Cumhuriyet Sci. J., 44(3) (2023) 470-473
DOI: https://doi.org/10.17776/csj.1195938

Cumhuriyet Science Journal

| csj.cumhuriyet.edu.tr | Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

Antioxidant Capacity of Essential Oils Obtained from *Myrtus communis* L. and *Citrus sinensis* (L.) Osbeck Plants Widely Consumed in Adana Region

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Research Article

History
Received: 28/10/2022
Accepted: 13/07/2023

ABSTRACT

Vitamin C, an antioxidant, is abundant in oranges (*Citrus sinensis* (L.) Osbeck), which are consumed worldwide. It has treated constipation, diarrhea, upper respiratory illnesses, obesity, menstruation issues, hypertension, and stress. Traditional medicine worldwide uses myrtle (*Myrtus communis* L.). Clinical and experimental studies show that the plant has a wide range of pharmacological and therapeutic activities, including antioxidant, anticancer, antidiabetic, antibacterial, antifungal, antiviral, pulmonary and skin diseases, dysentery, vomiting, rheumatism, sinusitis, leucorrhoea, and hair loss control. These plants contain flavonoids, terpenes, steroids, fatty acids, carbohydrates, volatile chemicals, carotenoids, and nutritional components. Plant antioxidants have gained popularity due to their health benefits. Many studies focused on medicinal organic antioxidants. The main goal of this research was to investigate the volatile components and antioxidant capacities of the essential oils of myrtle and orange, both of which are commonly utilized for medicinal purposes in the Adana region. Myrtle and orange extracts demonstrated substantial antioxidant properties when tested with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, a test based on the scavenging of the DPPH radical. It has been revealed that myrtle essential oil has a higher capacity in terms of antioxidant activity than orange essential oil.

Keywords: *Myrtus communis, Citrus sinensis*, Essential oils, Antioxidant activity, DPPH.

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Introduction

Plants have traditionally been a valuable source of sustenance and medicinal value in treating a wide range of human diseases. Recent investigations on the phytochemistry of medicinal plants have shown the efficacy of traditional remedies. Plants have long been used to treat a variety of illnesses and infections [1]. Natural products are a great source of molecules for medication development since they contain a wider range of size structures than synthetic ones. The discovery of new medicines will continue to rely heavily on natural products, which have historically been important sources of bioactive chemicals [2].

Only 10% of the plant kingdom has essential oils, which are pungent volatile chemicals. Because of their general safety, widespread consumer acceptability, and potential for several uses, essential oils, and their constituent parts can be very promising biological agents. Brittle secretory structures in plants, such as glands, secretory hairs, secretory ducts, secretory cavities, or resin ducts, are stored. Essential oils are intricate concoctions made up of numerous different components. They come from terpenes and their oxygenated compounds chemically. Essential oils are found in many families such as Lamiaceae, Rutaceae, and Myrtaceae [3, 4].

The family Rutaceae includes the genus *Citrus*, with an output of over 123 million tons per year in 2010, this genus is the most significant fruit tree crop in the entire planet. Asia is the original home of *C. sinensis*, which is now found across the Pacific and warm regions of the globe. *C. sinensis* is a flowering evergreen tree. Orange trees often reach a height of 9 to 10 m, and their branches have thick spines. *Citrus limon* (lemon), *C. medica* L. (citron), *C. x aurantium* L. (sour orange), *C. paradisi* Macfad. (grapefruit), *C. reticulata* Blanco (mandarin, tangerine), *C. clementina* (clementine), and *C. sinensis* (sweet orange) are useful plants in the genus. *C. sinensis* is widely consumed across the world as a top source of vitamin C, a potent natural antioxidant that strengthens the immune system. Traditional treatments for ailments such as colic, constipation, cramps, diarrhea, tuberculosis, bronchitis, cough, cold, menstrual disorders, obesity, angina, hypertension, anxiety, depression, and stress all involve the use of Citrus species. The secondary metabolites found in abundance in *C. sinensis* contribute to the pharmacological effects attributed to this plant. Flavonoids, steroids, alkanes, hydroxy amides and fatty acids, peptides, coumarins, carbohydrates, carbamates and alkylamines, volatile compounds, carotenoids, and nutritional components like potassium, calcium, magnesium, and sodium have all been found in fruits, peel, leaves, juice, and roots of *C. sinensis* [2, 5, 6].

The evergreen plant known as myrtle (*Myrtus communis*) is a member of the Myrtaceae family. This family of naturally occurring, up to 5 m tall, evergreen shrubs or small trees includes the genus, *Myrtus*. It naturally grows throughout the Mediterranean region and has been used for centuries as a spice, food, and medicine. Although clinical and experimental studies suggest it has a wider range of pharmacological and therapeutic effects, including antioxidative, anticancer, anti-diabetic, antiviral, antibacterial, antifungal, hepatoprotective, and neuroprotective activity, the herb is traditionally used to treat disorders like peptic ulcer, diarrhea, inflammation, hemorrhoid, pulmonary and skin diseases, vomiting, dysentery, rheumatism, sinusitis, leucorrhoea and cosmetic purposes like hair loss control. Among the substances thought to be the primary biologically active components are myrtucommulone, semimyrtucommulone, polyphenols, α-pinene, 1,8-cineole, myrtenyl acetate, linalool, limonene, and α-terpinolene [4, 7-9].

In this study, it was aimed to evaluate the volatile components and antioxidant capacities of essential oils of myrtle and orange that are frequently used therapeutically in Adana region. Terpene and essential oil content of *C. sinensis* and *M. communis* essential oils were analyzed qualitatively by their phytochemical reactions, and their antioxidant activity capacities were analyzed and compared with the 2,2-diphenyl-1-picrylhydrazine (DPPH) method.

Materials and Methods

Chemicals

2,2-diphenyl-1-picrylhydrazine (DPPH), ascorbic acid (AA, as a positive control), and ferric chloride were purchased from Sigma-Aldrich (USA). We used methanol (MeOH, as a solvent and negative control), chloroform, ferric chloride, and sulfuric acid from Merck (Germany). Every chemical was analytic reagent grade.

Essential Oil Samples

Orange essential oil (*C. sinensis*) and myrtle essential oil (*M. communis*) were acquired from Adana, Türkiye, a local market for use in this study. All samples of essential oils were verified and stored in the Analytical Chemistry Laboratory of the Faculty of Pharmacy, Cukurova University in Türkiye.

Extract Screening for Bioactive Agent

Following are some key constituents of the plants' essential oil samples that were subjected to phytochemical screening utilizing their respective qualitative methods descriptions [10, 11].

Test for Terpenoids

To dissolve the 2 mL of the plants' essential oil samples, 2 mL of chloroform was employed, and the mixture was then allowed to dry. After heating for approximately 2 minutes, another 2 mL of concentrated sulfuric acid was added. After adding, the sulfuric acid was heated for about 2 minutes. Terpenoids were evident because of the development of a greenish color [10].

Test for Essential Oils

A little amount of the extracts was dissolved in 90% alcohol, and two drops of $FeCl_3$ were added. There was a greenish color that also suggested the presence of essential oils [11].

DPPH Assay

An assay for free radical scavenging activity uses 1,1-diphenyl-2-picrylhydrazine (DPPH radical). The decrease in the stable free radical DPPH's absorbance at 517 nm can be used to assess the scavenging capacity of natural compounds. Scavenger combines with the purple free radical to produce the colorless product DPPH. A 0.1 M stock solution of DPPH in methanol was prepared. To prevent deterioration, this solution is stored in the refrigerator wrapped in foil. By adapting the methods of Brand-Williams et al. (1995) [12], and Blois (1958) [13], the DPPH test of the essential oil samples was investigated (2019). The essential oil samples were diluted with MeOH in twelve stages. A 96-well plate was loaded with 120 µL of the diluted essential oil samples and AA. To start the reaction, 40 µL of 0.1 M DPPH in MeOH was added. A UV spectrophotometer was used to test the reaction mixture's absorbance at 517 nm after it had stood at room temperature for 45 minutes (Thermo Scientific, Multiskan Sky Microplate Spectrophotometer, Waltham, MA, USA). The mean expression was calculated using three observations of the data. From the drop in absorbance, the % DPPH radical scavenging activity of samples and standards was determined. It was determined what percentage of DPPH was removed $((A_{Control}\ A_{Sample})/A_{Control})$ x 100). The value of the half-maximal inhibitory concentration (IC_{50}) is then determined. The values of effective concentration (EC_{50}), antiradical power (ARP), and ascorbic acid equivalent antioxidant capacity (AEAC) were determined using the IC_{50}, respectively [14-18].

Statistical Analysis

The mean and standard deviation (mean ± SD) of the IC_{50} was used to express all data. The data were compared using a one-way ANOVA analysis, and all tests were deemed statistically significant at $p < 0.05$.

Results

Test for Phytochemical Screening

This test was carried out to determine the volatile compounds found in essential oils of myrtle and orange and whether they contain terpenes. Results of phytochemical screening tests on *Citrus sinensis* and *Myrtus communis* samples showed in Table 1. The results show that the essential oils tested contain terpene and volatile substances.

Table 1. Phytochemical study of samples.

	Terpenoids	Essential oil
Citrus sinensis	+	+
Myrtus communis	+	+

Essential Oils Antioxidant Activities

The antioxidant capacity of the samples of essential oils rose as the IC_{50} value declined. The IC_{50} value displays the antioxidant activity when it is lower. If an antioxidant has low IC_{50}-EC_{50} and high ARP-AEAC values, it is known to be more potent. The essential oils (EO) were ranked in terms of antioxidant potency based on antioxidant criteria: AA (IC_{50}: 0.0111 ± 0.001 mg/mL)> *Myrtus communis* EO (IC_{50}: 2.810 ± 0.558 mg/mL)> *Citrus sinensis* EO (IC_{50}: 5.787 ± 0.994 mg/mL). AA has a stronger antioxidant potential when compared to the antioxidant characteristics of essential oils. These outcomes are those of nature. The antioxidant ascorbic acid is particularly effective. AA has the highest action as a result. However, it was discovered that myrtle and orange also showed antioxidant activity after statistical analysis. Results are given in Table 2.

Table 2. Antioxidant properties of the samples of essential oils.

	IC_{50} (mg/mL)	EC_{50} (mg/mL)	ARP (mL/mg)	AEAC
Ascorbic acid	0.0111 ± 0.001	0.289	345.260	-
***Citrus sinensis* EO**	5.787 ± 0.994^{a}	140.089	0.7138	192.329
***Myrtus communis* EO**	2.810 ± 0.558^{b}	70.831	1.411	396.080

EC_{50}: Effective concentration, ARP: Antiradical power, AEAC: Ascorbic acid equivalent antioxidant capacity. IC_{50} values expressed are means ± standard deviation of three measurements. The values of the superscript-containing essential oil samples differed considerably ($p \le 0.05$).

Overview

Turkey has a remarkable variety of plants, which differ by area. There are over 10,000 vascular plants in its flora, and nearly one-third (34.4%) of them are native to the nation. The utilization of ethnobotanical data gathered from studies on therapeutic plants has recently attracted interest on a global scale. For this reason, a great deal has been written about medicinal plants in our nation and recently, numerous ethnobotanical studies have been published. The public has utilized these medicinal plants for folk medicine for a long time, thus knowledge on how to use them to heal illnesses has been passed down the centuries [1].

Turkish people have used herbal medicine to cure various common illnesses for generations. A core of the Mediterranean region with a wide variety of plants is the Taurus Mountains in Adana. As a result, traditional herbal remedies are essential for maintaining human life [19].

Antioxidants are substances that interact with free radicals to neutralize them and avert or lessen the harmful effects they have on the body. Antioxidants, whether synthetic or natural, are frequently added to lipids, fatty foods, and cosmetics in order to stop oxidation. Due to their carcinogenicity, synthetic antioxidants are no longer allowed to be utilized in products for human consumption, which has greatly increased interest in antioxidants of natural origin [4].

The cause of many diseases is reactive stress. A lot of work has been done to find natural antioxidants that could be used as medicines. Antioxidative activity has been measured in many different ways [20]. Because secondary metabolites like phenylpropanoids and essential oils are active, aromatic, and medicinal plants like myrtle and orange are a good source of natural antioxidants. Since ancient times, these plant essential oils and extracts have been employed in medications, alternative remedies, and food preservation [4, 6]. In this study, different results were found compared to the literature data. Numerous studies have shown a substantial correlation between phenolic content and the ability of plant extracts to serve as antioxidants. Anthocyanins, flavonoids, and phenolic acids appear to be particularly important for the antioxidant capacity. However, since essential oils contain terpenes (Table 1) as the main compounds, the results in our study are very natural as in Table 2. Myrtle essential oil is found to have more antioxidant activity than orange essential oil, according to this research. This shows that the substances in myrtle essential oil are more active than the substances in orange essential oil. However, both essential oils do not have as strong antioxidant properties as the standard ascorbic acid. Future research will focus on what drug or substance family this characteristic comes from.

Conclusion

Even though essential oils are known to have antioxidant properties, their use may be constrained due to changes in their chemical composition. The fluctuations in their chemical makeup can be attributed to a variety of factors, including the aromatic plant's harvest time, climatic and agronomic circumstances, the plant's vegetative development, the portion of the plant employed, and the method of extraction. Due to its potential as a preservative, cosmetic, or nutraceutical, essential oils have been the subject of research in the food and cosmetic industries. Studies have also demonstrated that the essential oil's constituent parts work in concert because, when used as a benchmark, the active compounds had lower levels than the essential oil. Studies on synergism and antagonism should be created as a result.

Conflicts of interest

All authors declare that they have no conflict of interest.

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