrocatechol, (7) chlorogenic acid, (8) 4-OH-benzoic acid, (9) vanillic acid, (10) caffeic acid, (11) syringic acid, (12) vanillin, (13) salicylic acid, (14) *p*-coumaric acid, (15) rutin, (16) *t*-ferulic acid, (17) sinapic acid, (18) hesperidin, (19) isoquercitrin, (20) rosmarinic acid, (21) nicotiflorin, (22) *α*-coumaric acid, (23) rhoifolin, (24) quercitrin, (25) apigetrin, (26) coumarin, (27) myricetin, (28) fisetin, (29) cinnamic acid, (30) liquiritigenin, (31) quercetin, (32) luteolin, (33) naringenin, (34) apigenin, (35) hesperetin, (36) kaempferol, and (37) chrysin.

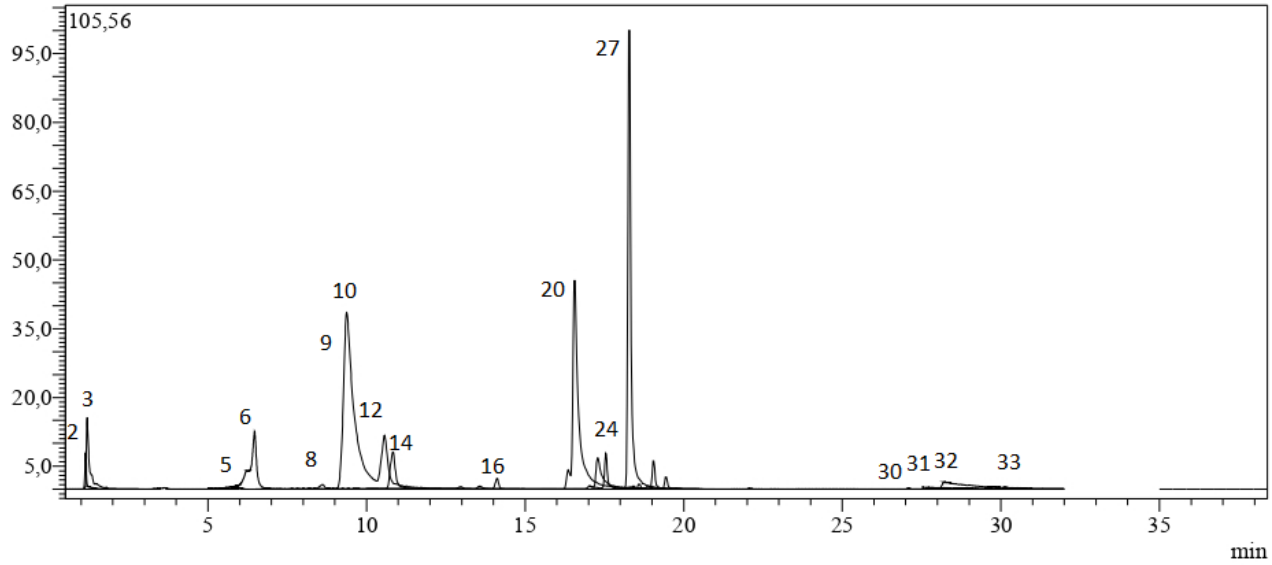

Figure S3. LC-MS/MS chromatograms of the *C. dimorpha* extract.

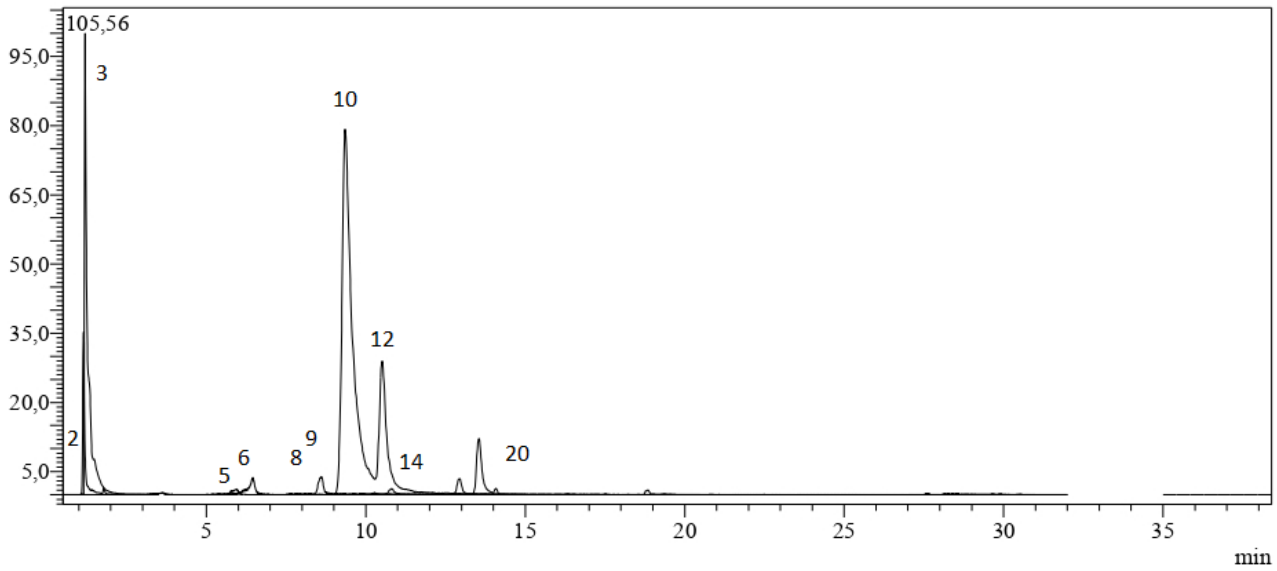

Figure S4. LC-MS/MS chromatograms of the of *C. tougourensis* extract.

TABLES

Table S1. Optimal conditions used for the analysis of 37 phenolic compounds in CP and CD extracts. Data were collected in negative ionization mode using the multi-reaction monitoring (MRM).

No.	Compounds	Retention Time (min)	Parent ion $[M-H]^-$ (m/z)	MS2 fragments or product ions (m/z)
1	Quinic acid	1.13	190.95	85.3-93.3
2	Malic acid	1.23	133.00	115.2-71.3
3	Fumaric acid	1.48	115.00	71.4
4	Gallic acid	3.00	168.85	125.2-79.2
5	Protocatechic acid	4.93	152.95	108.3
6	Pyrocatechol	6.48	109.00	108.35-91.3
7	Chlorogenic acid	7.13	353.15	191.2
8	4-OH-Benzoic acid	7.39	136.95	93.3-65.3
9	Vanillic acid	8.57	166.90	152.3-108.3
10	Caffeic acid	8.80	178.95	135.2-134.3
11	Syringic acid	9.02	196.95	182.2-167.3
12	Vanillin	10.87	151.00	1363-92.2
13	Salicylic acid	11.16	136.95	93.3-65.3
14	p-Coumaric acid	11.53	162.95	119.3-93.3
15	Rutin	12.61	609.05	300.1-271.1
16	tr-Ferulic acid	12.62	192.95	178.3
17	Sinapic acid	12.66	222.95	208.3-149.2
18	Hesperidin	12.67	609.00	301.1
19	Isoquercitrin	13.42	463.00	300.1-271.1
20	Rosmarinic acid	14.54	359.00	161.2-197.2
21	Nicotiflorin	14.68	593.05	285.1-255.2
22	α-Coumaric acid	15.45	162.95	119.4-93.3
23	Rhoifolin	16.11	577.05	269.2-211.1
24	Quercitrin	16.41	447.15	301.1-255.1
25	Apigetrin	16.59	431.00	268.2-239.2
26	Coumarin	17.40	147.05	91.0-103.2
27	Myricetin	18.72	317.00	179.2-151.3
28	Fisetin	19.30	284.95	135.2-121.3
29	Cinnamic acid	25.61	147.00	103.15-77.3
30	Liquiritigenin	25.62	254.95	119.3-135.1
31	Quercetin	28.17	300.90	151.2-179.2
32	Luteolin	28.27	284.75	133.2-151.2
33	Naringenin	30.68	270.95	151.2-119.3
34	Apigenin	31.43	268.95	117.3-151.2
35	Hesperetin	31.76	300.95	164.2-136.2
36	Kaempferol	31.88	284.75	255.1-117.3
37	Chrysin	36.65	252.95	143.3-119.4

Table S2. Total phenolic, flavonoid and flavonol contents of hydroalcoholic extracts of *C. dimorpha* and *C. tougourensis*

Samples	Total Phenols (mg GAE/g dry weight)	Total Flavonoids (mg QE/g dry weight)	Flavonol content (mg QE/g dry weight)
C. dimorpha	219.80 ± 0.47 *	82.81 ± 0.9 *	29.03 ± 0.20
C. tougourensis	191.90 ± 0.29	50.50 ± 0.06	46.32 ± 0.10 *

GAE: Gallic acid equivalent; QE: Quercetin equivalent. * P <0.05.

Table S3. Concentration range, linearity (R2), Limits of Detection (LODs), Limits of Quantification (LOQs) and percentages of recoveries resulting for tested compounds.

No.	Compounds	Conc. range (linearity range) (µg/ml)	R2	LOD (µg/ml)	LOQ (µg/ml)	Inter-day (n=3) RSD (%)	Intra-day (n=3) RSD (%)	Recovery% (n = 3)		U (%)
								Inter-day	Intra-day	
1	Quinic acid	0.250-10	0.996	0.075	0.079	0.259	0.274	100.28	98.77	0.0082
2	Malic acid	0.250-10	0.999	0.055	0.067	0.477	0.527	101.26	99.83	0.0113
3	Fumaric acid	0.10-5	0.997	0.028	0.034	0.536	0.460	99.74	99.86	0.0124
4	Gallic acid	0.250-10	0.998	0.095	0.106	1.601	01.443	100.00	100.45	0.0282
5	Protocatechic acid	0.100-5	0.995	0.028	0.031	1.236	1.296	99.40	101.07	0.0411
6	Pyrocatechol	1-20	0.996	0.261	0.278	1.313	1.339	99.98	99.93	0.0235
7	Chlorogenic acid	0.025-1	0.998	0.006	0.008	0.058	0.076	100.80	99.96	0.0069
8	4-OH-Benzoic acid	0.250-10	0.998	0.033	0.038	1.284	1.538	99.66	100.05	0.0289
9	Vanillic acid	0.1-20	0.999	0.122	0.139	0.528	0.619	100.09	104.09	0.0508
10	Caffeic acid	0.025-1	0.998	0.018	0.022	1.454	1.469	100.91	98.82	0.0354
11	Syringic acid	0.1-20	0.996	0.021	0.233	1.049	1.345	99.92	99.97	0.0238
12	Vanillin	0.250-10	0.998	0.044	0.053	0.696	0.793	99.67	99.61	0.0280
13	Salicylic acid	0.025-1	0.989	0.005	0.006	1.016	1.242	100.98	99.01	0.0329
14	p-Coumaric acid	0.025-1	0.992	0.007	0.009	1.820	1.727	100.61	101.22	0.0516
15	Rutin	0.025-1	0.997	0.005	0.006	0.473	0.624	100.99	98.01	0.0159
16	tr-Ferulic acid	0.250-10	0.997	0.036	0.042	0.708	0.619	99.98	100.28	0.0494
17	Sinapic acid	0.250-10	0.992	0.078	0.086	1.446	1.517	100.16	99.96	0.0281
18	Hesperidin	0.025-1	0.998	0.003	0.004	0.945	1.126	101.73	101.26	0.0262
19	Isoquercitrin	0.025-1	0.999	0.005	0.006	0.682	0.515	100.59	100.72	0.0133
20	Rosmarinic acid	0.100-5	0.994	0.006	0.008	2.014	1.751	99.20	103.43	0.0713
21	Nicotiflorin	0.100-5	0.991	0.022	0.025	0.737	0.875	102.55	100.97	0.0276
22	α-Coumaric acid	0.025-1	0.999	0.024	0.031	2.730	2.566	98.34	99.06	0.0513
23	Rhoifolin	0.100-5	0.999	0.023	0.027	0.747	1.528	101.04	101.73	0.0941
24	Quercitrin	0.100-5	0.999	0.022	0.025	1.528	2.320	99.72	100.62	2.0079
25	Apigetrin	0.025-1	0.993	0.005	0.006	1.797	1.607	101.39	100.41	0.0597
26	Coumarin	1-20	0.994	0.208	0.228	1.306	1.239	99.94	100.08	0.0237
27	Myricetin	0.250-10	0.999	0.053	0.057	0.652	0.711	99.98	100.04	0.0126
28	Fisetin	0.250-10	0.991	0.054	0.051	0.557	0.820	99.87	100.03	0.0148
29	Cinnamic acid	5-20	0.996	0.821	0.859	0.648	0.816	100.05	99.92	0.0143
30	Liquiritigenin	0.025-1	0.996	0.005	0.006	1.849	1.738	100.33	99.95	0.0341
31	Quercetin	0.100-5	0.990	0.023	0.028	1.589	1.360	98.47	100.10	0.0543
32	Luteolin	0.025-1	0.997	0.005	0.006	0.575	0.696	100.77	99.52	0.0174
33	Naringenin	0.025-1	0.995	0.005	0.006	2.054	2.019	99.88	101.00	0.0521
34	Apigenin	0.025-1	0.990	0.005	0.006	2.304	2.204	101.44	101.33	0.0650
35	Hesperetin	0.025-1	0.997	0.005	0.006	3.209	2.605	98.85	99.43	0.0562
36	Kaempferol	1-20	0.992	0.206	0.214	1.436	1.070	99.97	99.85	0.0209
37	Chrysin	0.025-1	0.993	0.005	0.006	0.490	0.630	100.33	100.43	2.0083

RSD %: relative standard deviation.

U (%): uncertainty percent at 95% confidence level (k = 2).

Table S4. Quantitative determination of secondary metabolites in the hydroalcoholic extracts of *C. dimorpha* and *C. tougourensis* by LC-MS/MS. Relative standard deviations (RSDs %) of three independent measurements were in a range from 0.90 to 3.15%.

N°	Compounds	MeOH 80% extracts (µg/g extract)	
		C. dimorpha	***C. tougourensis***
1	Quinic acid	20533.79	571641.3
2	Malic acid	2452.56	8402.23
3	Fumaric acid	ND	ND
4	Gallic acid	64.75	52.09
5	Protocatechic acid	82.2	137.49
6	Pyrocatechol	ND	ND
7	Chlorogenic acid	2689.66	3147.35
8	4-OH-Benzoic acid	168.11	397.12
9	Vanillic acid	ND	ND
10	Caffeic acid	60.37	5.11
11	Syringic acid	ND	ND
12	Vanillin	ND	ND
13	Salicylic acid	6.6	26.88
14	p-Coumaric acid	57.2	17.38
15	Rutin	3319.17	ND
16	tr-Ferulic acid	ND	ND
17	Sinapic acid	7.09	ND
18	Hesperidin	61.06	79.35
19	Isoquercitrin	301.18	ND
20	Rosmarinic acid	ND	ND
21	Nicotiflorin	5556.34	ND
22	α-Coumaric acid	ND	ND
23	Rhoifolin	206.24	ND
24	Quercitrin	ND	ND
25	Apigetrin	131.77	ND
26	Coumarin	ND	ND
27	Myricetin	ND	ND
28	Fisetin	ND	ND
29	Cinnamic acid	ND	ND
30	Liquiritigenin	ND	ND
31	Quercetin	ND	ND
32	Luteolin	ND	ND
33	Naringenin	ND	ND
34	Apigenin	ND	ND
35	Hesperetin	ND	ND
36	Kaempferol	ND	ND
37	Chrysin	ND	ND
Total of compounds		**16**	**10**

ND: Not detected.

Table S5. Antioxidant activity of the hydroalcoholic extracts of *C. dimorpha* and *C. tougourensis*. Results are presented as IC_{50} values in μg/mL and are expressed as mean ± SD of three parallel measurements. Superscript letters indicate significance of data (a > b > c > d).

	DPPH	**ABTS**	**FRAP**	**Phenanthroline**
C. dimorpha	8.79 ± 0.19 [b]	1.92 ± 0.03 [b]	2.44 ± 0.06 [a]	8.85 ± 0.15 [c]
C. tougourensis	27.77 ± 0.60 [c]	7.75 ± 0.36 [d]	6.87 ± 0.08 [b]	35.02 ± 0.29 [d]
BHA	6.14 ± 0.41[a]	1.81 ± 0.10 [b]	NT	0.93 ± 0.07 [a]
BHT	6.55 ± 0.59 [a]	1.29 ± 0.30 [a]	NT	2.24 ± 0.17 [b]
α-tocopherol	13.02 ± 5.17 [b]	2.33 ± 0.21 [c]	34.93 ± 2.38 [c]	NT
Ascorbic acid	8.12 ± 0.02 [b]	1.12 ± 0.01 [a]	6.77 ± 1.15 [b]	NT

BHA: butylhydroxyanisole; BHT: butylhydroxyltoluene; NT: Not tested