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The chemical composition of the essential oils of two Mediterranean species of Convolvulaceae: *Convolvulus althaeoides* subsp. *tenuissimus* collected in Sicily (Italy) and *Calystegia silvatica* collected in Algeria

Larbi Derbak[a*], Alessandro Vaglica[b*], Natale Badalamenti[b,c], Antonella Porrello[b], Vincenzo Ilardi[b], Khellaf Rebbas[a], Bendif Hamdi[a] and Maurizio Bruno[b,c,d]

[a]Laboratory of Ethnobotany and Natural Substances, ENS de Kouba, Algiers, Faculty of Sciences, University of M'sila, Algeria; [b]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy; [c]NBFC, National Biodiversity Future Center, Palermo, Italy; [d]Centro Interdipartimentale di Ricerca "Riutilizzo bio-based degli scarti da matrici agroalimentari" (RIVIVE), University of Palermo, Palermo

ABSTRACT

Convolvulus L. and *Calystegia* R.Br. are two closely related genera of the Convolvulaceae family distributed in Asia, Mediterranean, Macaronesia, East Africa, and Arabia, including about 210 and 30 accepted species, respectively, of flowering plants, present as trees, shrubs, and herbs. The ethnomedical use of *Convolvulus* species dates to 1730s as they displayed profuse medicinal properties. In the present study, the not previously investigated chemical compositions of the essential oils from aerial parts of *Convolvulus althaeoides* subsp. *tenuissimus* (Sm.) Bat., collected in Sicily, and *Calystegia sylvatica* (Kit.) Griseb., collected in Algeria, were evaluated by GC-MS. The main components of the essential oil of the first one were *β*-caryophyllene (28.68%), *γ*-muurolene (23.75%), and *γ*-elemene (17.55%), whereas the *C. silvatica* essential oil was shown to be rich of valeranone (10.77%), viridiflorol (9.45%), and germacrene D (8.61%). Furthermore, a complete literature review on the ethno-pharmacological uses of *Convolvulus* and *Calystegia* species was performed.

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CONTACT Antonella Porrello antonella.porrello@unipa.it; Hamdi Bendif hamdi.bendif@univ-msila.dz
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*These authors contributed equally to this work.

1. Introduction

Convolvulaceae is a family of plants from the order of Solanales that includes around 1900 species found on all continents except for circumpolar areas (Staples and Noltie 2007). *Convolvulus* L. is a genus of the Convolvulaceae family, and it is one of the most medicinally and economically important. According to POWO (2023) it has about 210 accepted species of flowering plants, present as trees, shrubs, and herbs. The greatest diversity of *Convolvulus* plants has been found in Western and Central Asia, Mediterranean, Macaronesia, East Africa, and Arabia (Carine et al. 2003; Ranjbar et al. 2017), and among them some species have a very large distribution such as *Convolvulus lineatus* L., *Convolvulus prostratus* Forssk., *Convolvulus althaeoides* L., *Convolvulus arvensis* L. and *Convolvulus pilosellifolius* Desr (POWO 2023).

The use of species of *Convolvulus* genus in traditional medicine dates back to 1730s. In general, *Convolvulus* plants displayed profuse medicinal properties (Salehi et al. 2020) and they also occupied a prominent position in Ayurvedic medicine, where they have been employed as brain tonic during hypotensive syndromes (Shah and Bole 1960).

Convolvulus arvensis L. (vernacular name: 'Leli', 'Hiran khari') is a very common plant in Pakistan. The whole plant is laxative. The powder of the plant is given in chronic constipation and in diabetes. Plant paste is applied on rat bite and inflammation. Its decoction is expectorant and used in cough, flu, and in jaundice. It is very good blood purifier, so plant is ground along with black pepper and eaten in leprosy and in other skin diseases and also in piles and diarrhoea. The smoke of the plant purifies the air. It is favourite fodder of cattle and it is specially given to buffaloes as this plant improves the milk production (Iqbal et al. 2011). Always in Pakistan the roots are dried, crushed and taken orally one or two spoon as purgative. The herb is used for skin infections. Leaves are used as poultice and as antiseptic. Two spoons of the leaf decoction are used daily to regulate the abnormal menstrual cycle in woman (Aziz et al. 2016; Tariq et al. 2018). Other uses of this species in Pakistan include its application to hair to treat dandruff (Bibi et al. 2017; Khan et al. 2017), as diuretic and antiangiogenic (Khan et al. 2017), for cancer, kidney-bladder stones and liver heat (Yaseen et al. 2015). In Ladakh (India) *C. arvensis*, where it is known as 'Khri khrik', is used against rheumatic pain, cuts and wounds (Rinchen and Pant 2014). This species is utilised in Turkey (vernacular name: 'Sarmaşık', 'Tarla sarmaşığı', and 'Bağırsak out') for constipation as laxative, for stomach-ache (Cakilcioglu and Turkoglu 2010; Cakilcioglu et al. 2011) and against haemorrhoids (Güneş et al. 2017). Also, in Tuscany (Italy) the decoction of this plant, known as 'vilucchio, viluppio, filucchio, filuppio, and viticchio' is drank as laxative (Uncini Manganelli et al. 2002). *Convolvulus scammonia* L. ('Saghmouneya') is used in Iran for abortion and fever, as anthelminthic (Esmaeili et al. 2015), and its macerated gum-resin is applied against acne (Pasalar et al. 2022). The whole organs of *Convolvulus soldanella* L. have been reported in China to have an antiscorbutic, diuretic, febrifuge, and purgative effect (Hassine et al. 2014) whereas in India one teaspoon of dried powder of *Convolvulus microphyllus* Sieber ex Spreng. ('Bhumari') is taken orally with water for constipation (Katewa et al. 2004). A similar utilisation has been reported for *Convolvulus glomeratus* Choisy ('Ullayq') both in Qatar (Phondani et al. 2015) and in Pakistan (Qasim et al. 2014). *Convolvulus virgatus* Boiss.

"Adlam', 'hub al-risha' is a perennial herb growing on dry sandy and gravelly soils in Qatar and Pakistan (Baluchistan), S. Iran, Muscat, Oman. Boiled in water it is taken internally as a tonic, aphrodisiac and expectorant. Dried leaves crushed with sugar are taken as a treatment for jaundice (Phondani et al. 2015). *C. prostratus* is astringent and has been claimed to relieve mental fatigue and stresses. The juice of white-flowered variety of *C. prostratus* is a hallucinogenic remedy for hemicrania. Powder or decoction of *C. prostratus* roots is used to treat ear diseases, rheumatism, chronic bronchitis, fevers, nervous disorders, dysentery, and hair tonic, and to normalise high blood pressure. Its laxative, diuretic, emetic, and antiperiodic properties make it effective in the treatment of medical conditions like urinogenital disorders and animal stings (Salehi et al. 2020). In Saudi Arabia it is used for brain related disease, improve memory and skin disease (Shahat et al. 2015). In Algerian traditional medicines, one glass of the decoction or infusion of the aerial parts of *Convolvulus sabatius* subsp. *mauritanicus* (Boiss.) Murb. is given on empty stomach daily during one month for thyroid disorders management (Taïbi et al. 2021). *Convolvulus austro-aegyptiacus* Abdallah & Sa'ad and *Convolvulus pilosellifolius* Desr. are mainly used in Saudi Arabia as folk medicine for treatment of ulcer diseases (Al-Rifai et al. 2017). In Syria, the decoction of *Convolvulus betonicifolius* Mill., known as 'Maddadeh byda' is used internally for fever, loss of memory, insomnia, and as antispasmodic, laxative, diuretic, whereas the infusion of the leaves of *Convolvulus cantabrica* L. ('Maddadeh') is utilised as diuretic, and for kidney stone (Khatib et al. 2021).

An extremely related genus to *Convolvulus* is *Calystegia* R.Br. and many species of this genus has been reported, in the past, belonging to *Convolvulus*. Actually, it is an accepted genus with, according to POWO (2023), about thirty species distributed in the same areas of *Convolvulus*.

The halophyte *Calystegia soldanella* (L.) Roem. et Schult is a perennial herb that grows on coastal sand dunes worldwide. This plant has been extensively used in oriental traditional medicine for general consumption and as a type of herbal treatment, since it is considered to confer bioactive effects against rheumatic arthritis, sore throat, dropsy, scurvy, fever and diarrhoea (Kim et al. 2022, Hotta et al. 1989). Extracts from *C. soldanella* have been also used in Portuguese ethno-medicine to cure hydrops, paralysis, rheumatism, and scurvy (Gaspar 1999).

The leaves of *Calystegia sepium* (L.) R.Br. ('Curriola, Velucchio') are topically used in Campania (Italy) as wound shealing (Menale and Muoio 2014) and as antirheumatic (Di Sanzo et al. 2013), whereas in Morocco (Tarbouche laghrabe) for hypertension control (Idm'hand et al. 2022). In Syria, where it is known as 'Leblab elsiaj', a decoction of roots and flowering herbs are used to treat constipation (Khatib et al. 2021). Various parts *Calystegia japonica* Choisy (syn. *Calystegia pubescens* Lindl.), a perennial herbaceous vine widespread throughout Southeast and East Asia, are used as diuretics and tonics in traditional Chinese medicine (Hotta et al. 1989). *Calystegia hederacea* Wall. is a plant growing in India and East Asia. All parts of this plant are used for the treatment of menoxenia, gonorrhoea, etc., (Hotta et al. 1989) or, in China (Dawanwanhua), as antiphlogosis (Gao et al. 2019). In Greece the tea made from the leaves of *Calystegia silvatica* (Kit.) Griseb. ('agrabi') is drunk as a diuretic; however large doses are said to be hallucinogenic (Brussel 2004). This species has been used for the treatment of fever, urinary tract disorders, constipation and reduced bile

production, for rheumatoid arthritis as an anti-inflammatory, pain killer and as an anti-tuberculosis treatment (Dehyab et al. 2020; Karaköse 2022). Furthermore, In Southern Italy ('Cancola') it is externally applied for pimples (Savo et al. 2011).

Extensive researches on the non-volatile metabolites of plants belonging to *Convolvulus* genus have shown complex chemical profiles including flavonoids, steroids, terpenoids, carbohydrate, amino acids, anthraquinones, anthocyanidins, phenylpropanoids, coumarins, lignans, resins tannins, saponins, alkaloids, lipids, and caffeoylquinic acid derivatives. Their occurrence as well as the biological properties have been recently reviewed (Salehi et al. 2020).

As for the phytochemical investigation of *Calystegia* species several bioactive resin glycosides were isolated from *C. sepium* (Liang et al. 2022; Lv et al. 2022), *C. japonica* , *C. hederacea* (Ono et al. 2020, 2021a, 2021b, 2023a, 2023b), and *C. soldanella* (Takigawa et al. 2011a, 2011b; Ono et al. 2014a, 2014b, 2015, 2017). Flavone glucosides were identified in the leaves of *C. japonica*, *C. hederacea* (Hattori and Shimokoriyama 1956), and *C. soldanella* (Murai et al. 2015), anthocyanin pigments in *C. silvatica* (Imbert 1969), *C. hederacea*, *C. japonica*, *C. sepium*, and *C. soldanella* (Tori et al. 2000; Tatsuzawa et al. 2004), and tropane alkaloids in *C. sepium* (Goldmann et al. 1990; Asano et al. 2001; Scholl et al. 2001). Recently, the methanol and *n*-hexane extract of *C. silvatica*, collected from the north coast of Egypt, showed the presence of several fatty acids with potential antitumor properties (Youssef et al. 2023).

On the other hands, quite few papers have been published on the essential oils (EOs) of *Convolvulus* species and they will be discussed later on, whereas, at the best of our knowledge, no one species of *Calystegia* has been investigated for its EO composition.

In the frame of our on-going research on Mediterranean plants (Badalamenti et al. 2021, Badalamenti et al. 2022, Di Napoli et al. 2022, Lauricella et al. 2022) we decided to investigate the EO composition of the aerial parts of *Convolvulus althaeoides* subsp. *tenuissimus* (Sm.) Batt. (syn. *C. elegantissimus* Mill.) collected in Sicily, Italy, a plant never previously chemically investigated and described by Battandier (1890), and of *Calystegia silvatica* (syn *Convolvulus silvaticus* Kit.), collected in Algeria. The botanical description and the distribution of both species are enclosed in Supplementary Material.

2. Results and discussion

Hydrodistillation of *C. althaeoides* subsp. *tenuissimus* (**CAT**) flowering aerial parts gave a pale-yellow EO. Overall, eight compounds were identified, representing 96.42% of total components, listed in Table S1 according to their linear retention indices on a DB-5 MS column and classified into four classes based on their chemical structures. Sesquiterpene hydrocarbons (82.71%) was, by far, the principal class with *β*-caryophyllene (28.68%), *γ*-muurolene (23.75%), and *γ*-elemene (17.55%), as the main constituents. Monoterpene hydrocarbons represented only by limonene (6.47%) whereas *trans*-chrysantenyl acetate (4.97%) was the only compound of the oxygenated sesquiterpenes. Only three other articles have been published on EO of *Convolvulus* species. The first one concerns the essential oil of *Convolvulus persicus* L., a plant distributed in the Southern coasts of Caspian Sea. It was dominated, as **CAT**, by

sesquiterpene hydrocarbons (55.0%), chiefly *β*-caryophyllene (47.0%). Other notable components included dodecanal (8.8%), caryophyllene oxide (5.7%), tetradecanal (4.4%) and dihydroedulan I (4.4%) (Dehghan et al. 2015). Investigation on the other subspecies of *C. althaeoides*, *C. althaeoides* subsp. *althaeoides*, collected in Tunisia showed of different profile of its essential oil with respect to **CAT** (Hassine et al. 2014). In fact, although the main class of metabolites was represented by sesquiterpene hydrocarbons (36.3%), a large amount of oxygenated sesquiterpenes (34.7%), totally absent in **CAT**, and monoterpenes (24.5%) was observed. Among the sesquiterpene hydrocarbons the main constituents were germacrene D (12.5%), (*E*,*E*)-*α*-farnesene (5.8%) and *δ*-cadinene (4.7%), whereas *β*-caryophyllene, the main metabolites of **CAT**, occurred in limited amount (3.5%). Among oxygenated sesquiterpenes it is noteworthy the presence of *τ*-cadinol (11.8%). Also the EO of *C. arvensis*, collected in Saudi Arabia (Salamatullah 2022), was very rich in sesquiterpene hydrocarbons (66.07%) with cuprenene (34.72%), himachalene (16.50%) and longifolene (10.21%), metabolites never detected in the other EOs of *Convolvulus* studied so far. Oxygenated monoterpenes (22.52%), the second class of the EO, was composed almost exclusively by thymol (20.10%).

Hydrodistillation of *Calystegia silvatica* (**CS**) flowering aerial parts gave a pale-yellow EO. Overall, sixty-two compounds were identified, representing 91.80% of total components, listed in Table S2 according to their linear retention indices on a DB-5 MS column and classified into ten classes based on their chemical structures. The EO of **CS** was extremely rich of sesquiterpenoids (77.54%). Oxygenated sesquiterpenes (42.60%) was the most abundant class with valeranone (10.77%), viridiflorol (9.45%) and palustrol (6.67%) as main metabolites. Sesqiterpene hydrocarbons occurred in similar amount (34.94%) being germacrene D (8.61%), *β*-cadinene (6.19%) and *α*-cadinene (4.81%) the more representative. It is noteworthy the presence of good quantity of diterpenes (6.33%) [sclareoloxide (4.16%) and phytol (1.56%)] whereas all the other classes of compounds were present in limited and seminal quantity. The chemical profile of **Cs** was quite similar with that of the oil of *C. althaeoides* subsp. *althaeoides*, another species collected also in North Africa; in fact, the percentages of oxygenated sesquiterpenes, sesquiterpene hydrocarbons and germacrene D were numerically very close.

3. Experimental

3.1. Plant material

Aerial parts of *Convolvulus althaeoides* subsp. *tenuissimus*, at full flowering stage, were collected on the carbonate cliffs in the Tardara Gorges between Menfi and Sambuca di Sicilia (Agrigento province), Sicily, Italy at about 240 m s/l, 37°36′55" longitude N and 13°03′17" latitude E, in May 2023. Samples of *C. althaeoides* subsp. *tenuissimus*, identified by Prof. Vincenzo Ilardi, have been stored in the Herbarium of the University of Palermo, Italy (Vouchers No. 109812).

The aerial parts of *Calystegia silvatica* were collected in May 2023 at Bejaia region, situated in the north-east of Algeria (36°35′36 N and 04°36′37 E at 836 m altitude). Samples of *Calystegia silvatica* (Vouchers No. KR0101) was identified and stored by

Prof. K. Rebbas from Natural and Life Sciences Department, University of M'Sila (Algeria).

3.2. Isolation of volatile components

Air-dried samples (aerial parts, leaves and flowers) were ground in a Waring blender and then subjected to hydro-distillation for 3 h, according to the standard procedure described in European Pharmacopoeia (2020). The EOs were dried over anhydrous sodium sulphate and stored in sealed vial under N_2, at −20 °C, ready for the GC and GC-MS analyses. The sample yielded 0.06% and 0.04% of oils (*w/w*) (CAT and CS, respectively).

3.3. GC and GC-MS analysis

Analysis of EOs were performed according to the procedure reported by Badalamenti et al. (2023). GC-MS analyses were performed using a Shimadzu QP 2010 plus equipped with an AOC-20i autoinjector (Shimadzu, Kyoto, Japan) gas chromatograph equipped with a FID, a non-polar capillary column (DB-5 MS) 30 m × 0.25 mm i.d., film thickness 0.25 μm and a data processor. The oven program was as follows: temperature increase at 40 °C for 5 min, at a rate of 2 °C/min up to 260 °C, then isothermal for 20 min. Helium was used as carrier gas (1 mL min^{-1}). The injector and detector temperatures were set at 250 °C and 290 °C, respectively. 1 μL of EO solution (3% EO/hexane *v/v*) was injected with split mode 1.0; MS range 40–600. The percentage in Tables S1 and S2 are calculated with the TIC from MS peaks. The settings were as follows: ionisation voltage, 70 eV; electron multiplier energy, 2000 V; transfer line temperature, 295 °C; solvent delay, 3 min. Linear retention indices (LRI) were determined by using retention times of *n*-alkanes (C_8-C_{40}) and the peaks were identified by comparison with mass spectra and by comparison of their relative retention indices with WILEY275, NIST 17, ADAMS, and FFNSC2 libraries.

4. Conclusion

In conclusion, it is the first time that qualitative compositional analyses have been carried out on essential oil samples from two different species, *Convolvulus althaeoides subsp. tenuissimus* collected in Sicily (Italy) and *Calystegia silvatica* collected in Algeria genera of the Convolvulaceae family, by GC and GC/MS analyses. The chromatographic analysis highlighted how the *Convolvulus althaeoides subsp. tenuissimus* essential oil was rich in terpenoids, mainly sesquiterpene hydrocarbons (82.71%) such as *β*-caryophyllene (28.68%), *γ*-muurolene (23.75%), and *γ*-elemene (17.55%), as the main constituents, while small percentages of compounds belonging to the monoterpene hydrocarbons and oxygenated sesquiterpenes' classes were revealed. Essential oil of *Calystegia silvatica* was also extremely rich in sesquiterpenoids (77.54%), mostly oxygenated sesquiterpenes (42.60%) with valeranone (10.77%), viridiflorol (9.45%), and palustrol (6.67%) as main metabolites. Sesquiterpene hydrocarbons occurred also in good amount (34.94%), but in this case it is worth noting the presence of several diterpene compounds (6.33%) like sclareoloxide (4.16%) and phytol (1.56%).

Disclosure statement

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