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Cistus salviifolius: Elucidation of Phytochemical Composition and Biological Activities for Potential Pharmacological Field

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Abstract: The plants of the *Cistus* genus are interesting species in terms of their distribution profiles and biological/pharmacological activity potentials. This study investigated the phytochemical composition, *in vitro* anti-inflammatory, antioxidant, cytotoxic, and enzyme-inhibitory activities of ethanolic (Cs-EtOH) and aqueous (Cs-dH_2O) extracts from *C. salviifolius*. Both extracts were rich in gallic acid, (+)-catechin and hyperoside. In the concentration range of 50-500 µg/mL, the activity of the two extracts in the anti-inflammatory test was 24.34-83.54% and 19.86-69.34%, respectively. All doses of the Cs-dH_2O showed high cell viability, and no cytotoxicity was observed. The Cs-EtOH showed higher antioxidant activity than Cs-dH_2O in all test systems. Both extracts also exhibited promising enzyme inhibitory activities on α-amylase, acetylcholinesterase (AChE), tyrosinase, butyrylcholinesterase (BChE), and α-glucosidase. *C. salviifolius* is considered an alternative reference source in industries such as medicine, food, and cosmetics.

Keywords: *Cistus salviifolius*; chemical composition; anti-inflammatory; cytotoxic; antioxidant; enzyme inhibitory

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1. Introduction

Members of the *Cistus* genus are interesting species in terms of their distribution profiles and biological/pharmacological activity potentials. It is known that five species of the *Cistus* genus (Cistaceae) are endemic to the Canary Islands. However, many other flowering and fruiting members of this genus are distributed in countries on the Mediterranean coast [1-5]. *Cistus salviifolius* L. is a white-flowered plant classified in the subgenus Leucocistus. This species is known to be quite common in Italy. However, it spreads from Portugal to the North African coasts (including European countries with a coast to the Mediterranean, Turkey, Israel, and North African countries) [6,7]. *C. salviifolius* usually blooms between March and June, does not shed its leaves, and is a perennial herb. It has adapted quite well to the maquis areas of the Mediterranean and some arid environments. It can also be found in degenerated forest areas. This species is found especially in parts of the Italian peninsula [8,9].

In studies with extracts obtained from various members of the *Cistus* genus, it has been reported that these species exhibit antiproliferative, antioxidant, cytotoxic, gastro-protective, antidiabetic, antimicrobial, and anti-ulcerogenic activities [10-22].

C. salviifolius is rich in sesquiterpenes, which exhibit interesting biological activities. Literature studies have revealed that these phytochemicals' biological activities (antioxidants, enzyme inhibition, etc.) are remarkable. *C. salviifolius* has also been reported to produce oleoresin, which has a strong odor [23]. In some Mediterranean countries, this species is used as cicatrizing and astringent agents due to its rich phenolic compounds compared to other members of the *Cistus* genus [9,24]. *C. salviifolius* has been reported to be used in the treatment of gout in Jordan [25]. In addition, some compounds of *C. salviifolius* have been reported to increase adipocyte glucose uptake by activating receptors (activated by peroxisome proliferator) [26]. Yeşilada *et al*. [27] reported that *C. salviifolius* can be used effectively in ulcer cases in Turkey. In addition to these reports, it is also known that this plant exhibits remarkable antimicrobial activity. Haouat *et al*. [28] reported that this plant exhibits strong anti-mycobacterial activity against some *Mycobacterium* species in Morocco.

This study aims to determine the phytochemical composition and to document the anti-inflammatory, antioxidant, cytotoxic, and enzyme inhibitory activities of water and EtOH extracts (Cs-dH_2O and Cs-EtOH) obtained from by ultrasonication from *C. salviifolius* (Cs). Although some studies in the literature have published some biological/pharmacological activities of this plant, the plant species in question has been subjected to a comprehensive activity screening with this study for the first time.

2. Materials and Methods

The experimental procedures in this section are briefly summarized due to the high similarity rates. Please refer to the supplemental file for details of all experimental processes in the material and method section.

2.1. Plant material and solvent extraction.

C. salviifolius was collected from the Ağva-Şile-İstanbul region (Turkey) in June-July 2021 and identified by Professor Mustafa Kargioglu and deposited in the herbarium of Afyon Kocatepe University (Herbarium no: AKU-10385).

The modified ultrasonic extraction method prepared aqueous and ethanolic extracts of *C. salviifolius.*

2.2. Phytochemical analysis.

The determination of the total phenolic and flavonoid contents of the extracts was carried out by the Folin&Ciocalteu reagent and aluminum chloride assay, respectively [29]. In addition, phytochemical ses of the extracts were performed using LC–ESI–MS/MS with a sensitive, fast, simple, and reproducible method previously developed and validated [30].

2.3. Anti-inflammatory capacity.

The anti-inflammatory capacities of the extracts were investigated using the modified inhibition of protein denaturation method [31,32].

2.4. Cytotoxicity assay.

In-vitro cytotoxicity tests of the extracts were performed on Human Dermal Fibroblast Cell Line (PCS-201-012), according to Kumar *et al*. [33].

2.5. Antioxidant and enzyme inhibitory activity.

Antioxidant capacities of Cs-EtOH and Cs-dH_2O were monitored with different test systems: Phosphomolybdenum [29], radical scavenging on DPPH and ABTS [34], ferrous ion chelating [35], CUPRAC and FRAP reducing power [36,27]. In addition, inhibition activity tests were performed on AChE, BChE, tyrosinase, α-amylase, and α-glucosidase enzymes [38].

3. Results and Discussion

3.1. Phytochemical analysis.

The phenolic composition of the extracts is given in Table 1 and Figure 1, while Figure 2 shows the extracts' total phenolic and flavonoid contents.

While no statistically significant difference was observed between the total phenolic contents of both extracts, it was determined that the total flavonoid content of the Cs-EtOH was 2-fold higher than that of the Cs-dH_2O.

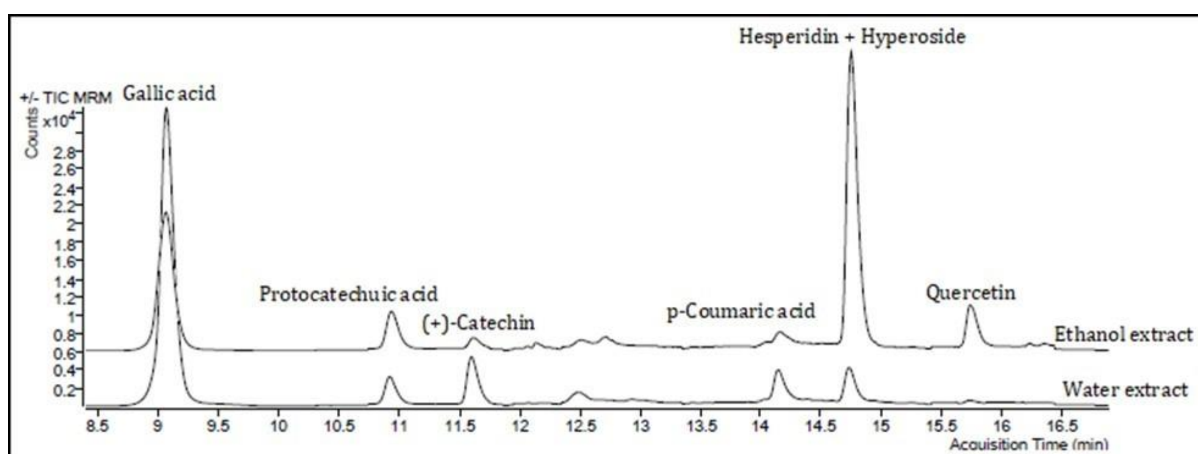

Figure 1. LC–ESI–MS/MS chromatograms of the extracts of *C. salviifolius*.

Table 1. Concentrations (µg/g extract) of selected phenolic compounds in the extracts of *C. salviifolius*.

Compounds	Water	Ethanol
Gallic acid	5463 ± 27^a	2443 ± 1^b
Protocatechuic acid	437 ± 1^b	531 ± 2^a
Pyrocatechol	44.8 ± 0.2^b	48.7 ± 0.2^a
3,4-Dihydroxyphenylacetic acid	13.2 ± 0.1^a	12.7 ± 0.1^b
(+)-Catechin	2930 ± 26^a	577 ± 2^b
Chlorogenic acid	15.3 ± 0.1^a	15.4 ± 0.1^a
2,5-Dihydroxybenzoic acid	210 ± 1^b	105 ± 1^a
4-Hydroxybenzoic acid	69.4 ± 0.2^a	45.6 ± 0.8^b
(-)-Epicatechin	nd	42.6 ± 2.7
Vanillic acid	161 ± 2^a	40.5 ± 3.1^b
Caffeic acid	30.4 ± 0.7^a	16.8 ± 0.2^b
Syringic acid	30.1 ± 1.0^a	31.0 ± 0.2^a
3-Hydroxybenzoic acid	nd	nd
Vanillin	19.2 ± 0.3^b	21.1 ± 0.2^a
Verbascoside	11.1 ± 0.1^b	11.6 ± 0.1^a

Compounds	Water	Ethanol
Taxifolin	27.1 ± 0.3^a	23.1 ± 0.9^b
p-Coumaric acid	220 ± 2^a	88.8 ± 0.3^b
Sinapic acid	11.5 ± 0.1^a	11.4 ± 0.1^a
Ferulic acid	23.0 ± 0.3^a	14.8 ± 0.8^b
Luteolin 7-glucoside	17.9 ± 0.2^b	22.2 ± 0.2^a
Hesperidin	817 ± 8^b	992 ± 6^a
Rosmarinic acid	12.7 ± 0.1^a	12.9 ± 0.1^a
Hyperoside	649 ± 12^b	6840 ± 13^a
Apigenin 7-glucoside	9.40 ± 0.56^b	15.3 ± 0.5^a
2-Hydroxycinnamic acid	11.9 ± 0.1^a	11.7 ± 0.1^a
Pinoresinol	17.6 ± 2.1^a	19.6 ± 0.8^a
Eriodictyol	13.6 ± 0.2^a	13.8 ± 0.1^a
Quercetin	56.0 ± 1.1^b	594 ± 2^a
Luteolin	22.7 ± 0.9^b	32.4 ± 0.6^a
Kaempferol	29.5 ± 0.8^b	151 ± 1^a
Apigenin	16.2 ± 0.2^b	43.3 ± 0.2^a

There is no statistical difference between values marked with the same superscripts on the same row.

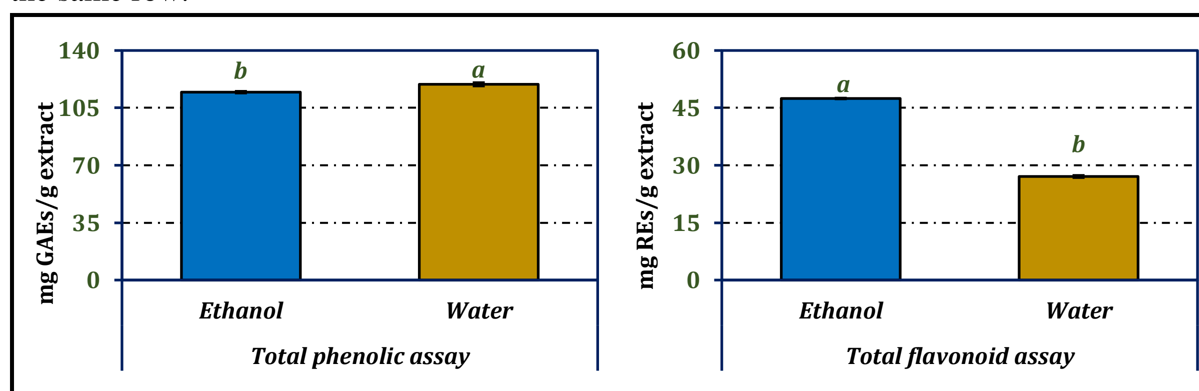

Figure 2. Total phenolics and flavonoids of the extracts of *C. salviifolius* (GAEs, REs: Gallic acid and rutin equivalents, respectively). There is no statistical difference between the values marked with the same superscripts on the bars.

According to the data in Table 1 and Figure 2, both of the extracts were rich in gallic acid (Cs-dH_2O:5463 µg/g and Cs-EtOH: 2443 µg/g), (+)-catechin (Cs-dH_2O:2930 µg/g and Cs-EtOH: 577 µg/g) and hyperoside (Cs-dH_2O: 649 µg/g and Cs-EtOH: 6840 µg/g). In addition, protocatechuic acid (531 µg/g), hesperidin (992 µg/g), and quercetin (594 µg/g) were found in high amounts in the Cs-EtOH.

Literature data show that members of the *Cistus* genus are rich in labdane-type diterpenes and clerodans, various alkaloids, flavonoids, ellagitannins, and phenylpropanoids [39-44]. According to Barrajon-Catalan *et al*.[39], although the phytochemical content of *Cistus* species is affected by some environmental factors such as soil type, locality, and altitude, *C. salviifolius* is particularly rich in ellagitannins. Similar findings suggesting that the plant species in question is rich in ellagitannins have also been reported by Lukas *et al*. [45]. Tomas-Menor *et al*. [46] reported that *C. salviifolius* contains significant amounts of galloylated flavanols and some specific flavonols.

Studies in the literature also qualitatively analyze the chemical composition of various extracts from *C. salviifolius* [24,47]. The chemical composition of the leaves and flower buds of *C. salviifolius* was investigated by El Euch *et al*. [24], and it was reported that flower buds

contain higher amounts of phenolic compounds and flavonoids than the leaves. However, leaves were also found to be richer in tannins and anthocyanins than flower buds.

As can be seen from the literature data detailed above, there is no similar study in the literature investigating the amounts of phenolic compounds presented in Table 1 in *C. salviifolius*. Therefore, the present study is the first one in which the amounts of the compounds in the table in this species were determined quantitatively.

3.2. Anti-inflammatory capacity.

The inhibition of protein denaturation determined the anti-inflammatory capacity of Cs-dH_2O and Cs-EtOH. Concentration-dependent % inhibition results are given in Figure 3. Diclofenac sodium, used as standard, showed 48.702-141.607% anti-inflammatory activity in the 50-500 µg/mL concentration range. In the same concentration range, Cs-dH_2O and Cs-EtOH showed 24.34-83.54% and 19.86-69.34% anti-inflammatory potential, respectively.

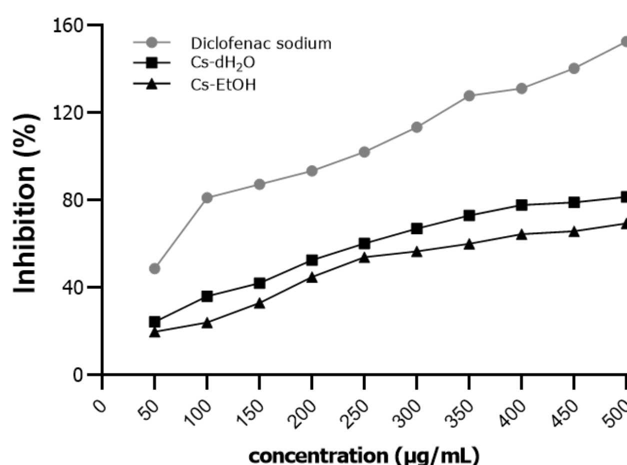

Figure 3. Anti-inflammatory activity of Cs-dH_2O and Cs-EtOH extracts and standard diclofenac sodium.

Anti-inflammatory capacity was determined to be inhibiting for the first time in the extracts of C. salviifolius by the inhibition of protein denaturation method. It is seen in the literature that various test methods have investigated the anti-inflammatory capacities of herbal extracts. Sayah *et al*. [48] investigated the anti-inflammatory capacity of *C. salviifolius* in rats using the carrageenan-induced paw edema test. These researchers reported that water extract of *C. salviifolius* (500 mg/kg) inhibited paw edema by 91.57%. Based on these results, Sayah *et al*. [48] suggested that *C. salviifolius* water extract has an anti-inflammatory capacity and can be used in traditional therapy in this respect. El Euch *et al*. [24], on the other hand, investigated the anti-inflammatory capacity of *C. salviifolius* through the anti-5-lipoxygenase (5-LOX) method. In this study, in which the activities of methanol extracts of the leaves and flower buds of the plant were compared, leaf extract has been reported to have 4 times stronger anti-inflammatory capacity than flower bud extract, as it is richer in condensed tannins and anthocyanins.

In addition to the literature data on *C. salviifolius*, it is useful to look at the biological/pharmacological activity potentials of major compounds given in Table 1 to evaluate their possible contributions to the activity. In a study by Ojeaburu and Oriakhi [54], gallic acid has been shown to down-regulate the expression of pro-inflammatory cytokines, interleukin 1 beta (IL-1B), interleukin 6 (IL-6), cyclooxygenase 2 (COX 2), cyclooxygenase 2 (COX 2), and tumor necrosis factor-alpha (TNF alpha) in male Wistar rats. According to Kuang *et al*. [55],

catechin is a potential anti-inflammatory compound. The network pharmacology analysis showed that catechin might mediate ferroptosis on macrophages to exhibit a significant anti-inflammatory effect on RAW264.7. In addition, hyperoside, another major compound in Table 1, shows anti-inflammatory activity by suppressing nuclear factor-kappa B activation in mouse peritoneal macrophages [56].

3.3. Cytotoxicity assay.

Cytotoxicity of Cs-dH_2O was evaluated on human dermal fibroblast cells. The cell viability was higher than the control group at all concentrations for all periods. All sample doses showed high cell viability, and no cytotoxicity was observed (Figure 4). In Cs-EtOH, the cell viability decreased as the concentration increased for all periods and 24 hours; higher cell viability was observed at a concentration of 375 µg/mL and lower concentrations compared to the control group. Cell viability was higher at the 48th and 72nd hour than the control group up to a 375 µg/mL concentration. Cell viability decreased when the concentration was above 375 µg/mL compared to the control group (Figure 5).

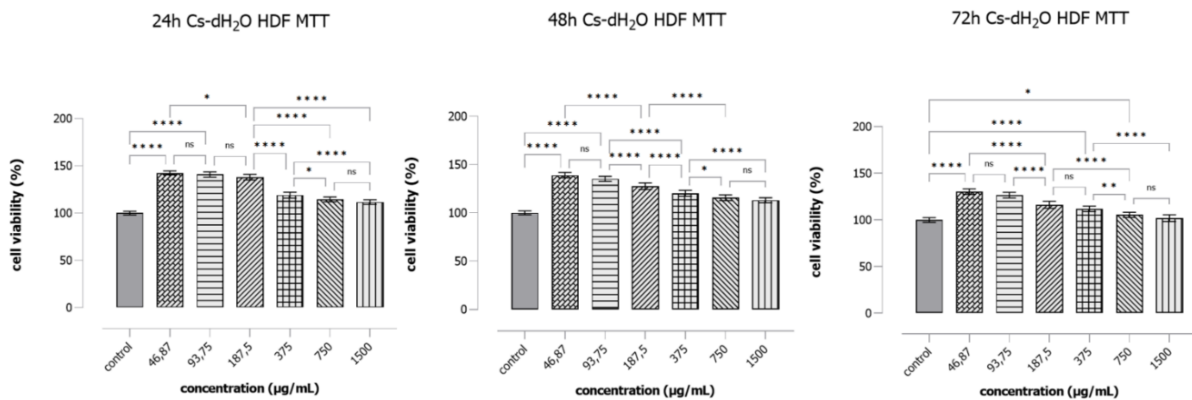

Figure 4. The cytotoxicity results of Cs-dH_2O extract on PCS-201-012 cells

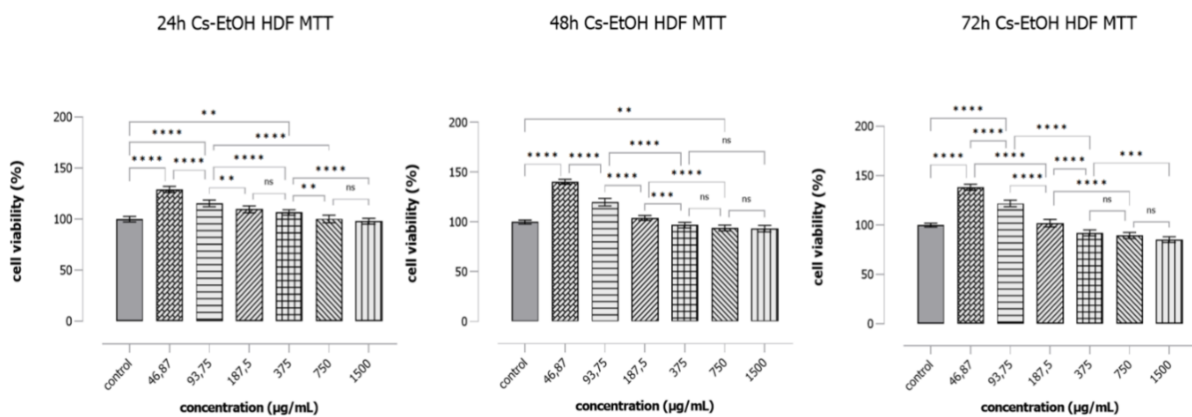

Figure 5. The cytotoxicity results of Cs-EtOH extract on PCS-201-012 cells.

As with the anti-inflammatory capacity, there are different methods to investigate the cytotoxic activities of the plant extracts. In a study investigating the cytotoxic effect of methanol extracts from leaves and flower buds of *C. salviifolius* on OVCAR and MCF-7 cells, It was concluded that the flower buds extract had a higher toxic effect on the cells in question than the leaf extract [24]. A group of researchers from Turkey analyzed the toxic effects of *n*-hexane, methanol, water, and *n*-butanol extracts from *C. salviifolius* on HepG2 cells with the

Alamar Blue fluorometric assay method for 24 and 48 hours [49]. They reported that, among the tested extracts, methanol extract significantly reduced cell viability within 24 hours.

Just as in section 3.2, it is useful to include literature data on the possible contributions of major compounds to cytotoxic activity. In a study by Cota and Patil [57], it was reported that gallic acid has a cytotoxic effect on human colorectal adenocarcinoma cells (Caco2, COLO.205, HT.29). In a study investigating the cytotoxic effect of catechin, another major compound, on Caco-2 cells, it was reported that catechin nanoliposomes were cytotoxic at a concentration of 0.025 mg/mL and this effect was higher at 36th hour [58]. In another study, it was reported that treatment with hyperoside made breast cancer cells more sensitive to paclitaxel by blocking TLR4 signaling [59]. These examples of literature data indicate that these compounds may be responsible for the cytotoxic activity of *C. salviifolius*.

In the literature, no study has investigated the toxic effects of *C. salviifolius* extracts on the Human Dermal Fibroblast Cell Line (PCS-201-012). For the literature, the results obtained from this study are, therefore, a new record.

3.4. Antioxidant capacity.

The antioxidant capacity results of the extracts analyzed using different test systems are given in Figure 6. Cs-EtOH had a higher antioxidant capacity than Cs-dH_2O in all test systems. A statistically significant difference was found between the antioxidant capacities of the extracts in all tests except the phosphomolybdenum test.

The antioxidant capacity of *C. salviifolius* was determined by some researchers using FRAP, ABTS, and DPPH tests [24,45,50-53]

Due to the different presentations of the antioxidant activity results of the plant in question in some of these studies, it is not reasonable to compare it with the data in the current study.

In some of these studies, it is not reasonable to compare the antioxidant capacity of the plant in question with the data in the current study due to different presentations of the results, such as fresh weight [51] or IC_{50} [24,53]. However, in a study conducted by Lukas *et al*. [45] on a dry weight basis, it was reported that the DPPH radical scavenging activity of *C. salviifolius* was 261.0 mg TEs/g. This data is lower than the data from the current study. It is thought that the phenolic compound composition of the plant probably causes this difference. Abu-Orabi *et al*. [50], on the other hand, compared the antioxidant activities of *C. salviifolius* essential oil and various extracts (water, methanol, and butanol extracts) obtained from this plant and reported that the extracts showed stronger antioxidant activity than the essential oil. This finding is a very important example of how antioxidant activities increase as the polarity of the compounds increases.

In addition to the literature data given above, there are some reports on the antioxidant activity of gallic acid, catechin, and hyperoside in some studies. In a study by Ojeaburu and Oriakhi [54], gallic acid significantly up-regulated the expression of antioxidant genes, superoxide dismutase, and catalase. Wu *et al*. [58] have evaluated the antioxidant activity of the catechin nanoliposomes, and they found that the antioxidant activities of catechin nanoliposomes for the scavenging of 2,2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid, hydroxyl, and 1,1-diphenyl-2-picrylhydrazyl radicals in vitro was obviously improved. Additionally, hyperoside has been reported to prevent oxidative damage induced by hydrogen peroxide in lung fibroblast cells. The literature data in question show that these compounds may contribute to the antioxidant activity of *C. salviifolius* Piao *et al*. [60].

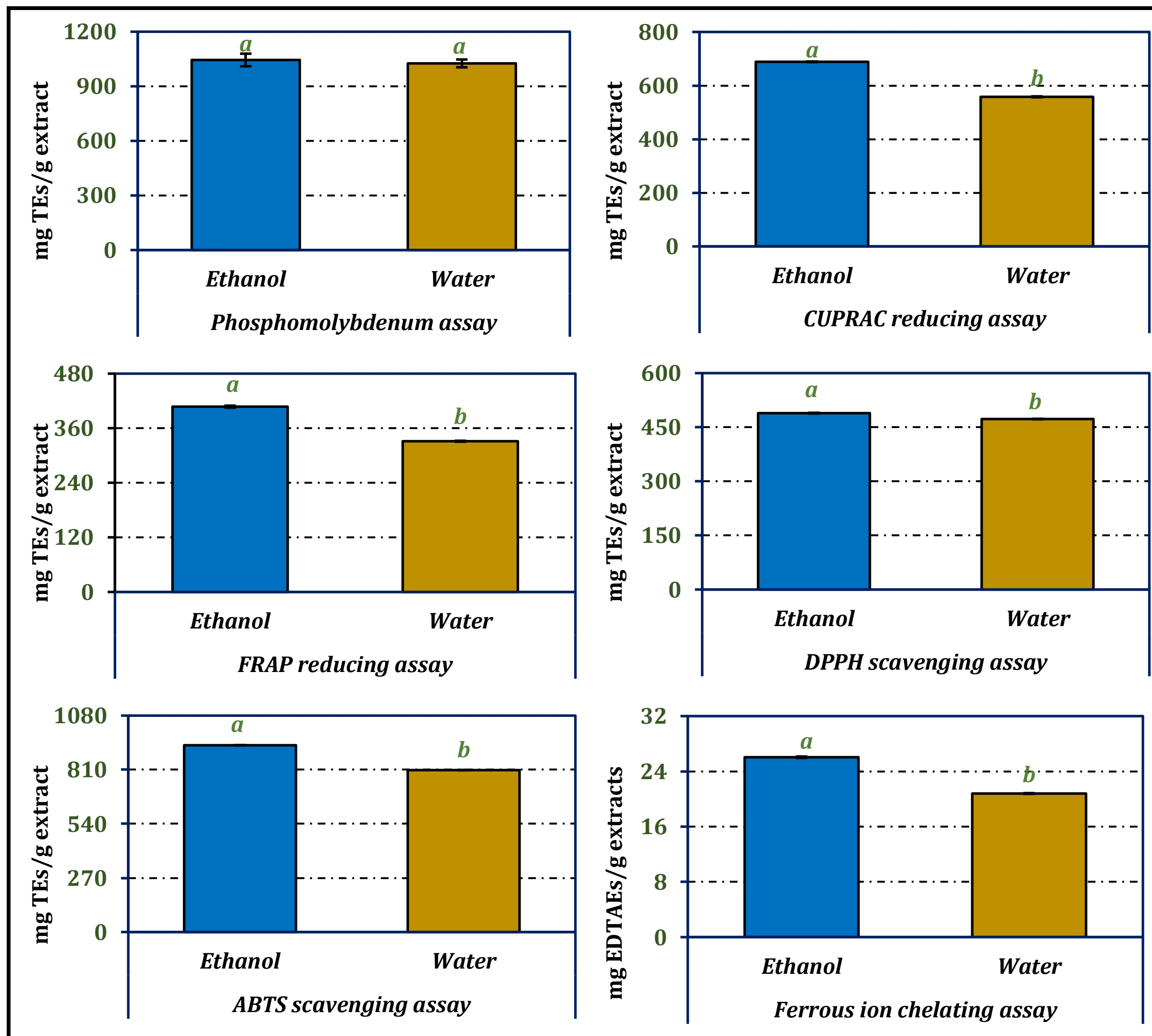

Figure 6. Antioxidant capacities of the extracts of *C. salviifolius* [TEs and EDTAEs: Trolox and ethylenediaminetetraacetic acid (disodium salt) equivalents, respectively]. There is no statistical difference between the values marked with the same superscripts on the bars.

3.5. Enzyme inhibitory capacity.

The performance of two extracts in cholinesterase, α-amylase, α-glucosidase, and tyrosinase inhibitory tests are given in Figure 7.

While no significant difference was found between the AChE inhibitory capacities of the extracts, it was determined that the Cs-dH_2O had a higher capacity in the BCHE inhibitory test.

No evidence of BChE inhibitory activity of *C. salviifolius* could be found in the literature. However, a study investigating the AChE inhibitory activity of 80% methanol extracts obtained from the leaves and flower buds of the plant species in question determined that the leaves exhibited stronger inhibitory activity than flower buds [24].

To determine the antidiabetic activity of *C. salviifolius* extracts, α-amylase and α-glucosidase inhibitory activity tests were also performed. Cs-dH_2O and Cs-EtOH had higher capacity in the α-amylase and α-glucosidase inhibitory activity tests, respectively. The extracts exhibited stronger inhibitory activity on α-glucosidase than on α-amylase.

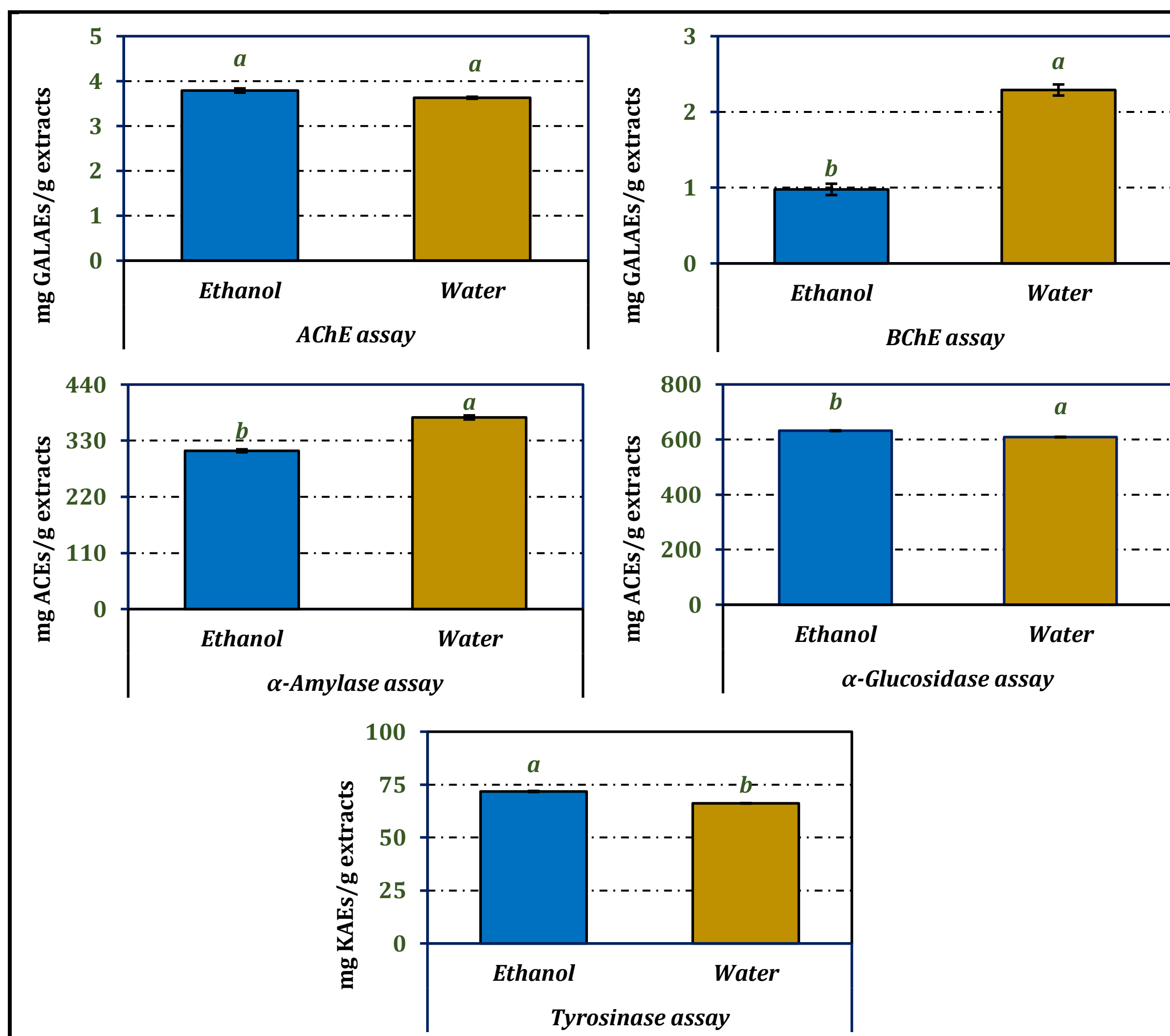

Figure 7. Enzyme inhibitory capacities of the extracts of *C. salviifolius* (ACEs, GALAEs, and KAEs: Acarbose, galanthamine, and kojic acid equivalents, respectively). There is no statistical difference between the values marked with the same superscripts on the bars.

In a study investigating the α-amylase and α-glucosidase inhibitory activities of water and hydromethanol extracts from the aerial parts of *C. salviifolius*, it was determined that the activities of the extracts were dose-dependent and were stronger than the reference compound acarbose [53]. However, the findings from the current study showed that the extracts were more effective on α-glucosidase, while the extracts showed higher inhibitory activity on α-amylase, according to the findings reported by Sayah *et al*. [53]. It is thought that this may be due to the difference in the chemical compositions of the tested extracts.

The Cs-EtOH (71.77 KAEs/g) exhibited higher inhibitory activity on tyrosinase than the Cs-dH_2O (66.17 mg KAEs/g).

No study in the literature investigates the tyrosinase inhibitory activity of *C. salviifolius*. The results from this study are, therefore, the first record for the literature.

4. Conclusions

This study concluded that Cs-EtOH and Cs-dH_2O extracts obtained from the aerial parts of *C. salviifolius* exhibited remarkable biological activities. The extracts exhibited significant anti-inflammatory activity, although not as much as the standard compound. Cs-EtOH extract was an effective antioxidant agent in all test systems. In addition, both extracts showed promising enzyme inhibitory activities. Cytotoxicity testing revealed that the Cs-dH_2O extract

provided high cell viability at all doses and was non-toxic. Therefore, *C. salviifolius* is considered an alternative reference source in industries such as medicine, food, and cosmetics.

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Conflicts of Interest

The authors confirm that there is no conflict of interest.

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Supplementary materials

Section S1. Analytical methods applied for phenolic composition, antioxidant and enzyme inhibitory activities.

Chemicals

Gallic acid, (+)-catechin, pyrocatechol, chlorogenic acid, 2,5-dihydroxybenzoic acid, 4-hydroxybenzoic acid, (−)-epicatechin, caffeic acid, syringic acid, vanillin, taxifolin, sinapic acid, p-coumaric acid, ferulic acid, rosmarinic acid, 2-hydroxycinnamic acid, pinoresinol, quercetin, luteolin and apigenin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Vanillic acid, 3-hydroxybenzoic acid, 3,4-dihydroxyphenylacetic acid, apigenin 7-glucoside, luteolin 7-glucoside, hesperidin, eriodictyol and kaempferol were obtained from Fluka (St. Louis, MO, USA). Finally, verbascoside, protocatechuic acid and hyperoside were purchased from HWI Analytik (Ruelzheim, Germany). Methanol and formic acid of HPLC grade were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany), respectively. Ultra-pure water (18 mΩ) was obtained using a Millipore Milli-Q Plus water treatment system (Millipore Bedford Corp., Bedford, MA).

Solvent extraction

The fresh leaves of C. salviifolius were dried in a dryer at 45°C, then fine powdered in a mill and stored in sterile black glass bottles at room temperature. The modified ultrasonic extraction method was used to prepare dH2O and EtOH extracts of C. creticus L. (Cs-dH2O and Cs-EtOH). 30 g powdered sample of C. salviifolius was prepared with 400 mL each of dH2O and EtOH ultrasonicated 1h, at room temperature. The available aqueous extract was filtered through Whatman filter paper No. 1. The extract was evaporated to dryness in a rotary evaporator (Heidolph) at 40°C and followed by freeze-drying.

Phytochemical analysis

An Agilent Technologies 1260 Infinity liquid chromatography system hyphenated to a 6420 Triple Quad mass spectrometer was used for quantitative analyses. Chromatographic separation was carried out on a Poroshell 120 EC-C18 (100 mm × 4.6 mm I.D., 2.7 µm) column. The mobile phase configuration (0.1% formic acid/methanol) was selected on the base of the better chromatographic resolution of isomeric compounds. On the other hand, the selected mobile phase configuration also provided higher sensitivity for many of the phenolic compounds. As a result, the mobile phase was made up from solvent A (0.1%, v/v formic acid solution) and solvent B (methanol). The gradient profile was set as follows: 0.00 min 2% B eluent, 3.00 min 2% B eluent, 6.00 min 25% B eluent, 10.00 min 50% B eluent, 14.00 min 95% B eluent, 17.00 min 95% B and 17.50 min 2% B eluent. The column temperature was maintained at 25°C. The flow rate was 0.4 mL min−1 and the injection volume was 2.0 µL.

The tandem mass spectrometer was interfaced to the LC system via an ESI source. The electrospray source of the MS was operated in negative and positive multiple reaction monitoring (MRM) mode and the interface conditions were as follows: capillary voltage of −3.5 kV, gas temperature of 300°C and gas flow of 11 L min−1. The nebulizer pressure was 40 psi.

Table S1. ESI–MS/MS Parameters and analytical characteristics for the Analysis of Target Analytes by MRM Negative and Positive Ionization Mode.

Target compounds	Rt (min)	Precursor ion	MRM1 (CE, V)	MRM2 (CE, V)
Compounds analyzed by NI mode				
Gallic acid	8.891	168.9 [M − H]−	125.0 (10)	–
Protocatechuic acid	10.818	152.9 [M − H]−	108.9 (12)	–
3,4-Dihydroxyphenylacetic acid	11.224	167.0 [M − H]−	123.0 (2)	–
(+)-Catechin	11.369	289.0 [M − H]−	245.0 (6)	202.9 (12)
Pyrocatechol	11.506	109.0 [M − H]−	90.6 (18)	52.9 (16)
2,5-Dihydroxybenzoic acid	12.412	152.9 [M − H]−	109.0 (10)	–
4-Hydroxybenzoic acid	12.439	136.9 [M − H]−	93.1 (14)	–
Caffeic acid	12.841	179.0 [M − H]−	135.0 (12)	–
Vanillic acid	12.843	166.9 [M − H]−	151.8 (10)	122.6 (6)
Syringic acid	12.963	196.9 [M − H]−	181.9 (8)	152.8 (6)
3-Hydroxybenzoic acid	13.259	137.0 [M − H]−	93.0 (6)	–
Vanillin	13.397	151.0 [M − H]−	136.0 (10)	–
Verbascoside	13.589	623.0 [M − H]−	461.0 (26)	160.8 (36)
Taxifolin	13.909	303.0 [M − H]−	285.1 (2)	125.0 (14)
Sinapic acid	13.992	222.9 [M − H]−	207.9 (6)	163.8 (6)
p-Coumaric acid	14.022	162.9 [M − H]−	119.0 (12)	–
Ferulic acid	14.120	193.0 [M − H]−	177.8 (8)	134.0 (12)
Luteolin 7-glucoside	14.266	447.1 [M − H]−	285.0 (24)	–
Rosmarinic acid	14.600	359.0 [M − H]−	196.9 (10)	160.9 (10)
2-Hydroxycinnamic acid	15.031	162.9 [M − H]−	119.1 (10)	–
Pinoresinol	15.118	357.0 [M − H]−	151.0 (12)	135.7 (34)
Eriodictyol	15.247	287.0 [M − H]−	151.0 (4)	134.9 (22)
Quercetin	15.668	301.0 [M − H]−	178.6 (10)	151.0 (16)
Kaempferol	16.236	285.0 [M − H]−	242.8 (16)	229.1 (18)
Compounds analyzed by PI mode				
Chlorogenic acid	11.802	355.0 [M + H]+	163.0 (10)	–
(−)-Epicatechin	12.458	291.0 [M + H]+	139.1 (12)	122.9 (36)
Hesperidin	14.412	611.1 [M + H]+	449.2 (4)	303.0 (20)
Hyperoside	14.506	465.1 [M + H]+	303.1 (8)	–
Apigenin 7-glucoside	14.781	433.1 [M + H]+	271.0 (18)	–
Luteolin	15.923	287.0 [M + H]+	153.1 (34)	135.1 (36)
Apigenin	16.382	271.0 [M + H]+	153.0 (34)	119.1 (36)

R_t, retention time; NI, negative ion; and PI, positive ion.

Table S2. Calibration curves and sensitivity properties of the method.

Compounds	Linearity and sensitivity characteristics				
	Range (µg/L)	Linear equation	R^2	LOD (µg/L)	LOQ (µg/L)
Gallic acid	5–500	y = 4.82x − 26.48	0.9988	1.46	4.88
Protocatechuic acid	2.5–500	y = 5.65x − 9.99	0.9990	1.17	3.88
3,4-Dihydroxyphenylacetic acid	5–500	y = 5.13x − 12.39	0.9990	1.35	4.51
(+)-Catechin	10–500	y = 1.45x + 1.95	0.9974	3.96	13.20
Pyrocatechol	25–400	y = 0.11x − 0.52	0.9916	9.62	32.08
Chlorogenic acid	1–500	y = 12.14x + 32.34	0.9995	0.55	1.82
2,5-Dihydroxybenzoic acid	5–500	y = 3.79x − 14.12	0.9980	2.12	7.08
4-Hydroxybenzoic acid	5–500	y = 7.62x + 22.79	0.9996	1.72	5.72
(−)-Epicatechin	5–500	y = 9.11x − 9.99	0.9971	1.85	6.18
Caffeic acid	5–500	y = 11.09x + 16.73	0.9997	3.15	10.50
Vanillic acid	10–500	y = 0.49x − 1.61	0.9968	2.56	8.54
Syringic acid	10–500	y = 0.74x − 1.54	0.9975	3.75	12.50
3-Hydroxybenzoic acid	5–500	y = 3.69x − 12.29	0.9991	1.86	6.20
Vanillin	50–500	y = 2.02x + 135.49	0.9926	15.23	50.77
Verbascoside	2.5–500	y = 8.59x − 28.05	0.9988	0.82	2.75
Taxifolin	5–500	y = 12.32x + 9.98	0.9993	1.82	6.05
Sinapic acid	5–500	y = 2.09x − 6.79	0.9974	2.64	8.78
p-Coumaric acid	5–500	y = 17.51x + 53.73	0.9997	1.93	6.44
Ferulic acid	5–500	y = 3.32x − 4.30	0.9992	1.43	4.76
Luteolin 7-glucoside	1–500	y = 45.25x + 156.48	0.9996	0.45	1.51
Hesperidin	5–500	y = 5.98x + 0.42	0.9993	1.73	5.77
Hyperoside	2.5–500	y = 16.32x − 1.26	0.9998	0.99	3.31
Rosmarinic acid	1–500	y = 9.82x − 17.98	0.9989	0.57	1.89
Apigenin 7-glucoside	1–500	y = 21.33x − 31.69	0.9983	0.41	1.35
2-Hydroxycinnamic acid	1–500	y = 16.72x − 26.94	0.9996	0.61	2.03
Pinoresinol	10–500	y = 0.80x − 2.69	0.9966	3.94	13.12

Compounds	Linearity and sensitivity characteristics				
	Range (μg/L)	Linear equation	R^2	LOD (μg/L)	LOQ (μg/L)
Eriodictyol	2.5–500	y = 14.24x − 0.50	0.9998	0.80	2.68
Quercetin	5–500	y = 14.68x − 18.25	0.9997	1.23	4.10
Luteolin	5–500	y = 8.96x + 26.80	0.9992	1.34	4.46
Kaempferol	10–500	y = 0.82x − 3.06	0.9959	3.30	10.99
Apigenin	2.5–500	y = 11.29x + 38.05	0.9987	0.96	3.20

LOD and LOQ: limit of detection and limit of quantification, respectively.

Antioxidant and enzyme inhibition activities

For total phenolic content, sample solution (0.25 mL) was mixed with diluted Folin-Ciocalteu reagent (1 mL, 1:9) and shaken vigorously. After 3 min, Na_2CO_3 solution (0.75 mL, 1%) was added and the sample absorbance was read at 760 nm after 2 h incubation at room temperature. Total phenolic content was expressed as equivalents of gallic acid.

For total flavonoid content, sample solution (1 mL) was mixed with the same volume of aluminium trichloride (2%) in methanol. Similarly, a blank was prepared by adding sample solution (1 mL) to methanol (1 mL) without $AlCl_3$. The sample and blank absorbance were read at 415 nm after 10 min incubation at room temperature. Absorbance of the blank was subtracted from that of the sample. Total flavonoid content was expressed as equivalents of quercetin.

Total antioxidant activity of the samples was evaluated by phosphomolybdenum method. Sample solution (0.2 mL) was combined with 2 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample absorbance was read at 695 nm after 90 min incubation at 95°C.

For 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, sample solution (1 mL) was added to a 4 mL of 0.004% methanol solution of DPPH. Sample absorbance was read at 517 nm after 30 min incubation at room temperature in dark.

For ABTS cation radical scavenging activity, briefly, $ABTS^{\cdot+}$ radical cation was produced directly by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand for 12-16 h in dark at the room temperature. Prior to beginning the assay, ABTS solution was diluted with methanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. Sample solution (1 mL) was added to ABTS solution (2 mL) and mixed. Sample absorbance was read at 734 nm after 7 min incubation at room temperature.

For metal chelating activity on ferrous ions, briefly, sample solution (2 mL) was added to $FeCl_2$ solution (0.05 mL, 2 mM). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). Similarly, a blank was prepared by adding sample solution (2 mL) to $FeCl_2$ solution (0.05 mL, 2 mM) and water (0.2 mL) without ferrozine. Then, the sample and blank absorbance were read at 562 nm after 10 min incubation at room temperature.

For cupric ion reducing activity (CUPRAC), sample solution (0.5 mL) was added to a premixed reaction mixture containing $CuCl_2$ (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM) and NH_4Ac buffer (1 mL, 1 M, pH 7.0). Similarly, a blank was prepared by adding sample solution (0.5 mL) to a premixed reaction mixture (3 mL) without $CuCl_2$. Then, the sample and blank absorbance were read at 450 nm after 30 min incubation at room temperature.

For ferric reducing antioxidant power (FRAP), sample solution (0.1 mL) was added to a premixed FRAP reagent (2 mL) containing acetate buffer (0.3 M, pH 3.6), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) (10 mM) in 40 mM HCl and ferric chloride (20 mM) in a ratio of

10:1:1 (v/v/v). Then, the sample absorbance was read at 593 nm after 30 min incubation at room temperature.

Inhibitory activity on α-amylase was performed using Caraway-Somogyi iodine/potassium iodide (IKI) method. Sample solution (25 µL) was mixed with α-amylase solution (50 µL) in phosphate buffer (pH 6.9 with 6 mM sodium chloride) in a 96-well micro plate and incubated for 10 min at 37°C. After pre-incubation, the reaction was initiated by the addition of starch solution (50 µL, 0.05%). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme solution (α-amylase). The reaction mixture was incubated 10 min at 37°C. The reaction was then stopped with the addition of HCl (25 µL, 1 M). This was followed by the addition of iodine-potassium iodide solution (100 µL). The sample and blank absorbance were read at 630 nm. Absorbance of the blank was subtracted from that of the sample.

For α-glucosidase inhibitory activity, sample solution (50 µL) was mixed with glutathione (50 µL), α-glucosidase solution (50 µL) in phosphate buffer (pH 6.8) and PNPG (50 µL) in a 96-well microplate and incubated for 15 min at 37°C. Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (α-glucosidase) solution. The reaction was then stopped with the addition of sodium carbonate (50 µL, 0.2 M). The sample and blank absorbance were read at 400 nm. Absorbance of the blank was subtracted from that of the sample.

Tyrosinase inhibitory activity was measured using a modified dopachrome method with L-DOPA as substrate. Sample solution (25 µL) was mixed with tyrosinase solution (40 µl) and phosphate buffer (100 µl, pH 6.8) in a 96-well microplate and incubated for 15 min at 25°C. The reaction was then initiated with the addition of L-DOPA (40 µl). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (tyrosinase) solution. The sample and blank absorbance were read at 492 nm after 10 min incubation at 25°C.

Cholinesterase (ChE) inhibitory activity was measured using Ellman’s method. Sample solution (50 µL) was mixed with DTNB (125 µL) and AChE (or BuChE) solutions (25 µL) in Tris-HCl buffer (pH 8.0) in a 96-well microplate and incubated for 15 min at 25°C. The reaction was then initiated with the addition of acetylthiocholine iodide (ATCI) or butyrylthiocholine chloride (BTCl) (25 µL). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme solutions (AChE or BuChE). The sample and blank absorbance were read at 405 nm after 10 min incubation at 25°C. Absorbance of the blank was subtracted from that of the sample.

The sample concentration, which decreases the initial concentration by 50% for enzyme inhibition, radical scavenging and metal chelation tests, was defined as IC_{50}, while the EC_{50} values were calculated as sample concentration providing 0.500 absorbance for reducing power and phosphomolybdenum assays, and inhibiting the initial concentration by 50% for radical scavenging and metal chelation tests. The biological activities of the extracts were expressed as mg standard equivalent/g extract and compared with those of the standards, including trolox, ethylenediaminetetraacetic acid (disodium salt) (EDTA), galanthamine, kojic acid, and acarbose, used as positive controls.

Anti-inflammatory activity

Anti-inflammatory activities of Cs-dH_2O and Cs-EtOH extracts were determined by the modified inhibition of protein denaturation method**.** The extract solution (500 µL) prepared at different concentrations was taken and mixed with 450 µL of 5% (w/v) BSA solution. The pH of the reaction medium was adjusted to 6,3 with 1N HCl. The samples were successively incubated at 37 °C for 30 minutes and at 57 °C for 3 minutes, and after cooling, 2,5 mL of PBS was added. The absorbance of the samples was measured at 660 nm and inhibition (%) was calculated by using an Equation 1, diclofenac sodium as a standard.

$$\%I = 100 - \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100 \quad (1)$$

Cytotoxicity assay

In-vitro cytotoxicity test of the extracts obtained by ultrasonic extraction method was tested with Human Dermal Fibroblast Cell Line (PCS-201-012) at different concentrations for 24, 48 and 72 h. Viability of the cells was investigated with MTT assay [33]. Briefly, cells at the 70-80% confluency were trypsinized and seeded on to a 96-well plate at the cell/well density of 1×10^4. After overnight attachment, the medium (DMEM complete with 10% FBS and 1% penicillin-streptomycin) (Sigma-Aldrich®, Germany) was replaced with an experimental medium that contained plant extracts incubated for 24,48 and 72 h at 37 ˚C in humidified air containing 5% CO_2. After the incubation period, the MTT(1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan, Thiazolyl blue formazan (Sigma-Aldrich®, Germany) reagent was added onto wells and incubated at 37 ˚C for two more hours at dark in 5% CO_2. After incubation, MTT reagent was removed from the cells, DMSO was added to each well, and the plate was shaken at low speed for 5 minutes at room temperature. Absorbance values were measured with Gen5 Biotek Microplate Reader (BioTek, Epoch, ABD) at wavelength of 570 nm and cell viability was calculated. Untreated cells (cells on wells without any sample but DMEM media) were used as a control and considered as 100% viable. All experiments were performed in triplicate, and statistical analysis of *in vitro* test data was performed via GraphPad Prism 9 with One-Way ANOVA and Tukey's multiple comparisons tests. Data were presented as a mean of 95 % confidence interval (CI). A,P value below 0.05 was considered statistically significant.