Bioactivities of Ethanolic Extract and its Fractions of Cistus laurifolius L. (Cistaceae) and Salvia wiedemannii Boiss. (Lamiaceae) Species

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ABSTRACT
Background: Cistus laurifolius L. (Cistaceae) and Salvia wiedemannii Boiss. (Lamiaceae) have been used for treatment of some illnesses in Turkish folk medicine. In the present study, the ethanolic extract and its fractions obtained using re-extraction by hexane (Hx), chloroform (CHCl₃), butanol, and remaining-water (r-H₂O) of C. laurifolius were screened for their in vitro bioactivities. Materials and Methods: Activities were determined against both standard and the isolated strains of Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus, Enterococcus faecalis, as well as yeasts such as Candida albicans and Candida parapsilosis by microdilution method. Also, antiviral activity of C. laurifolius and S. wiedemannii extracts were tested on herpes simplex virus-1 (HSV-1) and parainfluenza-3 (PI-3) using Madin-Darby bovine kidney and vero cell lines. Results: Tested extracts of C. laurifolius (minimum inhibitory concentration 32 μg/mL) exerted a strong antimicrobial activity against Gram-negative bacteria of E. coli, P. mirabilis, K. pneumoniae, and A. baumannii. Conclusion: The Hx extract of C. laurifolius (cytopathogenic effect of 32–8 μg/mL) had antiviral activity on PI-3. Also, the r-H₂O, CHCl₃, and ethanol extracts (16–0.25 μg/mL) of S. wiedemannii had significant antiviral activity on HSV-1, same as control. Key words: Antibacterial activity, antifungal activity, antiviral activity, Cistus laurifolius, cytotoxicity, Salvia wiedemannii

SUMMARY
• The objective of this study was to evaluate the bioactivity of plant extracts used in folk medicine

INTRODUCTION
Resistances to current antimicrobiological agents have been increasing. Therefore, there is a need for developing new antimicrobial drugs. Plants can be a source of these new medications given the fact that plants have been used as active substance of numerous antibiotics by industry[1-2] and as "anti-infection drugs" by people in folk medicine.

Five Cistus species belonging to Cistaceae family grow naturally in Turkey. The genus Salvia widely distributed in Turkey is represented by 94 taxa belonging to 89 species, with a 50% ratio of endemism.[3] Cistus and Salvia species have been used for high fever, rheumatic pain, peptic ulcer, stomachache, urinary inflammations, catarrh, cold, wounds, stomachache, flatulence, constipation, rheumatic pain, wards, sunstroke, and hemorrhage in Turkish folk medicine.[4-6] It has been shown that Cistus and Salvia species have some activities, including antimicrobial,[11-14] antioxidant,[15-18] anti-inflammatory,[19-21] analgesic,[19,20,21] and antiviral[14,21-25] activities. In our previous studies, we demonstrated in vitro inhibitory effects of Cistus laurifolius leaves on interleukin-1 alpha and beta (IL-1α and IL-1β) and tumor necrosis factor (TNF) biosynthesis. The extract and fractions obtained from the leaves were found to be ineffective on TNF inhibition, but effective on IL-1 inhibition. The methanolic extract and the hexane (Hx) and chloroform (CHCl₃) fractions were found to have remarkable IL-1α inhibitory effects in high concentrations.[27] The active compounds were isolated from the CHCl₃ extract of C. laurifolius. Of these three compounds, quercetin 3-methyl ether exhibited the highest inhibitory effect on H. pylori at the concentration of 3.9 μg/mL.[31] Likewise, analgesic activity of the same extract was also demonstrated. C. laurifolius CHCl₃ extract and its precipitated fraction exhibited central analgesic effect in mice.[30] These studies support folkloric utilization of the plant. In addition to these confi m activities, we aimed to evaluate bioactivities of these plants.

In this study, antibacterial and antifungal activities of the ethanol (EtOH), Hx, CHCl₃, butanol (BuOH), and remaining-water (r-H₂O) extracts of C. laurifolius were assessed against Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacterbaumannii, Staphylococcus aureus, Enterococcus faecalis, as well as yeasts such as Candida albicans and Candida parapsilosis by microdilution method. Also, antiviral activity of C. laurifolius and S. wiedemannii extracts were tested on herpes simplex virus-1 (HSV-1) and parainfluenza-3 (PI-3) using Madin-Darby bovine kidney and vero cell lines. Results: Tested extracts of C. laurifolius (minimum inhibitory concentration 32 μg/mL) exerted a strong antimicrobial activity against Gram-negative bacteria of E. coli, P. mirabilis, K. pneumoniae, and A. baumannii. Conclusion: The Hx extract of C. laurifolius (cytopathogenic effect of 32–8 μg/mL) had antiviral activity on PI-3. Also, the r-H₂O, CHCl₃, and ethanol extracts (16–0.25 μg/mL) of S. wiedemannii had significant antiviral activity on HSV-1, same as control. Key words: Antibacterial activity, antifungal activity, antiviral activity, Cistus laurifolius, cytotoxicity, Salvia wiedemannii

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also evaluated the efficacies of C. laurifolius and Salvia wiedemannii extracts (EtOH, Hx, CHCl₃, BuOH, and r-H₂O) on Herpes simplex virus-1 (HSV-1) and Parainfluenza-3 (PI-3) viruses. The effects of these extracts were compared to reference drugs (i.e. ampicillin, oll xacin, levofl xacin, ketocanozole, fluconozole, acyclovir, and oseltamivir).

MATERIALS AND METHODS

Plant material

The leaves of C. laurifolius and the aerial parts of S. wiedemannii were collected in the vicinity of Kurtboğazi (Ankara-May 2005) and Yunak (Ankara-May 2003) in Turkey, respectively. Voucher specimens were identified by a comparison with authentic specimens that had already been identified by Prof. Dr. Ekrem Sezik. Authenticated voucher specimens (C. laurifolius-GUEF No: 97B002, S. wiedemannii-GUEF No: 2379) were stored in the herbarium of Faculty of Pharmacy at Gazi University, Ankara, Turkey.

Preparation of the extract and its fractions

The air-dried and powdered plant (C. laurifolius; 510 g, S. wiedemannii; 360 g) was macerated in 95% EtOH (2200 mL EtOH) for 6 h in water bath adjusted to 40°C. Then, the macerate was filtered, and this procedure was repeated three times. The combined extracts were evaporated to dryness in vacuo using a rotary evaporator (C. laurifolius EtOH extract 32.35%, S. wiedemannii EtOH extract 31.38%). The EtOH extract was redissolved in 90% MeOH/H₂O (1000 mL) and re-extracted with portions of n-Hx (3 x 300 mL) for 6 h in water bath adjusted to 40°C. The combined Hx fraction was evaporated to dryness to obtain a sticky residue (the Hx fraction for C. laurifolius and S. wiedemannii; 4.5% and 3.61%, respectively). Then, the MeOH/H₂O extract was concentrated under reduced pressure in a rotary evaporator and dissolved in H₂O (150 mL). The water extract was re-extracted with portions of CHCl₃ (3 x 400 mL) for 6 h in water bath adjusted to 40°C. The combined CHCl₃ fraction was evaporated in a rotary evaporator (CHCl₃ fraction for C. laurifolius and S. wiedemannii; 10.98%, and 10.27%, respectively). The aqueous extract was then re-extracted with BuOH saturated with distilled H₂O for 6 h in water bath adjusted to 40°C and evaporated to dryness (BuOH fraction for C. laurifolius and S. wiedemannii; 6.27%, and 7.77%, respectively). The remaining aqueous part (r-H₂O) was lyophilized (r-H₂O fraction for C. laurifolius and S. wiedemannii; 9.6% and 8.61%, respectively).

Bioactivities assay

The ethanolic extract, and Hx, CHCl₃, BuOH, and r-H₂O fractions dissolved in dimethylsulfoxide (DMSO, 80%) and EtOH (20%) at a final concentration of 512 µg/mL. The extracts were sterilized by filtration using 0.22 µm millipore used as the stock solutions. Reference pharmaceutical agents were purchased from Sigma Chemical Co., and dissolved in phosphate buffer solution (ampicillin, pH: 8.0; 0.1 mol/mL), DMSO (ketocanozole) or in water (oill xacin, levoill xacin, and fluconozole). As the standards, Gram-negative strains of E. coli ATCC 35218, P. aeruginosa ATCC 10145, P. mirabilis ATCC 7002, K. pneumoniae RSKK 574, A. baumannii RS KK 0206, and as Gram-positive strains of S. aureus ATCC 25923, and E. faecalis ATCC 29212, and its isolates were used for the determination of antibacterial activity. C. albicans ATCC 10231 and C. parapsilosis ATCC 22019 were used for determination of antifungal activity. Mueller Hinton Broth (MHB; Difco) and Mueller Hinton Agar (MHA; Oxoid) were applied for growing and diluting of the bacteria suspensions. The synthetic medium RPMI-1640 with L-glutamine was buffered to pH: 7 with 3-(N-morpholino)-propanesulfonic acid and culture suspensions were prepared as described previously. The microdilution method was employed for antibacterial and antifungal activity tests as described previously.

Vero cell line and Madin-Darby bovine kidney cell cultures were grown in Eagle’s Minimal Essential Medium (Seromed; Biochromin) enriched with 10% fetal calf serum (Biochrom, 100 mg/mL of streptomycin; 100 IU/mL of penicillin) in a humidified atmosphere of 5% carbon dioxide at 37°C as described previously. In order to determine the antiviral activity of the extracts, HSV-1, and PI-3 were used. Maximum cytopathogenic effect (CPE) concentrations as the indicator of antiviral activities of the extracts were determined with maximum no-toxic concentrations (MNTCs) as described previously.

RESULTS

The results of comparative antibacterial and antifungal activities of the extracts of C. laurifolius, which assayed in vitro antibacterial and antifungal activity against standard strains of E. coli P. aeruginosa, P. mirabilis, K. pneumoniae, A. baumannii, S. aureus, E. faecalis and their drug-resistant strains as well as yeasts C. albicans, and C. parapsilosis are presented in Table 1.

As shown in Table 1, all of the extracts of C. laurifolius showed the same activity against standard Gram-negative bacteria (E. coli, P. mirabilis, K. pneumoniae, and A. baumannii) with minimum inhibitory concentrations (MICs) value of 32 µg/mL. The efficacies of the extracts were lower against resistant isolates (ESBLs enzyme positive; E. coli, trimethoprim-sulfamethoxazole, tazobactam-resistant P. aeruginosa, trimethoprim-sulfamethoxazole, ceftriaxon-resistant P. mirabilis/K. pneumonia, and trimethoprim-sulfamethoxazole-resistant A. baumannii). MICs for all microbes were two times higher for all extracts. The extracts of C. laurifolius showed some degree of antibacterial activity against standard and isolated strains of P. aeruginosa at MIC values of 64, 128 µg/mL, respectively. Interestingly, the extracts were found to have good activity against isolated Gram-negative strains of E. coli, which showed a close effect to those of the references; ampicillin (MIC; 64 µg/mL).

As for Gram-positive bacteria, MIC values of 64 µg/mL and 128 µg/mL were determined for standard (S. aureus ATCC25923, and E. faecalis ATCC29212) and drug-resistant strains (methicillin-resistant S. aureus, and cephalosporin-resistant E. faecalis), respectively. All extracts had similar MICs values (8 µg/mL) on C. albicans and C. parapsilosis compared to the references drugs employed (ketocanozole and fluconozole).

The results of antiviral activities of the extracts of C. laurifolius, and S. wiedemannii are presented in Table 2. As shown in Table 2, the H₂O, CHCl₃, and EtOH extracts (16–<0.25 µg/mL) of S. wiedemannii had significant antiviral activity on HSV-1 with a MNTC of 16 µg/mL similar to that of acyclovir. The BuOH extract of S. wiedemannii had remarkable inhibitory activity on HSV-1 (32–<0.25 µg/mL). On the other hand, the BuOH and Hx extracts of C. laurifolius had less antiviral activity (32–16 µg/mL) on HSV-1 with the MNTC of 32 µg/mL. In particular, the Hx extract of C. laurifolius (CPE of 32–8 µg/mL) had significant antiviral activity on PI-3 with a MNTC of 32 µg/mL similar to that of oseltamivir (CPE of 32–<0.25 µg/mL). Regarding PI-3, the EtOH, BuOH extracts of C. laurifolius, and the BuOH extract of S. wiedemannii showed the same inhibitory activity (CPE of 64–16 µg/mL).

DISCUSSION

In a previous study, in vitro bioactivities of the aqueous extracts of C. laudanifer and C. populifolius leaves were studied.
**Table 1:** Antimicrobial activity of the *C. laurifolius* extracts and references expressed as minimum inhibitory concentrations (MICs; μg mL⁻¹)

| Extracts | *E. coli* ATCC | Gram negative bacteria | *P. aeruginosa* ATCC | *P. mirabilis* ATCC | *K. pneumoniae* ATCC | *A. baumannii* ATCC | *S. aureus* ATCC | Gram positive bacteria | *E. faecalis* ATCC | Yeasts | *C. albicans* ATCC | *C. parapsilosis* ATCC |
|----------|----------------|------------------------|----------------------|---------------------|---------------------|---------------------|------------------|----------------------|---------------------|--------|------------------|------------------|
|          | ATCC | Isol | ATCC | Isol | ATCC | Isol | ATCC | Isol | ATCC | Isol | ATCC | Isol |
| EtOH     | 32   |       | 64   | 64   | 128  | 32   | 64   | 64   | 32   | 64   | 32   | 64   | 32   | 64   | 64   | 128  | 64   | 128  | 8    | 8    |
| r-H₂O    | 32   | 64   | 64   | 64   | 128  | 64   | 64   | 64   | 64   | 64   | 32   | 64   | 64   | 64   | 64   | 128  | 64   | 128  | 8    | 8    |
| CHCl₃    | 32   | 64   | 64   | 64   | 128  | 32   | 64   | 64   | 64   | 32   | 64   | 32   | 64   | 32   | 64   | 32   | 64   | 32   | 64   | 8    | 8    |
| BuOH     | 32   | 64   | 64   | 64   | 128  | 32   | 64   | 64   | 64   | 32   | 64   | 32   | 64   | 32   | 64   | 32   | 64   | 32   | 64   | 8    | 8    |
| Hexane   | 32   | 64   | 64   | 64   | 128  | 32   | 64   | 64   | 64   | 32   | 64   | 32   | 64   | 32   | 64   | 32   | 64   | 32   | 64   | 8    | 8    |
| AMP      | 2    | 64   | -    | -    | 2    | 4    | 4    | 2    | 4    | 2    | 4    | <0.12 | 8    | 0.5  | 1    | NT    | NT    |
| OFX      | 0.12 | 1    | 1    | 4    | <0.12 | 1    | 0.12 | 2    | 0.5  | 4    | 1    | 2    | NT   | NT   |
| LVX      | <0.12 | 0.25 | 1    | 2    | 0.12 | <0.12 | 0.12 | 2    | 0.5  | 4    | 0.5  | 2    | NT   | NT   |
| KET      | 0.1  | NT   | NT   | NT   | 0.1  | NT   | NT   | NT   | NT   | NT   | NT   | NT   | NT   | NT   | 1    | 1    |
| FLU      | 0.1  | NT   | NT   | NT   | 0.1  | NT   | NT   | NT   | NT   | NT   | NT   | NT   | NT   | NT   | 4    | 4    |

**Table 2:** Antiviral activity together with CPEs of the extract of the *C. laurifolius* and *S. wiedemannii* and references as MICs (μg mL⁻¹) values

| Extracts | MDBK cells (mg mL⁻¹) | MNTC³ (mg mL⁻¹) | CPE² inhibitory concentration | Vero cells (mg mL⁻¹) | MNTC³ (mg mL⁻¹) | CPE² inhibitory concentration |
|----------|----------------------|----------------|--------------------------------|----------------------|----------------|--------------------------------|
|          | HSV-1 Max. | HSV-1 Min. | HSV-1 Max. | HSV-1 Min. | HSV-1 Max. | HSV-1 Min. | HSV-1 Max. | HSV-1 Min. | HSV-1 Max. | HSV-1 Min. | HSV-1 Max. | HSV-1 Min. |
| Cl-2EtOH | 32      | 64    | 32      | 64    | 32      | 64    | 32      | 64    | 32      | 64    | 32      | 64    |
| Cl-r-H₂O | 32      | 64    | -       | -     | 32      | 64    | -       | -     | 32      | 64    | -       | -     |
| Cl-CHCl₃ | 32      | -     | 32      | 64    | 32      | -     | 32      | 64    | -       | -     | 32      | 64    |
| Cl-BuOH  | 32      | 32    | 16      | 64    | 64      | 16    | 64      | 16    | 64      | 16    | 32      | 32    |
| Cl-Hexane| 32      | 32    | 16      | 64    | 64      | 16    | 64      | 16    | 64      | 16    | 32      | 32    |
| Sw-BuOH  | 32      | 32    | <0.25   | 64    | 64      | 16    | 64      | 16    | 64      | 16    | 32      | 32    |
| Sw-H₂O   | 16      | 16    | <0.25   | 64    | 64      | 16    | 64      | 16    | 64      | 16    | 32      | 32    |
| Sw-CHCl₃ | 16      | 16    | <0.25   | 64    | 64      | 16    | 64      | 16    | 64      | 16    | 32      | 32    |
| Sw-EtOH  | 16      | 16    | <0.25   | 64    | 64      | 16    | 64      | 16    | 64      | 16    | 32      | 32    |
| Sw-Hexane| 46      | 64    | 64      | 64    | 64      | 64    | 64      | 64    | 64      | 64    | 64      | 64    |
| Acyclovir| 16      | 16    | <0.25   | NT    | NT      | NT    | NT      | NT    | NT      | NT    | 16      | 16    |
| Oseltamivir | NT         | NT      | NT      | 32      | 32    | <0.25   | 32      | 32    |

MNTC: Maximum non-toxic concentration; CPE: Cytopathogenic effect; Cl-: Cisto laurifolius; SW: Salvia wiedemannii; *: No activity observed; NT: Not tested

exhibited antimiobic activity.

C. *incanus* extract which was rich in polyphenolics exerted antiviral activity against influenza A virus (H1N1) *in vitro* and *in vivo*. Relevantly, antiviral activity of *Salvia* species has been evaluated. Ogutcu et al. studied the methanol, Hx, and dichloromethane extracts of *S. limbata* and *S. sclarea*. Anti-influenza drug rimantadine and (E)-5-(2-bromovinyl)-2′-deoxyuridine hydrochloride were used as the references. The dichloromethane extract of *S. sclarea* and the methanol extract of *S. limbata* showed high anti-influenza virus activity while the methanol extract of *S. sclarea* had a limited antiherpetic activity.[14] The inhibitory activity of the aqueous extract of *S. officinalis* on HSV-1 and HSV-2 was tested using RC-37 cells (African green monkey kidney cells). In the study, acyclovir was the positive control. The aqueous extracts of *S. officinalis* had significant antiviral activity on HSV-1 (0.777 μg/mL) and HSV-2 (1.359 μg/mL). In another study, antiviral activities of seven extracts of *S. miltiorrhiza* were evaluated.[14] The ethyl acetate and water extracts of the plant inhibited viral RNA synthesis; however, the other extracts did not have any protective activity. Fifty percentage inhibitory concentrations of the ethyl acetate and water extracts on *Enterovirus* 71 were 0.742 ± 0.042 mg/mL and 0.585 ± 0.018 mg/mL, respectively. Likewise, another study showed antiviral activity (anti-human deficie cy virus) with no cytoxicity of two compounds (lithospermic acid and lithospermic acid B) isolated from the roots of *S. miltiorrhiza*. Th Th C100 doses for lithospermic and lithospermic acid B were >297 μM and >223 μM, respectively.

Recently, resistance to conventional antiviral drugs is on rise. Therefore, there is a strong need for novel effective substances synthesized or isolated from plants. Th s study shows for the f st time that *C. laurifolius* and *S. wiedemannii* are very promising plants regarding their antiviral and cytotoxic effects. However, further studies are necessary on the isolation and mechanism of the active component(s) of *C. laurifolius* and *S. wiedemannii*.

Financial support and sponsorship
Nil

Conflicts of interest
There are no confl its of interest.

REFERENCES
1. Mathur A, Bhat R, Prasad GBKS. Antimicrobial activity of plants traditionally used as medicines against some pathogens. Rasayan J Chem 2010;3:615-20.
2. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol 2005;100:90-4.
3. Davis PH. Flora of Turkey and the East Aegean Islands. Edinburgh University press. Scotland, 1996.
4. Yøjilada E, Honda G, Sezik E. Traditional Medicine in Turkey. Vol. 1. Folk Medicine in the Inner Taurus Mountains. J Ethnopharmacol 1995;46:133-52.
5. Honda G, Yøjilada E, Tabata M, Sezik E, Fujita T, Takeda Y, et al. Traditional Medicine in Turkey XII. Folk Medicine in Anatolia: Erzurum, Erzincan, Ağrı, Kars, Iğdır provinces. J Ethnopharmacol 1996;53:75-87.
6. Tabata M, Sezik E, Honda G, Yøjilada E, Fukui H, Goto K, et al. Traditional medicine in Turkey III. Folk medicine in East Anatolia, Van and Bitlis provinces. Int. J. Pharmacogn 1994;32:3-12.
7. Fujita T, Sezik E, Tabata M, Yøjilada E, Honda G, Takeda Y, et al. Traditional medicine in Turkey XVII. Folk medicine in Middle and West Black Sea Regions. Econ Bot 1996;49:405-22.
8. Sezik E, Yøjilada E, Honda G, Takaishi Y, Takeda Y, Tanaka T. Traditional medicine in Turkey XV. Folk medicine in Central Anatolia. J Ethnopharmacol 2001;75:95-116.
9. Yøjilada E, Honda G, Sezik E, Tabata M, Goto K, Ikeshiro Y. Traditional medicine in Turkey IV. Folk medicine in the Mediterranean Subdivision. J Ethnopharmacol 1993;39:31-8.
10. Sezik E, Yøjilada E, Tabata M, Honda G, Takaishi Y, Fujita T. Traditional medicine in Turkey VIII. Folk medicine in East Anatolia: Erzum, Erzincan, Ağrı, Kars, Iğdır provinces. Econ Bot 1997;51:195-211.
11. Catalán EB, Amoros SE, Saura D, Guillen E, Guiebierre FA, Carretero SA. Cistaceae aqueous extracts containing ellagitannins show antioxidant and antimicrobial capacity, and cytotoxic activity against human cancer cells. Food Chem Toxicol 2010;48:2273-82.
12. Güvenç A, Yıldız Ş, Öksüz AM, Erdurak CS, Çağırın M, Yılmaz G, et al. Antimicrobial Studies on Turkish Cistus Species. Pharm Biol 43;2005:178-83.
13. Ustün O, Özçelik B, Akyol Y, Abbasoglu U, Yojilada E. Flavonoids with anti-Helicobacter pylori activity from Cistus laurifolius leaves. J Ethnopharmacol 2006;108:467-61.
14. Ogutçu H, Sokmen A, Sokmen M, Polissoiu M, Serkedjıeva J, Daferera D, et al. Bioactivities of the various extracts and essential oils of Salvia sclarea. J Ethnobot Med 2008;52:181-192.
15. Sadhu SK, Ouyama E, Fujimoto H, Ishibashi M, Yojilada E. Prosstaglandin inhibitory and antioxidant components of Cistus laurifolius, a Turkish medicinal plant. J Ethnopharmacol 2008;108:371-78.
16. Örhan I, Kartal M, Naz Q, Ejaz A, Yılmaz G, Kar Y, et al. Antioxidant and anticholinesterase evaluation of selected Turkish Salvia species. Food Chem 2007;103:1247-54.
17. Yojilada E, Ustün Ö, Sezik E, Takaishi Y, Ono Y, Honda G. Inhibitory effects of Turkish remedies on inflammatory cytokines: Interleukin-1α, Interleukin-1β and Tumor Necrosis Factor α. J Ethnopharmacol 1997;58:59-73.
18. Küpeli E, Yojilada E. Flavonoids with anti-inflammatory and anticoagulant activity from Cistus laurifolius L. leaves through bioassay-guided procedures. J Ethnopharmacol 2007;112:524-30.
19. Küpeli Akkol E, Goger E, Kosar M, Başer KHC. Phenolic composition and biological activities of Salvia haluphiæ and Salvia virgata from Turkey. Food Chem 2008;108:942-49.
20. Arı M, Ustün Ö, Yojilada E. Analgesic activity of Cistus laurifolius in mice. Pharm Biol 2004;42:176-78.
21. Amabeoku GJ, Eagles P, Scott G, Mayeng I, Springfield E. Analgesic and antipyretic effects of Dodonaea angustifolia and Salvia africana-lutea. J Ethnopharmacol 2001;75:117-124.
22. Droebner K, Ehhardt C, Pretter A, Ludwig S, Planz O. CYSTUS056, a polyphenol-rich plant extract, exerts anti-influenza virus activity in mice. Antiviral Res 2007;76:1-10.
23. Nollemper S, Reichling J, Stintzing FC, Carle R, Schützler R. Antiviral effect of aqueous extracts from species of the Lamiaceae family against Herpes simplex virus type 1 and type 2 in vitro. Pflanz Med 2006;72:1378-1382.
24. Wu BW, Pei TL, Leu YL, Chang YK, Tai PJ, Lin KH, et al. Antiviral effects of Salvia miltiorrhiza (Danshen) against enterovirus 71. Am J Chin Med 2007;35:153-168.
25. Abd-Elaezrn IS, Chen HS, Bates RB, Huang RC. Isolation of two highly potent and non-toxic inhibitors of human immunodeficiency virus type 1 (HIV-1) integrase from Salvia miltiorrhiza. Antiviral Res 2002;55:91-106.
26. Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS), National Committee for Clinical Laboratory Standards, Performance standards for antimicrobial susceptibility testing; CLSI document M7-A7, PA, USA, 2006.
27. Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeast; approved standard- third edition. CLSI document M27-A3, Clinical and Laboratory Standards Institute, PA, USA, 2008.
28. Özçelik B, Koza U, Kaya DA, Şekeroglu N. Evaluation of the in vitro bioactivities of Mahaleb Cherry (Prunus mahaleb) L. Rom Biotech Lett 2012;7:7863-72.
29. Piras A, Rosa A, Marongiu B, Forcedda S, Falconieri D, Dessì MA, et al. Chemical composition and in vitro bioactivity of the volatile and fixed oils of Nigella sativa L. extracted by supercritical carbon dioxide. Ind Crops and Prod 2013;34:317-323.

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