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Article *in* Natural Product Research · September 2016
DOI: 10.1080/14786419.2016.1236095

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Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

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NATURAL PRODUCT RESEARCH, 2016
http://dx.doi.org/10.1080/14786419.2016.1236095

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Comparison of essential oils from *Cistus* species growing in Sardinia

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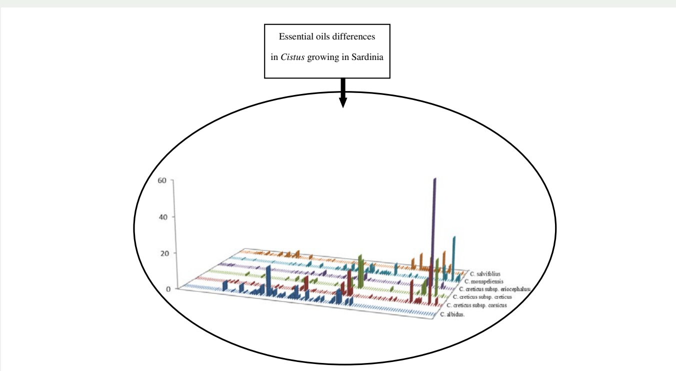
ABSTRACT

Cistus genus is present in Sardinia with large populations of *C. monspeliensis*, *C. salvifolius*, *C. creticus* subsp. *eriocephalus* and few stations of *C. albidus*, *C. creticus* subsp. *creticus* and *C. creticus* subsp. *corsicus*. No chemical studies are currently being carried on *Cistus* species of Sardinia. The essential oils have shown six different profiles. *C. creticus* subsp. *eriocephalus* showed a high amount of manoyl oxide and its isomer (70%). *C. salvifolius* has pointed out the group of labdans, (20%); another consistent percentage is made of perfumed molecules as ionone and its derivate. Several linear hydrocarbons were produced by *C. monspeliensis*, and the heneicosane was the most represented element. In *C. albidus* no labdane-type diterpenes were identified. Analysis of *C. creticus* subsp *creticus* revealed several oxygenated sesquiterpenes and labdane-type diterpenes, especially manoyl oxide. *C. creticus* subsp. *corsicus* was qualitatively very similar to *C. creticus* subsp. *creticus*, notably concerning the labdane-type compounds.



ARTICLE HISTORY
Received 16 February 2016
Accepted 8 August 2016

KEYWORDS
Cistus; essential oil; labdane; manoyl oxide

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Supplemental data for this article can be accessed here at http://dx.doi.org/10.1080/14786419.2016.1236095.

1. Introduction

Cistaceae family and its perennial shrubs grow mostly in the Mediterranean semi-arid ecosystem. Some of the species of this family are rich in flammable resins, and their overgrowth can lead to major environmental problems and wildfires (Ferrandis et al. 1999). These plants may serve as a good source of potential ingredients for further industrial applications. This family includes *Cistus* genus and some species are much appreciated in the perfume industry, and others have been used since ancient times in traditional folk medicine, notably in preventing and treating microbial disorders and illnesses.

The ancient ethnobotanical uses reveal that *Cistus* plants are an excellent remedy for various microbial infections (Bouamama et al. 2006); they are also well known and used as anti-inflammatory and antiulcerogenic (Ustün et al. 2006), wound healing and vasodilator remedies (Belmokhtar et al. 2009). All *Cistus* species secrete essential oils in different amounts that are mostly composed of monoterpene, sesquiterpene, and diterpene compounds, but not all of them are fully characterised. For example, the essential oil of *C. albidus*, completely characterised, showed a high content of sesquiterpenes bearing the bisabolene and cadinene skeleton (Paolini et al. 2008). Various reports have demonstrated the strength and effectiveness of the antimicrobial activity of *Cistus* essential oils (Bouamama et al. 2006). The antimicrobial activity of the essential oil of *C. salvifolius*, one of the extracts obtained by the use of organic solvents from *C. creticus* (Güvenç et al. 2005) and by *C. ladanifer* (Ferreira et al. 2012) has been fully documented and scientifically proven. The main part, responsible for the antimicrobial capacity of *Cistus* plants, seems to be the volatile fraction containing essentially terpenes, although relevant activity has also been reported regarding the polyphenolic fraction (Barrajón-Catalán et al. 2011). Currently, numerous manufacturers offer in the market herbal infusions (as '*Cistus* tea') as useful anti-oxidant supplement to prevent chronic diseases (Riehle et al. 2013) and *Cistus* essential oil is now officially approved by the Food & Drugs Administration as a food additive and flavouring agent (European Commission). Moreover, a new hypothesis on the use of *C. ladanifer* L. essential oil is to use it as herbicide because the oil showed herbicidal activity in *in vitro* bioassays (Verdeguer et al. 2012).

Cistus genus is widespread in Sardinia with different species that include large population of *C. salvifolius* L., *C. monspeliensis* L., *C. creticus* subsp. *eriocephalus* (Viv.) Greuter & Burdet. *C. albidus* L., *C. creticus* L. subsp. *creticus* and *C. creticus* L. subsp. *corsicus* (Loisel.) Greuter & Burdet. (endemic in Sardinia and Corsica) are present in few stations characterised by small populations and respectively situated in central, north-oriental and central-western Sardinia (Atzei 2003; Camarda & Valsecchi 2008; Arrigoni 2010).

The locals in Sardinia have traditionally used *Cistus* species as natural remedy in folk medicine. The leaves and flowers of these plants are traditionally used as anti-inflammatory, antiulcerogenic, wound healing, analgesic. These plants are ingested as decoctions and infusions in order to treat gastrointestinal problems. They are also made into poultices and ointments and applied directly on the infected wound or skin disease (Atzei et al. 1991; Atzei 2003).

These species are pioneering plants capable of withstanding high concentrations of metals and living in nutrient-starved environments, and their use in phytoremediation is of considerable interest (Jiménez et al. 2011). Most studies regarding *Cistus* species have been made on the essential oils composition of these shrubs in various Mediterranean regions, but no previous phytochemical researches have been reported on *Cistus* species growing in Sardinia. Only a previous study (Paolini et al. 2009) has been published on the chemical composition of *C. creticus* subsp. *eriocephalus* collected in North Sardinia. The aim of the present study is to analyse chemical composition of essential oils obtained from the *Cistus* plants growing in Sardinia.

2. Results and discussion

A yellow oil is derived from the hydrodistillation of the aerial parts of *Cistus* species collected in Sardinia. Overall, numerous compounds were identified for every species, representing from a minimum amount of 81.27% to a maximum of 99.43% of the total composition. The components, listed in Table S1 (see supplementary materials) according to their Retention index on a AT-5 column were further divided into classes on the basis of their chemical structures. Another large amount of secondary metabolites has been monitored in essential oils of the *Cistus* species used in this study; the samples have shown six profiles extremely diversified among them, characteristic of each species and subspecies.

C. salvifolius (53 components, amounting to 81.27% of the oil) showed a high quantity of norisoprenoids (10.44%), which were absent in the oils of the other *Cistus* we analysed, with members of the ionone family and β-damascenone as the most important components. Moreover, it presents the presence of cistodiol a clerodane diterpene. Among the diterpenes, the principal one seems to be the manoyl oxide (11.98%), which is also the main compound of the oil. Similarly, diterpenes were also a significant fraction of the metabolites identified in *C. salvifolius* populations in Tunisia and Sicily (Loizzo et al. 2013) and in Cretan populations with *cis*-ferruginol, manoyl oxide and 13-epi manoyl oxide (Demetzos et al. 2002). Sesquiterpene hydrocarbons are also represented by geranyl-p-cymene (9.61%), its most abundant compound. Finally, *C. salvifolius* showed the presence of several hydrocarbons and carbonyl compounds, but those were in low concentrations. Oils from *C. salvifolius* of plants growing at Aeolian Islands (Sicily) are very rich in sesquiterpenes (hydrocarbons 12.5%, and oxygenated 31.5%). Among the sesquiterpene hydrocarbons, the principal one was germacrene D (9.1%), also the main compound of the oil (Loizzo et al. 2013).

Among the Sardinian species, a total of 40 constituents (representing 92.15% of the total oil) have been identified from the essential oil extracted from *C. monspeliensis*. Linear hydrocarbons (41.36%) represent the main fraction of the oil and heneicosane (25.20%) was the principal compound. Sesquiterpenes are also present in good amount (14.53%). Diterpenes were present in quite low amount and only the phytol (4%) has been found in significant concentration. The essential oil of *C. monspeliensis* from Sardinia presented peculiar qualitative and quantitative results, compared to the other oils isolated from plants from different regions (Angelopoulou et al. 2002; Anghelopoulou et al. 2001; Jemia et al. 2013).

Chemical analysis of *C. albidus* essential oil preparations in Sardinia showed high content of sesquiterpenes (42%); in particular β-caryophyllene, β-bourbonene, ar-curcumene (16%) constituted the most acterised by a high content of sesquiterpenes with abundant molecules. Several oxygenated sesquiterpenes were also identified; *cis*-cadin-4-en-7-ol (7.43%) and τ-cadinol (6.22%) were the principal ones. The labdane-type diterpenes were absent, although they were present in all the other oils. The same, *C. albidus* tissue and essential oils preparations in North-eastern Spain, France and Italy showed high content of sesquiterpenes. In fact principal compound of *C. albidus* gathered in Spain, was identified as zingiberene (14.8%) (Palá-Paúl et al. 2005). The essential oil of *C. albidus* obtained from plants growing wild in Provence (France) is characterised by a high content of sesquiterpenes with α-zingiberene (12.8%) (Paolini et al. 2008; Maccioni et al. 2007). *Cistus albidus* is the only species of *Cistus* genus containing α-zingiberene that is also the main component. For this reason, this compound may be considered characteristic for this species (Robles & Garzino 1998).

Thirty-seven constituents, representing an amount of 94.15% of the total oil, have also been identified during the analysis of the essential oil of Sardinian *C. creticus* subsp. *eriocephalus*. The most peculiar characteristic of this essential oil is the presence of a consistent quantity of labdane-type diterpenes with manoyl oxide (64%) and its isomer (4.4%), which represent the most abundant components.

The oxygenated labdane diterpenes are considered chemotaxonomic markers and were found abundant in *C. creticus* subsp. *corsicus* (Paolini et al. 2009). Similarly, high concentration was measured for the labdane-type compound (manoyl oxide (9.9%) and 13-epi manoyl oxide (3.4%) in Cretan *C. creticus* subsp. *eriocephalus* leaf extract (Demetzos et al. 1997). Maggi et al. (2016) have found these compounds only in scant amounts (1.4%). Paolini et al. (2009) reported that no essential oil was obtained from the samples of *C. creticus* subsp. *eriocephalus* from different regions of Corsica and North Sardinia; the volatile fraction, instead, which was analysed by HS-SPME, was characterised by a significant amount of monoterpene hydrocarbons, especially myrcene and limonene.

Twenty components were identified in essential oil of *C. creticus* subsp. *creticus*, representing 99.26% of the total oil. Labdane-type diterpenes (38.70%) were a significant fraction of metabolites identified with manool (20.26%) and manoyl oxide (17%) being the most abundant. Oxygenated sesquiterpenes identified accounted for a total content of 49%, including selina-3,11-dien-6-α-ol (18%) and cis-cadin-4-en-7-ol (15.55%). Only a small proportion of sesquiterpenes but no monoterpenes (hydrocarbons or oxygenated) were identified. Several monoterpenes were present in essential oil of Cretan *C. creticus* subsp. *creticus*, but in small concentration (Demetzos et al. 1997; Demetzos et al. 1999). Further studies conducted on Cretan plants revealed the presence of several sesquiterpenes and oxygenated sesquiterpenes and labdane-type diterpenes, especially manoyl oxide and 13-epi-manoyl oxide. *C. creticus* subsp. *corsicus* (37 components, amounting to 94.6% of the total composition of the oil) is the only Sardo-Corse endemic species; the essential oil showed a similar amount and profile compared to *C. creticus* subsp. *creticus*. Similarly, diterpenes are present in good quantity (44.25%), with manool (21%) and 13-oxo-15,16-bis-nor-ent-labd-8(17)-ene (11.66%) as principal components. Several hydrocarbon sesquiterpenes and oxygenated sesquiterpenes were identified with τ-cadinol (13.63) being the most abundant. In comparison, the essential oils of *C. creticus* subsp. *corsicus* from Corsica were qualitatively rather similar, but different in the amounts of their major components (Paolini et al. 2009). In Table 1 is reported a comparison among the main components present in the analysed *Cistus* species.

We carried out an analysis of principal components (PCA), using the analytical data derived from essential oil. In Figure 1 are reported the score plot (a) and the loading plot (b). The Score plot shows a cluster formed by the three *C. creticus* subspecies. *C. salvifolius* remains very close to that cluster, this fact could be justified from that reported by Vogt et al. (1987). In that paper, the authors suggest possible hybrid origin of *C. creticus* subsp. *eriocefalus* with *C. salvifolius*. In the loading plot is noteworthy the distribution of the essential oils' components that are mostly in agreement with the position of the *Cistus* species in the score plot.

Table 1. Comparison among main constituents of the essential oils of *Cistus* species growing wild in Sardinia.

RI	Components	*C. albidus*	*C. creticus* subsp. *corsicus*	*C. creticus* subsp. *creticus*	*C. creticus* subsp. *erio-cephalus*	*C. monspeliensis*	*C. salvifolius*
1481	α-curcumene	16.01				0.72	
1640	τ-cadinol	6.22	13.63		3.47	3.37	
1644	selina-3,11-dien-6-α-ol		4.23	18.04			
1660	selin-11-en-4-α-ol			15.55	0.69	1.55	
1846	sclareol oxide (*cis*-A/B)		0.40	2.07	1.10		6.07
1965	geranyl-p-cymene						9.61
1972	cembrene A (3*E*)						8.55
1983	13-oxo-15,16-bis-nor-ent-labd-8(17)-ene		11.66	1.46	0.36		
1998	manoyl oxide	0.18	2.47	8.17	64.00		11.98
2021	3-methyl eicosane					8.11	4.67
2057	manool		21.07	20.26	2.82		
2100	heneicosane					25.20	0.38

3. Experimental

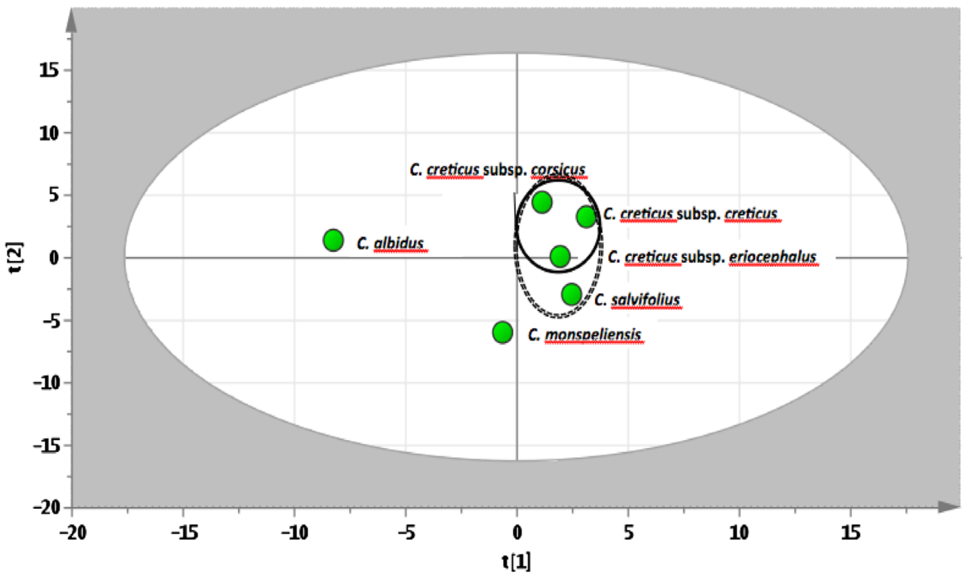
3.1. Plant material

The plant materials were obtained from several locations during the months of May and June 2014. We collected the plants all at the same vegetation stage (anthesis). We collected the aerial part of plants making sure to take plant material around all plants collecting material from the top, from the sides and from the base of scrubs. In the laboratory, the plant material was cleaned from other foreign plants. Very woody branches were removed and the samples were made as uniform as possible. The fresh material (aerial part) of *C. salvifolius* and *C. albidus* was collected from plants growing on Monte Ortobene, Nuoro (40°19′19.8″N 9°22′16.6″E, very close to the Park on the top of Monte Ortobene), while biomass (aerial part) of *C. monspeliensis* was collected from plants growing on Monte Albo, Oliena (40°30′7″N 9°35′24″E, at the base of M. Albo). The aerial parts of *C. creticus* subsp. *creticus* and *C. creticus subsp. eriocephalus* were harvested in Calagonone North-Oriental Sardinia (40°16′50″N e 9°37′47″E, the material has been picked up along the road leading to the inhabited centre) and *C. creticus subsp. corsicus* (aerial part) was collected in Central-Western Sardinia near Baratz Lake (40°40′51.24″N 8°13′32.88″E).

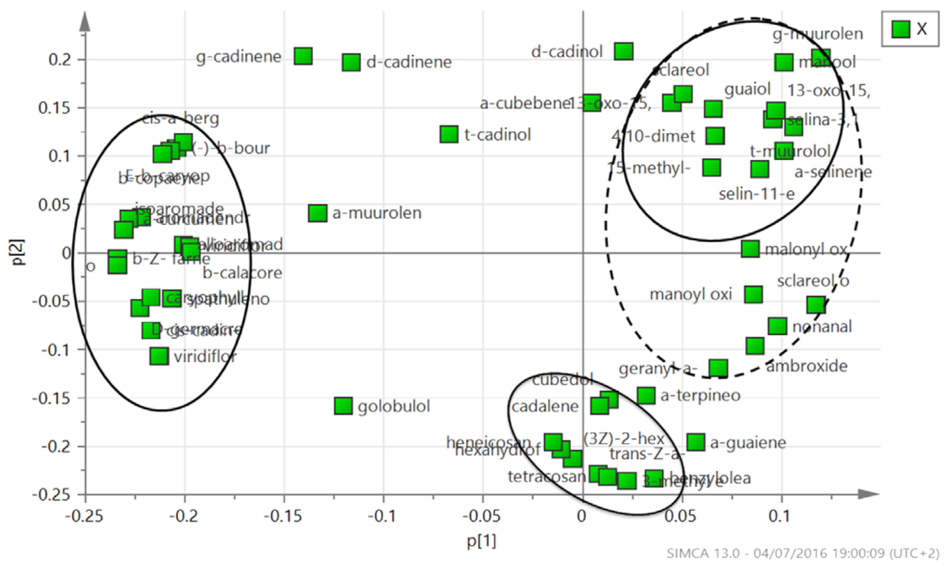
All species were identified by Prof. A.D. Atzei and a voucher specimen was deposited in the SASSA Herbarium of Department of Chemistry and Pharmacy – University of Sassari (collective numbers: *C. creticus* subsp *eriocephalus* n°132; C. *albidus* n°133; *C. creticus* subsp. *corsicus* n°134; .*C. salvifolius* n°134 bis, *C. monspeliensis* n°135; *C. creticus* subsp. *creticus* n°135 bis).

3.2. Oil distillation and yield

The essential oil samples were isolated from the fresh material (aerial parts) by hydro-distillation for 4 h using a Clevenger-type apparatus. The extraction yield calculated in mg of oil for 100 g of fresh material is reported in Table 2. The oils were stored in sealed vials, at −20 °C, ready for the chemical analysis.



Score plot of PCA (a)



Loading Plot of PCA (b)

Figure 1. PCA analysis and loading plot of PCA of essential oil of listed *Cistus* species.

3.3. Oil analyses

GC: Three replicates of each oil sample were analysed with the help of a Hewlett-Packard Model 5890A GC equipped with a flame ionisation detector and fitted with a 60 m × 0.25 µm AT-5 fused silica capillary column (Alltech Associates Inc., Deerfield, IL, US). Injection port and detector temperature were kept at 280 °C. The oven temperature program was set to rise from 50° to 135 °C at a rate of 3 °C min^{-1}, to further increase to 225 °C at a rate of

Table 2. Essential oil yields of listed *Cistus* species (mg/100 g) compared with literature data.

	C. monspeliensis	*C. salvifolius*	*C. albidus*	*C. creticus* subsp. *corsicus*	*C. creticus* subsp. *eriocephalus*	*C. creticus* subsp. *creticus*
Fresh plant	0.382	1.363	6.592	23.656	34.621	162.000
Dry plant	1.42	5.92	26.38	94.60	133.15	648.00
Robles & Garzino 2000	21					
Angelopoulou et al. 2002	450					
Loizzo et al. 2013	70	70			120	90
Robles & Garzino 1998			130			
Maggi et al. 2016					30	
Demetzos et al. 2002						1600
Paolini et al. 2009				300		

5 °C min^{-1}, then to remain at this temperature for 5 min, to increase until 260 °C at the same rate, and finally held for 10 min at the highest temperature. Samples of 0.2 µL (volume injection) were analysed, diluted in hexane using 2,6-dimethylphenol as internal standard. Injection was performed using a split/splitless automatic injector HP 7673 and helium as carrier gas. Several measurements of peak areas were performed with a HP workstation with a threshold set to 0 and peak width to 0.02. The quantisation of each compound was expressed as absolute weight percentage using internal standard and response factors (RFs). The detector RFs were determined for key components relative to 2,6-dimethylphenol and assigned to other components on the basis of functional group and/or structural similarity. *GC* checked the standards purity. Several RF solutions were prepared with only four or five components (plus 2,6-dimethylphenol) in order to prevent potential interferences from trace impurities.

GC/MS: MS analyses were carried out with a Agilent Technologies model 7820A connected with a MS detector 5977E MSD (Agilent), and using the same conditions and column described above. The column was connected with the ion source of the mass spectrometer. Mass units were monitored from 10 to 900 at 70 eV. The identification of compounds was based on comparison of their retention times with those of authentic samples and/or by comparison of their mass spectra with those of published data (Nist Library Mass spectra) or on the interpretation of the EI-fragmentation of the molecules.

4. Conclusion

In this work, we wanted to focus for the first time on the composition of the essential oils of the *Cistus* species spontaneously growing in Sardinia. *C. salvifolius* showed a high quantity of norisoprenoids, which were absent in the oils of the other *Cistus* we studied, with presence of members of the ionone family. Moreover, it presents the presence of cistodiol a clerodane diterpene. In Sardinian *C. salvifolius* are present similar metabolites identified in *C. salvifolius* populations in Tunisia and in Cretan populations. The essential oil of *C. monspeliensis* from Sardinia (which shows a high amount of heneicosane) is very different from that studied in other different regions (Angelopoulou et al. 2002). *C. albidus* showed high content of sesquiterpenes (42%), the labdane-type diterpenes were absent.

Looking at the three subspecies of *C. creticus* growing in Sardinia we found that *C. creticus* subsp. *eriocephalus* showed high quantity of labdane-type diterpenes similar to the composition reported for Cretan *C. creticus* subsp. *eriocephalus* but extremely different from that of Corsica.

C. creticus subsp. *creticus*, in its labdane-type diterpenes and oxygenated sesquiterpenes groups, has several monoterpenes similar to those present in essential oil of Cretan *C. creticus* subsp. *creticus*, but in smaller concentration (Demetzos et al. 1997).

C. creticus subsp. *corsicus* is the only Sardo-Corse endemic species and its essential oil showed a similar amount and profile to that of *C. creticus* subsp. *creticus*. Its profile is qualitatively rather similar, but different in the amounts of major components from that of Corsica.

The essential oils obtained by hydrodistillation have shown six profiles, extremely diversified among them but, there are some analogies between plants growing in Sardinia and that from Corse as regards *C. creticus* subsp. *corsicus* with plant growing in Crete with respect to *C. creticus* subsp. *Creticus*.

Acknowledgements

We thank Ms. Barbara Sechi for the technical assistance and Dott.ssa Gaia Trebini for critical revision of English.

Disclosure statement

No potential conflict of interest was reported by the authors.

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