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Chemical Properties of Wild *Cistus creticus* L. and *Cistus salviifolius* L. Species at Different Development Stages

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Abstract: The seasonal variations are important exogenous factors that cause differences in the chemical properties of the plants. The primary objective of this study was to assess the chemical properties of two Cistus species (*Cistus creticus* L. and *Cistus salviifolius* L.), which are widely utilized in the pharmaceutical, food, cosmetic, and veterinary industries, taking into account seasonal variations. The *C. creticus* and *C. salviifolius* plant samples were collected during the early flowering, full flowering, and fruit maturity stages. Then, the 50% EtOH extract of each plant was prepared, and their total phenolic and flavonoid contents were determined by the Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively. In the early flowering stage period the *C. creticus* plant's both total phenol (CC1: 105.695 ± 2.657 mg/g, CC2: 120.260 ± 3.986 mg/g, CC3: 123.739 ± 2.677 mg/g) and the flavonoid (CC1: 8.298 ± 0.481 mg/g, CC2: 10.116± 1.659 mg/g, 11.935 ± 1.120 mg/g) contents were the highest. The *C. salviifolius* plant's total phenol content was the highest in the fruit maturity stage period (CS1: 151.565 ± 3.549 mg/g, CS2: 138.304 ± 2.551 mg/g, CS3: 132.652 ± 3.779 mg/g) while the total flavonoid content was the highest in the early flowering stage period (CS1: 10.896 ± 1.179 mg/g, CS2: 10.246 ± 1.196 mg/g, CS3: 9.077 ± 1.981 mg/g). To the best of our knowledge, the current study is the first to demonstrate the effect of seasonal variations on the total phenolic and total flavonoid compounds of *C. creticus* and *C. salviifolius*, specifically during the periods when they contained the highest levels.

Keywords: *Cistus creticus* L.; *Cistus salviifolius* L.; quality control; phenolic compounds; flavonoids.

1. Introduction

Cistus L., one of the important genera of the Cistaecae family, is represented by 68 species in the world; The *Cistus* species is distributed commonly in Mediterranean countries, and there are consistently about 30 species. Five species of *Cistus* species naturally show distribution in our country in bush form [1-3]. It has been determined that three of these species (*Cistus creticus* L., *Cistus salviifolius* L., and *Cistus laurifolius* L.) spread in the Western Mediterranean region. Five species (*C. creticus, Cistus parviflorus* Lam.*, C. salvifolius, Cistus monspeliensis* L., and *C. laurifolius*) grow naturally in maquis forests, especially in the coastal areas of Türkiye. *Cistus* species; It has been reported to have an effective

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that can be used in traditional medicine, perfumery industry, various medicines, food and many other supplementary industries, and even in the treatment of today's most epidemic disease, COVID-19. Today, the use of food supplements containing *Cistus* species (especially in upper respiratory diseases) is increasing rapidly [2, 4-7]. In studies conducted on different *Cistus species*, it was determined that *C. creticus* and *C. salviifolius* essential oils were rich in nonterpene components, *C. creticus* and *C. monspeliensis* species were rich in labdane-type diterpene components, and the chemical composition of the *C. creticus* plant included tannins, heterosides, triterpenes, flavonoids, and saponosides [8-12].

Cistus creticus L., also known as *Cistus incanus* subsp. *creticus* (L.) Heywood and *Cistus villosus* subsp. *creticus* (L.) Nyman is a very important plant for Türkiye. The plant known as hairy rock rose by local people [13, 14]. The upper stems, leaves, and their extracts were used in traditional folk medicine to treat conditions such as fever, rheumatism, eczema, abscesses, cancer, and diarrhea. Today, herbal preparations from *C. creticus* are being used to treat respiratory system illnesses, including influenza [15-17]. Recent studies have shown the antioxidant, antiviral, anti-inflammatory, antibacterial, antifungal, cytotoxic, and anticancer activities of *C. creticus* [15, 17-19].

Cistus salviifolius L., rich in ellagitannins and flavonoid compounds, has been used in traditional medicine for the prevention and treatment of digestive system disorders, cancer, diarrhea, and diabetes. The botanical epithet name "*salviifolius*" is attributed to a distinct aroma of fresh leaves that resembles sage, along with observable morphological similarities in the leaves [20, 21]. This plant has not been adequately studied for its medical and pharmacological potential. However, the antihyperglycemic, anti-inflammatory, analgesic, antioxidant, and antimicrobial properties have been demonstrated in previous studies [22-25].

The harvest date has a significant effect on the chemical content in medicinal and aromatic plants. Although the flowering period has been reported as the most suitable harvest time for many medicinal plants, this may not always be the case. *Cistus*, which is not cultivated in Türkiye, is collected from natural flora. Therefore, harvesting *Cistus* plants at the most suitable time in terms of chemical content is crucial for optimal productivity. In this study, the effects of different harvest periods on phenolic and flavonoid contents in two *Cistus* species were investigated. *Cistus* species, which are native to the Mediterranean climate, have not been introduced to agricultural production so far, although they are widespread in Türkiye's natural flora. In Europe, some *Cistus* species (*C. creticus* L., *C. ladaniferus* L.) are commercially cultivated and produced [26, 27]. Although suitable areas exist for cultivating the *Cistus creticus* and *Cistus salviifolius* species, which are suitable for medical use in Türkiye, their production is almost non-existent, and the required plant material is collected from the wild. For this reason, studies on the protection of plant genetic resources and their cultivation and introduction into agriculture are of priority. It is believed that the results obtained from this study will contribute to the cultivation studies that will be carried out later.

Propagation of *Cistus* species can be achieved through both seed germination and vegetative methods. The seeds of *Cistus* typically exhibit physical dormancy due to a hard seed coat. Therefore, pre-germination treatments such as dry or wet heat shocks and boiling water scarification significantly enhance germination. For instance, in *Cistus creticus*, germination rates of up to 90% have been reported after appropriate heat treatments [28]. Vegetative propagation is favored for maintaining genetic fidelity and enabling rapid multiplication. Semi-hardwood cuttings, when treated with rooting hormones like IBA and placed in a humid, controlled environment, root successfully [29]. These propagation strategies support the cultivation of *Cistus* for ornamental, medicinal, and ecological purposes.

2. Materials and Methods

2.1. Plant Materials

In the study, *Cistus creticus* L. and *Cistus salviifolius* L. species naturally growing in the Akdeniz University campus (coordinates 37.896.455.867.107.754N- 30.642.669.224.192.026W) were used as plant material. The plants grow in Mediterranean red soil (Terra Rossa) at a depth of 20-30 cm in Mediterranean climate conditions.

Firstly, the plants belonging to both species to be studied in the study area were marked. The experiment was established with two species, three harvest periods and three replications. Plant samples were taken during the early flowering (22.04.2022), full flowering (23.05.2022) and fruit maturity

(26.07.2022) stages. Plant samples of both species were taken by cutting from marked plants on three different harvest dates. The samples were dried at room temperature for chemical analysis. Plant samples are coded as *C. creticus* (CC1, CC2, CC3) and *C. salviifolius* (CS1, CS2, CS3).

2.2. Chemicals and Reagents

Gallic acid (≥97.5%), quercetin (≥95%), Folin & Ciocalteu′s phenol reagent (FCR), aluminum chloride ($AlCl_3$, anhydrous powder, 99.99%), sodium carbonate (Na_2CO_3, anhydrous powder, 99.99%) ammonium acetate (CH_3COONH_4, reagent grade, 98%) were delivered from Sigma-Aldrich (Germany).

2.3. Preparation of Extracts

Air-dried and powdered both *C. creticus* and *C. salviifolius* plant samples (10 g) were extracted with 100 mL ethanol-water (50%) for 24 hours at room temperature. Then the extracts were filtered with Whatman® Grade 1 filter paper, and the filtrates were collected.

2.4. Analysis of Total Phenolic and Total Flavonoid Contents of the Extracts

The Folin-Ciocalteu method was used to determine the total phenolic content of *C. creticus* and *C. salviifolius* extracts. The total phenolic content is expressed as gallic acid equivalents [30, 31]. The total flavonoid content of the *C. creticus* and *C. salviifolius* extracts was analysed by using the aluminium chloride colorimetric method. The total flavonoid contents are expressed as quercetin equivalents [31, 32].

3. Results and Discussion

The total phenol and flavonoid amounts in the plant materials were determined as gallic acid equivalents (mg/g extract) and quercetin equivalents (mg/g extract), respectively. The results were shown in Table 1. According to the results, in the early flowering stage period the *C. creticus* plant's both total phenol (CC1: 105.695 ± 2.657 mg/g, CC2: 120.260 ± 3.986 mg/g, CC3: 123.739 ± 2.677 mg/g) and the flavonoid (CC1: 8.298 ± 0.481 mg/g, CC2: 10.116± 1.659 mg/g, 11.935 ± 1.120 mg/g) contents were the highest. The *C. salviifolius* plant's total phenol content was the highest in the fruit maturity stage period (CS1: 151.565 ± 3.549 mg/g, CS2: 138.304 ± 2.551 mg/g, CS3: 132.652 ± 3.779 mg/g) while the total flavonoid content was the highest in the early flowering stage period (CS1: 10.896 ± 1.179 mg/g, CS2: 10.246 ± 1.196 mg/g, CS3: 9.077 ± 1.981 mg/g).

The chemical composition of plants varies mainly due to two different groups of factors, exogenous and endogenous. The endogenous factors are intrinsically associated with the morphological and physiological characteristics of plants, while the exogenous factors are environmental conditions such as light, precipitation, and the growing site. The seasonal chemical variations are comprised of a mixture of exogenous factors, including precipitation, radiation, and temperature. Recent studies in this field mostly focus on maximizing yields and harvesting times based on biologically active, poisonous, or valuable chemicals [33]. For instance, Santos-Gomes and Fernandes-Ferreira revealed that the oxygenated monoterpene content of *Salvia officinalis* L. essential oil decreased in winter. In contrast, the monoterpene hydrocarbon content increased during the same period [34]. In another study, Demirbolat et al. investigated the chemical compounds of *Artemisia vulgaris* L. essential oils during the pre-flowering, initial flowering, and post-flowering growth stages. Before the flowering stage, the predominant components of the essential oil were α-thujone (30.68%) and β-caryophyllene (22.05%). Concurrent with plant development, sesquiterpene hydrocarbons (predominantly β-caryophyllene) diminished, whereas a rise in oxygenated monoterpenes, particularly α-thujone, was observed [35].

Both *C. creticus* and *C. salviifolius* plants are rich in phenolic and flavonoid compounds (especially myricetin, quercetin and kaempferol derivates) and their biological activities are attributed to these compounds. According to the literature survey, the gallic acid, rutin, chlorogenic acid, caffeic acid, myricetin, myrcetin-rutinoside, quercetin, quercetin-rhamnoside, quercetin-3-glucoside, quercetin-pentoside, kaempferol, kaempferol-3-glucoside, kaempferol-3-rutinoside and kaempferol-rhamnosyl-hexoside were detected as the major compounds of the *C. salviifolius* [20, 23, 36].

Table 1. Total phenol and flavonoid contents of the *Cistus creticus* L. and *Cistus salviifolius* L. plant materials.

State of Growth	Harvesting Date	Sample	Total phenol[a] contents ± S.E.M.[b]	Total flavonoid[c] contents ± S.E.M.[b]	Sample	Total phenol[a] contents ± S.E.M.[b]	Total flavonoid[c] contents ± S.E.M.[b]
Early Flowering Stage	22.04.2022	CC1	105.695 ± 2.657	8.298 ± 0.481	CS1	111.130 ± 4.195	10.896 ± 1.179
	22.04.2022	CC2	120.260 ± 3.986	10.116 ± 1.659	CS2	106.130 ± 2.205	10.246 ± 1.196
	22.04.2022	CC3	123.739 ± 2.677	11.935 ± 1.120	CS3	83.739 ± 2.609	9.077 ± 1.981
		Mean	116.565	10.116	**Mean**	100.333	10.073
Full Flowering Stage	23.05.2022	CC1	53.521 ± 4.861	4.987 ± 0.552	CS1	90.695 ± 3.205	9.532 ± 1.176
	23.05.2022	CC2	50.043 ± 4.002	5.831 ± 0.739	CS2	102.000 ± 2.802	10.701 ± 1.224
	23.05.2022	CC3	79.173 ± 2.781	8.493 ± 1.541	CS3	84.173 ± 2.998	7.974 ± 0.541
		Mean	60.913	6.437	**Mean**	92.289	9.337
Fruit Maturity Stage	26.07.2022	CC1	103.695 ± 3.762	5.636 ± 0.864	CS1	151.565 ± 3.549	10.376 ± 0.289
	26.07.2022	CC2	130.913 ± 4.190	12.519 ± 1.017	CS2	138.304 ± 2.551	10.441 ± 1.013
	26.07.2022	CC3	99.173 ± 3.143	6.285 ± 0.194	CS3	132.652 ± 3.779	9.272 ± 0.886
		Mean	111.927	8.147	**Mean**	140.840	10.030

[a] Data expressed in mg equivalent of gallic acid to 1 g of plant.

[b] Standard error mean ($n = 3$, $p<0.05$).

[c] Data expressed in mg equivalent of quercetin per 1 g of plant.

The rutin, luteolin, luteolin 7-(2″-*p*-coumaroylglucoside), quercitrin, myricetin, kaempferol, kaempferol-3-glucoside, kaempferol-3-rutinoside, kaempferol-rhamnosyl-hexoside, myricetin rhamnoside, myrcetin-rutinoside, quercetin, quercetin-rhamnoside, and gallic acid were measured as the major compounds of the *C. creticus* [15, 23, 37]. In the literature, the total phenolic and flavonoid amounts of the *C. creticus* and *C. salviifolius* plants were determined. For instance, Carev et al. collected the *C. creticus* and *C. salviifolius* plants in June 2015 and prepared aqueous extracts from the aerial parts of the plants. The total phenolic content of the plants was detected as 209.27 ± 18.5 mg of GAE/g and 161.09 ± 7.2 mg of GAE/g, respectively [23]. In another study conducted by Yagı et al., the *C. creticus* and *C. salviifolius* leaf-methanol extracts' total phenolic extracts were determined as 90.53 ± 0.12 mg of GAE/g and 97.08 ± 1.08 mg of GAE/g, while the total flavonoid contents were measured as 36.21 ± 0.16 mg of RE/g and 49.60 ± 0.38 mg of RE/g, respectively [38]. Waed et al. collected the *C. creticus* and *C. salviifolius* plants in May 2014 and prepared methanol extracts from the aerial parts of the plants. The total phenolic content of the plants was determined as 69.34 ± 4.68 mg of GAE/g and 75.22 ± 4.79 mg of GAE/g, respectively. Additionally, the flavonoid content was detected at 11.56 ± 0.32 mg of RE/g and 12.50 ± 0.25 mg of RE/g [39]. As seen above, our results concurred with those of previous studies on the *Cistus* species. However, studies in the literature focus on the total phenolic and flavonoid amounts of *C. creticus* and *C. salviifolius*, the effect of seasonal variations on the chemical properties of the plants has not been considered. In the current study, it was investigated whether seasonal variations cause differences in the chemical properties of both *C. creticus* and *C. salviifolius*. The *C. creticus*'s total phenol and flavonoid contents were higher in the early flowering stage period than in the full flowering and fruit maturity stages. The fruit maturity stage period of *C. salviifolius* had the highest total phenol concentration, whereas the early flowering stage period had the highest total flavonoid amount. *Cistus* species are very important for their biological activities and usages in the pharmaceutical, food, cosmetic, and veterinary sectors. The use of *Cistus* species is particularly common in upper respiratory diseases, such as lozenges or herbal teas/infusions, in the pharmaceutical sector due to their strong antiviral and antioxidant properties [18, 40, 41]. They are also excellent new sources of natural antioxidant ingredients in the food industry [41]. For instance, *C. incanus* extract was used as an innovative functional additive to wheat bread by Cacak-Pietrzak et al. According to the results, bread containing *C. creticus* was characterized by a markedly higher total phenolic content and significantly higher antioxidant activity compared to the control bread. Also, the incorporation of 3% *C. creticus* extract into bread resulted in a product with favourable attributes that was also preferred by consumers [42]. The extract and essential oil of *C. creticus* and *C. ladaniferus*, with the extract of *C. monspeliensis*, are used in the cosmetic industry for fragrance and skin conditioning with antioxidant and astringent effects according to the CosIng - Cosmetics Ingredients, European Commission [43]. The *Cistus* species are also used in the veterinary industry as feed additives. For instance, *C. ladanifer* was used as a feed additive in the lambs by Dentinho et al., and according to the results, it improved protein efficiency and the lamb's growth rate [44]. For this reason, it is necessary to determine the phytochemical constituents and biological activities of the *Cistus* species. *Cistus* species are promising due to their biological activities and applications in various sectors. *Cistus* species have not been introduced into agricultural production, despite being widespread in Türkiye's natural flora. Consequently, research focused on the protection of plant genetic resources and their cultivation and introduction to agriculture is of paramount importance. The findings from the current study are anticipated to enhance future cultivation research.

4. Conclusion

C. creticus and *C. salviifolius* are traditionally utilized for medicinal purposes. Most of their medicinal effects are attributed to the presence of phenolic and flavonoid compounds. Seasonal variations are significant exogenous factors that affect the chemical properties of these plants. This study demonstrates the impact of seasonal variations on the total phenolic and total flavonoid compounds of two *Cistus* species (*C. creticus* and *C. salviifolius*) and identifies the period when their levels are highest. Determining the developmental stage with high phenolic content in these Cistus species is believed to encourage researchers regarding future studies on biological activity and applications in the pharmaceutical, food, cosmetic, and veterinary industries.

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