Araştırma Makalesi – Original Article

The Antimicrobial, Antioxidant and Anti-Inflammatory Evaluation of *Marrubium cuneatum* Banks & Sol. Extracts & Fractions*

Marrubium cuneatum Banks & Sol. Özüt ve Fraksiyonlarının Antimikrobiyal, Antioksidan ve Antienflamatuvar Etkilerinin Değerlendirilmesi

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ABSTRACT

Objective: This study aimed to determine the antimicrobial, antioxidant, and anti-inflammatory potential of the *Marrubium cuneatum* aqueous and extracts, in addition to the fractions acquired from the liquid-liquid fractionation, respectively.

Method: The plant extracts were obtained using maceration, which were separated into their fractions based on polarity using the liquid-liquid fractionation method. The *in vitro* antimicrobial activity was evaluated using the microdilution method. The antioxidant activity was assessed using DPPH•, $ABTS^{+}$, and CUPRAC assays. The anti-inflammatory activity of the extracts and fractions were evaluated by examining their effects on lipoxygenase (5-LOX) enzyme inhibition.

Results: In the antioxidant assays, the *n*-butanol fraction of the methanolic extract and the ethyl acetate fraction of the aqueous extract showed activity in all tests, followed by the ethyl acetate fraction of the methanolic extract, respectively. In the antimicrobial assays, no remarkable inhibitions were observed compared to the reference compounds. It was noticed that the extracts and fractions showed no effect on the inhibition of the lipoxygenase enzyme.

Conclusion: The ethyl acetate and *n*-butanol fractions showed relatively strong antioxidant effects, however, no significant antimicrobial activity was observed. In terms of anti-inflammatory activity, it was determined that the plant did not exhibit any anti-inflammatory effect through the lipoxygenase enzyme suggesting further detailed research on other fractions or purified compounds of the plant material.

Keywords: *Marrubium*, Antioxidant, Antimicrobial, Lipoxygenase, Ethnopharmacology

ÖZ

Amaç: Bu çalışmada *Marrubium cuneatum* bitkisinin sulu ve metanol özütleri ile bu özütlerin sıvı-sıvı fraksiyonlanması sonucu elde edilen fraksiyonlarının antimikrobiyal, antioksidan ve antienflamatuvar etkilerinin belirlenmesi amaçlanmıştır.

Yöntem: Bitki özütleri maserasyon yöntemi ile elde edilmiştir. Elde edilen özütler, sıvı-sıvı fraksiyonlama tekniği kullanılarak polaritelerine göre ayrılmıştır. Özüt ve fraksiyonların antimikrobiyal etkisi mikrodilüsyon yöntemi ile değerlendirilmiştir. DPPH•, $ABTS^{+}$ ve CUPRAC yöntemleri ile antioksidan etki, lipoksijenaz enzimi inhibisyonu yöntemi ile ise antienflamatuvar etki değerlendirilmiştir.

Bulgular: Antioksidan etki deneylerinde metanol özütünün *n*-bütanol fraksiyonu ile sulu özütün etil asetat fraksiyonu çalışılan tüm yöntemlerde etkileri ile öne çıkmıştır. Bu iki fraksiyonu metanol özütünün etil asetat fraksiyonu takip etmiştir. Antimikrobiyal etki deneylerinde, kullanılan referans bileşiklere kıyasla dikkate değer bir etki elde edilemediği gözlenmiştir. Çalışılan örneklerin lipoksijenaz enziminin inhibisyonu üzerinde herhangi bir etkisinin olmadığı gözlenmiştir.

Sonuç: Bitkinin etil asetat ve *n*-bütanol fraksiyonlarının güçlü antioksidan etkisi tespit edilmiştir ancak dikkate değer bir antimikrobiyal aktivitesi olmadığı görülmüştür Antienflamatuvar aktivitede ise lipoksijenaz enzimi üzerinden bitkinin antienflamatuvar bir etkisinin olmadığı belirlenmiştir. Buna bağlı olarak bitkinin farklı ekstre ve maddeleriyle araştırmalar yapılması önerilmektedir.

Anahtar Kelimeler: *Marrubium*, Antioksidan, Antimikrobiyal, Lipoksijenaz, Etnofarmakoloji

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Introduction

Marrubium L. a member of the Lamiaceae family (Lamioideae subfamily), contains approximately forty species indigenous to the Mediterranean, Asia, and Europe. There are 27 taxa that represent the genus in Türkiye, of which 17 are endemic.[1,2] The literature reports that several *Marrubium* species are employed in traditional medicine to cure various kinds of illnesses.[3-5] Undoubtedly the best known species of the genus is *Marrubium vulgare*, which is also included in monographs. *M. vulgare* is used as an expectorant, digestive stimulant, diuretic, asthma and flu remedies and has anti-inflammatory properties for liver issues in traditional medicine.[6-8] Studies have exhibited the anti-inflammatory, antimicrobial and antioxidant properties of the extracts obtained from various parts of *M. vulgare*.[7,9,10] Scientific results on *Marrubium* species have suggested a potential source for medicinal use of the plants.

Upon reviewing the literature on *M. cuneatum*, it becomes evident that research on this plant is relatively scarce. However, studies focusing on the analysis of the essential oil composition have drawn attention. The volatiles of the plant from Lebanon was analyzed, and germacrene D (15.6%) and spathulenol (6.5%) were identified as its major components. The antimicrobial effect of the derived essential oil was also assessed, and a negligible activity was observed.[11] The essential oil obtained from the plant grown in Iran was analyzed, and bicyclogermacrene (37.9%) and germacrene D (24.1%) were identified as the major components.[12] The essential oil of the plant collected from Türkiye was analyzed by our research group and the main compounds were determined to be β-caryophyllene (9.0%), caryophyllene oxide (9.8%) and linalool (29.7%).[13] Although there are few studies on *M. cuneatum*, according to reports it is used traditionally among the public. The infusion prepared from the leaves of *M. cuneatum* was used to treat abdominal pain in Malatya (Türkiye),[14] in Mardin (Türkiye) an infusion of made from flowers was used to cure coughs and the common cold,[15] in Lebanon the decoction of the flowering parts was utilized to cure haemorrhoids by compressing.[16] Although it is used for medical purposes among the public, the fact that there are few studies on it stands out as a deficiency in the literature. Therefore, the *in vitro* anti-inflammatory, antioxidant and antimicrobial activities of the plant were evaluated. To obtain a more specific study and evaluate the possible activity more accurately, not only the crude extracts but also the effects of their fractions varying according to polarity were analyzed. The purpose of this study was to close the gap in the literature specially for *M. cuneatum* and to investigate new potential medicinal plants. To the best of our knowledge, this study represents the first activity research based on extracts and fractions obtained from the aerial parts of *M. cuneatum* extracts.

Material and Method

Plant Material

Aerial parts of *M. cuneatum* were collected from Yeşilyurt, Malatya province of Türkiye, in 2013. Prof. Dr. Turan Arabacı (Department of Pharmaceutical Botany, Inonu University) recognized the plant and a voucher specimen was deposited in the herbarium of Faculty of Pharmacy, Inonu University. The aerial parts were dried in shade. The dried plant was powdered, weighed in 2x100 grams, and extracted separately with methanol (MeOH) or water (H_2O). After the crude extracts were obtained. The methanol extract was respectively fractionated with, *n*-hexane (*n*-Hex), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH) and the aqueous extract was respectively fractionated with ethyl acetate and *n*-butanol. The remaining aqueous ($R.H_2O$) fractions of both extracts were also evaluated. The data obtained in the extraction process are presented in ***Table 1***.

Antioxidant Activities

With a few minor adjustments, the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay from Brand-Williams et al.,[17] the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radical cation decolourization assay from Re et al.,[18] and the cupric reducing antioxidant capacity (CUPRAC) from Apak et al.[19] were used to assess the antioxidant activities of extracts and fractions.

In the DPPH radical scavenging assay, 50 µL of DPPH solution was added to 150 µL of each diluted (7.81-500 µg/mL) extracts and fractions in a 96-well microplate with 3 repetitions. The reaction mixture was slightly shaken and kept in dark for 30 min at room temperature before the spectrophotometric reading process. Following incubation, each concentration's absorbance at 517 nm was determined in relation to a blank absorbance. The graphical plot of the percent inhibition against extract concentration was used for determining the inhibitor concentration (IC_{50}). Butylated hydroxytoluene (BHT) was used as the reference.

In the ABTS radical cation decolorization method, radical cation was produced by adding 7mM ABTS, which solved in water to 2.45 mM potassium persulfate and carefully shaken for 12 hours at room temperature in the dark. In a 96-well microplate, 150 µL of each diluted extracts and fractions (7.81-500 µg/mL) was added to 50 µL of ABTS solution with 3 repetitions. After incubation, each concentration's absorbance at 734 nm was determined in relation to a blank absorbance. The IC_{50} was calculated using a graphical plot of the percent inhibition against extract concentration. Gallic acid (GA) was used as the reference in the ABTS assay.

In the CUPRAC assay, an ammonium acetate aqueous buffer at pH 7.00, copper (II) chloride solution, 7.5 mM alcoholic neocuproine solution, and distilled water added on the diluted extracts and fractions (7.81-500 µg/mL) in a 96-well microplate and shaken while incubating for 30 minutes in the dark. Following incubation, absorbance was measured at 450 nm. GA equivalent (mg GA/g E) was used to indicate cupric reducing antioxidant activity (E = Extract weight).

Anti-Inflammatory Activity

The anti-inflammatory potential of the extracts and fractions were determined by the Soya bean origin [*Glycine max* (L.) Merr.] lipoxygenase enzyme (5-LOX). With a few slight modifications, the spectrophotometric technique developed by Baylac and Racine[20] was utilized, with linoleic acid acting as the substrate. By measuring spectrometric kinetic absorbance at 234 nm/min for 10 min, enzyme inhibition (%) was determined using the formula below:

Inhibition % = [(A-B)/A] x 100

A: (3. min abs - 1. min abs) Control

B: (3. min abs - 1. min abs) Extract/Fraction

abs = absorbance

Antimicrobial Activity

Microbial strains

Klebsiella pneumonia NCTC 9633, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 19111, *Escherichia coli* NRRL B-3008, *Salmonella typhimurium* ATCC 13311, *Candida albicans* ATCC 90028 and *C. krusei* ATCC 6258 standard strains were obtained from the American Type Culture Collection (ATCC). All strains were stored at −85°C in 15% glycerol prior to use. Mueller Hinton agar and Mueller Hinton Broth was used for bacterial strains as growth medium for 24 hours at 37 °C. *Candida* strains were cultured on

Potato Dextrose Agar plates and RPMI medium at 37 °C. Afterwards, all microorganisms were standardized versus McFarland No: 0.5 (1 x 106 CFU/mL for *Candida* sp. and 1 x 10^8 CFU/mL for bacteria).[21-23]

***In vitro* microdilution method**

Stock solution of the test samples was prepared using methanol (MC-H_2O, MC-H_2O/R.H_2O and MC-MeOH/R.H_2O was prepared with sterile distilled water) and diluted with sterile distilled water from 5 mg/mL to 0.04 mg/mL in 96 well microtiter format. 100 µL, 1/100 diluted bacterial suspensions[24] and 1/1000 diluted *Candida* suspensions[21] were after added to each well. After incubation for 18-24 h at 37 °C, for spotting of viable microorganisms, 20 µL 0.01 % resazurin solution was added to all of the plate. The lowest sample concentration at which no discernible bacterial growth occurs is known as the minimum inhibitory concentration (MIC).[25,26] The last row containing medium with microorganism was used as negative control and medium served as a growth control. Chloramphenicol, amphotericin B, amoxicillin and Nystatin were used as reference antimicrobial agents at concentration range 0.062-32 µg/mL.

Statistical Analysis

Every sample was repeated three times for every experimental technique. Results from studies were expressed as standard error of mean (±SEM). To determine whether there were any significant differences, the information was contrasted using one-way analysis of variance (ANOVA) and Tukey's test.

Results and Discussion

The results obtained from the extraction process showed that the yield of the aqueous extract was slightly higher than the methanol extract. In the liquid-liquid fractionation stage, for both extract the amount of polar fractions, *n*-BuOH and R.H_2O fractions, attracted attention (***Table 1***).

Table 1. Yields of the fractions acquired from *M. cuneatum*

***Marrubium cuneatum* (100g)**	**Extracts yields (g)**	**Liquid-Liquid fractionation yields (g)**				
		***n*-Hex**	**CH_2Cl_2**	**EtOAc**	***n*-BuOH**	**R.H_2O**
MeOH	13.81	1.30	1.94	0.95	4.72	3.87
H_2O	17.76	-	-	0.87	2.01	11.02

Table 2 shows the antioxidant activity of the extracts and fractions from *M. cuneatum*. According to the findings, the highest activity in the ABTS experiment was observed in the MeOH/EtOAc fraction (IC_{50} =17.12 µg/mL). The MeOH/*n*-BuOH fraction (IC_{50} =36.78 µg/mL) and the H_2O/EtOAc fraction (IC_{50} =41.27 µg/mL) came after this activity. The IC_{50} value of GA used as reference was determined as 8.45 µg/mL. In the DPPH assay, the highest inhibitor activity was obtained from the MeOH/*n*-BuOH fraction (IC_{50} =60.21 µg/mL), the H_2O/EtOAc fraction (IC_{50} =67.07 µg/mL) came after this activity. BHT was used as reference and its inhibitor concentration was measured as 28.97 µg/mL. In the CUPRAC assay, as in the DPPH assay MeOH/*n*-BuOH fraction (110.05 mg GA/g E) and the H_2O/EtOAc fraction (99.00 mg GA/g E) exhibited the strongest antioxidant activity, respectively. When the 3 experiments were evaluated together, it was observed that the most effective fraction was the MeOH/*n*-BuOH fraction. This fraction is followed by the H_2O/EtOAc fraction and the EtOAc fraction of the methanol extract. Although it is known that *n*-BuOH fractions contain more polar phytochemicals than EtOAc fractions, we need to emphasize that they are close in polarity. They may even contain some of the same phytochemicals because they are consecutive fractions. Detailed analytical content analyses are needed to identify the phytochemistry. It has been was reported that the fractions generally contain flavonoids, phenols, tannins, anthocyanins, and triterpenes.[27]

Table 2. Antioxidant activity evaluation of the extracts and fractions.

Extracts/ Fractions /References	**ABTS IC_{50} (µg/mL)**	**DPPH IC_{50} (µg/mL)**	**CUPRAC (mg GA/g E)**
MC-H_2O	95.63±7.79	NA	41.58±1.35
MC-H_2O/EtOAc	41.27±1.88	67.07±1.13	99.00±3.74
MC-H_2O/*n*-BuOH	81.39±5.91	164.12±0.76	43.82±3.74
MC-H_2O/R.H_2O	272.06±24.57	-	25.52±0.67
MC-MeOH	99.37±3.03	125.94±3.65	22.17±0.62
MC-MeOH/*n*-Hex	1335.86±57.12	-	48.58±1.09
MC-MeOH/CH_2Cl_2	97.02±2.43	314.35±7.25	34.92±1.15
MC-MeOH/EtOAc	17.12±0.08	106.45±2.50	49.31±0.40
MC-MeOH/*n*-BuOH	36.78±0.40	60.21±1.25	110.05±0.96
MC-MeOH/R.H_2O	296.10±13.26	-	14.29±0.32
BHT		28.97±0.77	
GA	8.45±0.72		

-: No activity

In 5-LOX inhibition experiments it was observed that the extracts and fractions acquired from the aerial parts of *M. cuneatum* were not effective. Although it has been observed that the extracts and fractions have no effect on the inhibiton of 5-LOX enzyme, It would be inaccurate to state that they have no anti-inflammatory effects. It is known that anti-inflammatory effects can occur through many different pathways. Therefore, not having an effect on one of these, the LOX enzyme, does not mean that it has no anti-inflammatory effects. Rigano and colleagues[28] emphasized that the activity obtained in their *in vivo* anti-inflammatory experiment of *M. globosum* subsp. *libanoticum* acetone extract was a result of the inhibition of COX-2 and iNOS activities, which was due to marrulibanoside, a labdane diterpene that they isolated from the acetone extract.[28] This study supported that extracts and fractions from *M. cunetaum* showed no anti-inflammatory effect by inhibiting the 5-LOX enzyme. More specific studies may reveal whether anti-inflammatory effects are through other pathways.

Table 3. Antimicrobial evaluation of extracts and fractions

	***Escherichia coli* NRRL B-3008**	***Staphylococcus aureus* ATCC 6538**	***Salmonella typhimurium* ATCC 13311**	***Listeria monocytogenes* ATCC 19111**	***Klebsiella pneumoniae* NCTC 9633**	***Candida albicans* ATCC 90028**	***C. krusei* ATCC 6258**
MC-H2O	-	-	-	-	-	-	-
MC-H2O/EtOAc	-	2.5	1.25	2.5	2.5	-	-
MC-H2O/*n*-BuOH	-	-	-	-	-	-	-
MC-H2O/R.H2O	-	-	-	-	-	-	-
MC-MeOH	-	-	-	-	-	-	1.25
MC-MeOH/*n*-Hex	-	-	-	-	-	0.31	1.25
MC-MeOH/CH2Cl2	-	5	2.5	5	5	0.16	0.04
MC-MeOH/EtOAc	-	-	-	-	-	-	0.02
MC-MeOH/*n*-BuOH	-	-	-	-	-	-	0.01
MC-MeOH/R.H2O	-	-	-	-	-	-	-
Chloramphenicol	8	8	8	4	8		
Amoxicillin	0.25	>32	<0.062	<0.062	<0.062		
Amphotericin B						0.125	0.125
Nystatin						1	0.25

-: No activity

MIC range of extracts and fractions: (0.005-5 mg/mL)

MIC range of reference antimicrobials: (0.062-32 µg/mL)

The antibacterial activity study showed that the fractions and extracts were ineffective against *E. coli* strain at the tested concentration. Only the H_2O/EtOAc fraction and the MeOH/CH_2Cl_2 fraction were effective against other bacterial species at the concentration range of 5-1.25 mg/mL. The best anticandidal effect was observed in the MeOH/*n*-BuOH fraction at a concentration of >0.01 mg/mL against *C. krusei* strain. The H_2O/EtOAc fraction, which was found to have antioxidant effect, showed the highest antimicrobial activity. This effect has raised curiosity on major compounds in the fraction, which may be subject to another study.

The effects of the essential oil obtained from *M. cuneatum*, whose essential oil composition was provided above and collected from Lebanon, were evaluated on certain bacterial strains, and it was reported that no significant effect was observed.[11] The effects of the methanol extract prepared from the aerial parts of *M. vulgare* on certain bacteria were evaluated, and it was reported to have a moderate effect.[10] In addition to the limited studies conducted on *Marrubium* species, there are almost no studies on the reasons behind the observed activity. Therefore, compounds present in active extracts should be isolated, characterised and activity studies should be evaluated using pure compounds. This study may form the basis of further studies aimed at elucidating the phytochemical content of *M. cuneatum*.

Conclusion

In this study, anti-inflammatory, antioxidant and antimicrobial effects of the extracts and fractions of *M. cuneatum* were evaluated, in order to reveal its medical potential. In the antioxidant tests, significant effects of *n*-BuOH and EtOAc fractions of both extracts were observed. In terms of anti-inflammatory effect, it has been shown that neither extracts nor fractions inhibit the 5-LOX enzyme and do not provide an anti-inflammatory effect through this pathway. In terms of antimicrobial activity, only the H_2O/EtOAc fraction and the MeOH/CH_2Cl_2 fraction were found to be moderately effective. It was found that not a single extract or fraction were effective on *E. coli*. Confirming the medical impact of the extracts and fractions will require more thorough *in vitro* and *in vivo* investigations.

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References

1. Kharazian N, Hashemi M. Chemotaxonomy and morphological studies in five *Marrubium* L. Species in Iran. Iran J Sci Technol Trans A Sci 2017;41:17–31.
2. Celep F, Dirmenci T. Systematic and Biogeographic overview of Lamiaceae in Turkey. Volatiles Essent Oils 2017;4(4):14–27.
3. Erarslan ZB, Çolak R, Kültür Ş. The preliminary ethnobotanical survey of medicinal plants in Develi (Kayseri/Turkey). İstanbul J Pharm 2021;51:263–70.
4. Bulut G, at al. An ethnobotanical study of medicinal plants in Acipayam (Denizli-Turkey). J Herb Med 2017;10:64–81.

5. Meyre-Silva C, Cechinel-Filho V. A Review of the Chemical and Pharmacological Aspects of the Genus *Marrubium*. Curr Pharm Des 2010;16:3503–18.
6. Henderson MS, McCrindle R. Premarrubiin. A Diterpenoid from *Marrubium vulgare* L. J Chem Soc C Org 1969;15:2014–5.
7. Sahpaz S, et al. Isolation and pharmacological activity of phenylpropanoid esters from *Marrubium vulgare*. J Ethnopharmacol 2002;79:389-92.
8. Lorenzi H, Matos FJA. Plantas Medicinais Do Brasil: Nativas e Exóticas. Nova Odessa: Instituto Plantarum, Sao Paulo, 2008.
9. VanderJagt TJ, et al. Comparison of the total antioxidant content of 30 widely used medicinal plants of New Mexico. Life Sci 2002;70:1035–40.
10. Masoodi MH, et al. Antibacterial activity of whole plant extract of *Marrubium vulgare*. African J Biotechnol 2008;7:86–7.
11. Grassia A, et al. Chemical composition and antimicrobial activity of the essential oils from aerial parts of two *Marrubium* sp. (Lamiaceae) growing wild in Lebanon. Pol J Chem 2006;80:623–28.
12. Baher Nik Z, Mirza M, Shahmir F. Essential oil of *Marrubium cuneatum* Russell and its secretory elements. Flavour Fragr J 2004;19:233–35.
13. İçen MS, et al. Chemical profile of the volatile compounds from *Marrubium cuneatum* Banks & Sol. Nat Volatiles Essent Oils 2021;8:1–6.
14. Tetik F, Civelek S, Cakilcioglu U. Traditional uses of some medicinal plants in Malatya (Turkey). J Ethnopharmacol 2013;146:331–46.
15. Kılıç M, Yıldız K, Kılıç FM. Traditional uses of wild plants in Mardin central district and attached villages (Turkey). Indian J Tradit Knowl 2021;20:784–98.
16. El Beyrouthy M, Dhifi W, Arnold-Apostolides N. Ethnopharmacological survey of the indigenous Lamiaceae from Lebanon. Acta Hortic 2013;997:257–76.
17. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol 1995;28:25–30.
18. Re R, et al. Antioxidant activity applying an improved abts radical cation decolorization assay. Free Radic Biol Med 1997;26:1231–37.
19. Apak R, et al. Methods of measurement and evaluation of natural antioxidant capacity/activity. Pure Appl Chem 2013;85:957–98.
20. Baylac S, Racine P. Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. Int J Aromather 2003;13:138–42.
21. Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi, Approved Standard-Second Edition Method. M38-A2. CLSI 2002;22:1-27.
22. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Seventh Edition. M7-A7. CLSI 2006;26(2).
23. Demirci B, et al. Anticandidal pimaradiene diterpene from *Phlomis* essential oils. Comptes Rendus Chim 2009;12:612–21.
24. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. M100-S17. CLSI 2007;27(1).
25. Demirci F, et al. Anti-inflammatory and antibacterial evaluation of *Thymus sipyleus* Boiss. subsp. *sipyleus* var. *sipyleus* essential oil against rhinosinusitis pathogens. Microb Pathog 2018;122:117–21.
26. Kırmızıbekmez H, et al. Characterization of *Sideritis trojana* Bornm. essential oil and its antimicrobial activity. Marmara Pharm J 2017;21(4):860-65.
27. Etame RME, et al. Effect of fractioning on antibacterial activity of n-butanol fraction from *Enantia chlorantha* stem bark methanol extract. BMC Complement Altern Med 2019;19:1–7.
28. Rigano D, et al. Phytochemical and pharmacological studies on the acetonic extract of *Marrubium globosum* ssp. *libanoticum*. Planta Med 2006;72:575–78.