Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

Phytochemical Analysis, Antibacterial Activity of Marrubium vulgare L against Staphylococcus aureus in vitro

Mohammad Bokaeian,[1] Elham Saboori,[2] Saeide Saeidi,*[3] Abbas Ali Niazi,[4] Negar Amini-Borojeni,[5] Hamde Khaje,[6] Saphora Bazi[6]

1. Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, Iran
2. Department of Agricultural Biotechnology, Zabol University, Zabol, Iran
3. Department of Biology, Faculty of Sciences, Science and Research Branch, Islamic Azad University, Kerman, Iran
4. Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, Iran
5. Agricultural Biotechnology Research Institute, Zabol University, Zabol, Iran
6. Department of Biology, Faculty of Sciences, Payam-e-Noor University, Isfahan, Iran

Article information

Article history:
Received: 2 July 2013
Accepted: 20 July 2013
Available online: 10 Sep 2013
ZJRMS 2014 Oct; 16 (10): 60-64.

Keywords:
Phytochemical analysis
Antibacterial activity
Marrubium vulgare L

*Corresponding author at:
Department of Biology, Faculty of Sciences, Science and Research Branch, Islamic Azad University, Kerman, Iran.
E-mail:
s.saeedi12@yahoo.com

Abstract:

***Background*:** Herbal medicines are the major remedy in traditional medical systems and made a great contribution in maintaining human health and in preventing many infectious diseases. The present study was carried out to determine the potential antibacterial effect of ethanol extracts and essential oil of *Marrubium vulgare L.* against *Staphylococcus aureus* which is antibiotic resistant.

***Materials and Methods*:** All 17 strains of *S. aureus* isolated from nose and throat sample from 160 healthy subjects, hospital staffs and inpatient in the city of Zabol (Amir Al-Momenin hospital, Zabol, south-eastern Iran) were screened during years 2010-2011. In this study, the essential oil of *Marrubium vulgare* L. obtained by hydrodistillation was analyzed by gas chromatography coupled to mass spectrometry (GC-MS) in order to determine their chemical composition. The minimum inhibitory concentrations were investigated to characterize the antimicrobial activities of this essential oil and extract.

***Results*:** Thirty-one components in the oil of *Marrubium vulgare* were identified. The results demonstrated that the major components of the essential oil were γ-Eudesmol (11%), Germacrene (10%), D-Citronelly formate (10%), β-Citronellol (8%), Geranyl tiglate (7.1%), Geranyl formate (6.02%). The least MIC value of extract *M. vulgare* was 2.5 mg/mL and the highest MIC value of essential oil *M. vulgare* was 2.5 mg/mL.

Conclusion: This investigation showed that the *M. vulgare* essential oil and extract has a potent antimicrobial activity against *S. aureus.* The present studies confirm the use of this essential oil and extract as antibacterial agent. Further research is required to evaluate the practical values of therapeutic applications.

Introduction

The Lamiaceae plants was considered as one of the large plant families used as a framework to evaluate the occurrence of typical secondary metabolites [1]. Among them, *Marrubium vulgare* L. is a perennial herb of the Labiatae family which is commonly known as "horehound" in Europe, or "Marrubia" in Tunisia, and it is naturalized in North and South America, the latter and Western Asia. Marrubium is an aromatic plant and native in Iran, which has been widely distributed in Azarbaijan, Golestan, Kerman, Kurdestan, Qazvin and Tehran provinces. In Iran herbal medicine, Marrubium has been known as a tonic, stomachic tonic, anti-pyretic in external use, and it has been used as an anti-septic and healing agent [2]. It is helpful for bronchial asthma and nonproductive cough. It was formerly much esteemed in various uterine, visceral and hepatic affections and in phthisis [3]. In the Mediterranean region, *Marrubium vulgare* is frequently used in traditional medicine to cure a variety of diseases. The plant is reported to possess hypoglycemic [4], vasorelaxant, antihypertensive [5], analgesic [6], anti-inflammatory [7], antioxidant activity, antierdematogenic activity, and many other biological activities. The study of Megre- Silva et al. showed that *Prteus vulgaris* and *Eschericha coli* are sensitive about *Marrubium vulgare* [8]. In the study of Kahloche- Riachi et al., the *Marrubium vulgare* L extracts showed higher antibacterial activity of *E. coli*, the area of inhibition were 14.33+3.51 mm [9]. Bacterial infectious diseases represent by important cause of morbidity and mortality worldwide. An antibiotic resistant bacterium is a threat which is increasing currently.

Staphylococcus aureus infections are widespread in the USA, and commonly manifest as minor skin and soft tissue infections such as boils, abscesses, furuncles, folliculitis, and cellulitis. Complications of infection include bloodstream infections, surgical wound infections, urinary tract infections, and pneumonia. Due to the importance of these strains, numerous studies have been conducted on their prevalence in hospitals and communities of, Germany [10], Latvia [11], Iran [12], and Switzerland [13]. An increasing proportion of *S. aureus* infections have got resistant to antibiotics such as methicillin, penicillin, and cephalexin; these infections, known as methicillin-resistant *S. aureus* (MRSA), are often referred as a 'superbug' by the media. The Centers

for Disease Control (CDC) estimate that about 25-30% of the US population is colonized with *S. aureus*, while about 1-5% harbor MRSA; however, carriage rates can vary by geographic location and the specific population being sampled [14]. The present study was carried out to determine the potential antibacterial effect of ethanol extracts and essential oil of *Marrubium vulgare* L. against antibiotic resistant *S. aureus*. In recent years, essential oils of plants and their other products from secondary metabolism have been in high demand by the manufacturers of foods flavoring, fragrance, cosmetics, and pharmaceutical industries due to the growing interest of consumers in ingredients from natural sources.

Materials and Methods

Plant materials: The leaf *M. vulgare* was collected in the region of Iran (Zahedan and Kerman, south-eastern, Iran) and planted in Kerman Azad university herbarium received approval and dried at room temperature. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Distillation of essential oil: The leaf *M. vulgare* was ground prior to the operation and then 300 g of ground rosemary was submitted to water distillation for 4 h using a Clevenger apparatus. The distilled essential oil was dried over anhydrous sodium sulfate, filtered and stored at 4ºC.

Preparation of extracts: Plant was properly dried and pulverized into a coarse powder as reported by Hanafy and Hatem [15]. Each of 20 g grinded powders was soaked in 60 mL ethanol 95 %, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman No. 1 filter paper) .Then the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 40ºC in air tight screw-cap tube.

GC/MS (Gas chromatography/ mass spectrometry) analysis conditions: The essential oil was analyzed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and HP-5MS capillary column (30 m×0.25 mm, film thickness 0.25 µm; Agilent-Technologies, Little Falls, CA, USA). The injector and detector temperatures were set at 250 and 280ºC, respectively. The column temperature was programmed from 35 to 250ºC at a rate of 5ºC/min, with the lower and upper temperatures being held for 3 and 10 min, respectively. The flow rate of the carrier gas (helium) was 1.0 mL/min. A sample of 1.0 µL was injected, using split mode (split ratio, 1:100). All quantifications were carried out using a built-in data-handling programme provided by the manufacturer of the gas chromatograph. The composition was reported as a relative percentage of the total peak area. The identification of the essential oil constituents was based on a comparison of their retention times to n-alkanes, compared to published data and spectra of authentic compounds. Compounds were further identified and authenticated using their mass spectra compared to the Wiley version 7.0 library.

***Staphylococcus aureus* isolation and culturing:** cross-sectional study was performed that seventeen nose and throat sample from 160 healthy subjects: (80 samples) hospital staffs (40 samples) and inpatient (40 samples) in the city of Zabol (Amir al-Momenin hospital, Zabol, south-eastern Iran) were screened during years 2010-2011. Samples were collected from infected quarter just on time from each suffering man. An aliquot (10 µL) from each sample was spread over blood agar (Merck, Germany) (pH=6.5) plate and incubated at 370ºC for 24 h. Isolated Gram and catalase positive cocci were further tests for biochemical characterization viz. carbohydrates fermentation followed by urease, vogues-proskauer, arginine ultilization, lysostaphin sensitivity, coagulase, clumping factor thermonuclease, haemolysin tests [16].

Agar disk diffusion assay: The susceptibility of all antibiotics was carried out using disc diffusion method on Muller-Hinton agar as recommended by CLSI [17]. The procedure followed is briefly described here. *S. aureus* isolated plates were grown overnight on blood agar and colony suspension was prepared using the sterile salin water equivalent to a 0.5 McFarland standard. Suspension (100 µL) was spread over the media plate and antibiotic disc was transferred aseptically on the surface of inoculated media plate. Isolated plates were tested with different antibiotics and their concentration shown in parenthesis viz. tetracyclin (30 µg), ampicillin (10 µg), trimethoprim-sulfamethoxazol (1.25+23.15 µg), erythromycin (15 µg), ceftazidime (30 µg), penicillin (10 µg), amikacin (10 µg), ceftriaxon (30 µg).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plant extracts: The broth microdilution method was used to determine MIC and MBC according to Yu et al. [18]. All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 0.3 mg/mL to 10.00 mg/mL. To each well, 10 µL of indicator solution (prepared by dissolving a 10 mg extract in 2 mL of DMSO) and 10 µL of Mueller Hinton Broth were added. Finally, 10 µL of bacterial suspension (106 CFU/mL) was added to each well to achieve the concentration of 104 CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37ºC for 18-24 h. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC and MBC values for the tested extracts. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed.

Table 1. Compared to standard antibiotic discs inhibitory effect on pathogenic strains isolated (%)

	Trimethoprim	Ampicillin	Ceftazidime	Tetracycline	Erythromycin	Penicillin	Ceftriaxone	Amikacin
R	70.6	70	29.4	23.5	82.4	88.2	23.5	5.9
S	23.5	17.6	52.9	29.4	5.9	5.9	5.9	94.1
I	5.9	11.8	17.6	47.1	11.8	5.9	70.6	0

S= Sensitive, I= Intermediate, R= Resistant

Statistical treatment of the results: The mean values were analyzed with the MINITAB Release 13.20 program statistically by the general one-way analysis of variance (ANOVA) to find out the most effective plants and the most sensitive test organisms.

Results

Staphylococcus aureus constituted 17 (10.62%) of the 160 isolated plates. The majority of isolated plates were throat. Antibiotic resistant *S. aureus* isolates were obtained from both sources of nose and throat, but with a significant difference in the prevalence of resistant isolates between the hospital and control group. Table 1 shows the antibiotic resistance pattern of the isolates.

Table2. Chemical compositions and retention indices (RI) of the M. vulgare essential oil

Chemical composition	RI	%
Camphene	790	3.15
N,N- bistrimethylsilyl	862	0.77
α -Pinene	935	2.16
1-Vinylcyclohexane	960	0.50
Geraniol	1022	3.70
α -Thujone	1131	2.45
1,8-Cineole	1151	3.75
Camphor	1188	1.03
Iso menthon	1192	0.67
Borneol	1212	0.62
β -Citronellol	1256	8
Trans- caryophyllene	1290	2.34
Citronellyl formate	1343	10
Geranyl formate	1367	6.02
α -Copaene	1422	1.35
β -Bourbonene	1456	1.80
α-Humulene	1462	0.52
α -Muurolene	1458	0.63
α-Amorphene	1492	0.88
Neoalloocimene	1495	0.98
Germacrene- D	1510	10
Neryl acetate	1515	3.41
γ-Eudesmol	1530	11
Ledene	1539	5.15
β-bisabolene	1544	0.77
δ-Cadinene	1565	3.35
α-Agarofuran	1589	0.42
Furan-2-one, 4-phenyltetrahydro	1623	1.44
Geranyl tiglate	1639	7.1
β -Cubebene	1662	3.30
Cyclononasiloxane, octadecamethyl	1689	4.3

Chemical composition: The percentages and the retention indices of the identified components are listed in table 2 in the order of their elution on the HP-5MS column. GC-MS analysis of *Marrubium vulgare* essential oil led to the identification of thirty four (34) compounds, accounting for 100% of the total oil.

Yield of essential oil obtained by hydrodistillation from aerial part of plant was 0.34%. Indeed, the oil contains γ-Eudesmol (11%), Germacrene (10%), D- Citronelly formate (10%), β -Citronellol (8%), Geranyl tiglate (7.1%), Geranyl formate (6.02%) which all of them were presented in fairly good amounts. On the other hand, Lendene (5.15%), Cyclononasiloxane- Octadecamethyl (4.3), 1, 8-Cineole (3.75%), Geraniol (3.70%), Neryl acetate (3.41), γ- Cadinene (3.35) and B- Cubebene (3.30).

Antibacterial activity: Different inhibitory effects of alcoholic extract and essential oil from *M. vulgare* against most *S. aureus* isolates were demonstrated in table 3, 4.The results in tables 3 and 4 showed that ethanol extract and essential oil of *M. vulgare* had inhibitory effect against most isolated plates. The least MIC value of alcoholic extract of *M. vulgare* was 2.5 mg/mL and the highest MBC value of alcoholic extract of *M. vulgare* were 5 mg/mL and 10 mg/mL (Table 3). The least MIC and MBC value of essential oil were 0.3 mg/mL and 0.62 and 1.25 mg/mL respectively and the highest MIC and MBC value of essential oil of *M. vulgare* were 2.5 mg/mL and 5 mg/mL respectively (Table 4). No significant difference was noted between the extract and essential oil of *M. vulgare* (p<0.05).

Table 3. Minimum inhibitory concentration of M. vulgare extract against S. aureus

M. vulgare concentration (mg/mL)	0.3	0.62	1.25	2.5	5	10
MIC	0	0	0	11.76	70.58	0
MBC	0	0	0	0	41.17	41.17

Table 4. Minimum inhibitory concentration of M. vulgare essential oil against S. aureus

M. vulgare concentration(mg/mL)	0.3	0.62	1.25	2.5	5	10
MIC	5.88	5.88	5.88	47.05	17.64	17.64
MBC	0	5.88	5.88	11.76	47.05	26.41

Discussion

In the study show that thirty-one components in the oil of *Marrubium vulgare* were identified. The results demonstrated that the major components of the essential oil were γ-Eudesmol (11%), Germacrene (10%), D- Citronelly formate (10%), β -Citronellol (8%), Geranyl tiglate (7.1%), Geranyl formate (6.02%). The least MIC value of extract *M. vulgare* was 2.5 mg/mL and the highest MIC value of essential oil *M. vulgare* was 2.5 mg/mL.

Staphylococcus aureus is a prevalent pathogen worldwide, which is usually multi- resistant in hospital. In this study, the prevalence of *S. aureus* was 10.62%. In the study of Ekrami and Kalantar in a burn hospital in Iran,

the result shows that prevalence of *S. aureus* was 20.2% [19]. In our study 58 % of *S. aureus* MRSA were methicillin resistant. This pathogen has been reported as a major cause of nasocomial infection in Europe [20, 21], plant essential oils and extracts are potentially useful sources of antimicrobial compounds. Essential oils are natural products extracted from vegetal materials which, because of their antibacterial, antifungal, antioxidant and anti-carcinogenic properties, can be used as natural additives in many foods [22]. Results show the oil contains γ-Eudesmol (11%), Germacrene (10%), D-Citronelly formate (10%), β - Citronellol (8%), Geranyl tiglate (7.1%), Geranyl formate (6.02%) which were presented in fairly good amounts and Lendene (5.15%), Cyclononasiloxane- Octadecamethyl (4.3), 1, 8-Cineole (3.75%), Geraniol (3.70%), Neryl acetate (3.41), γ-Cadinene (3.35) and β-Cubebene (3.30) which were presented in this study. Morteza and Saeedi, reported that the major constituents of the essential oil of *M. vulgare* from Iran were β-bisabolene (20.4%), 8-cadinene (19.1%) and isocaryophyllene (14.1%) [23]; Khanavi et al. showed that the major component of *Marrubium vulgare* from other region of Iran were bisabolene (25.4%), β-caryophyllene (11.6%), germacrene (9.7%) and E-B-farnesene (8.3%) [24], and Asadipour et al. found that caryophyllene oxide (18.7%), β-caryophyllene (12.8%) and germacrene D (10.0%) were the major compounds of *Marrubium vulgare* collected from another region of Iran [25]. The antimicrobial activities of *Marrubium vulgare* essential oil and extract against *S. aureus* examined in the present study and their potency was qualitatively and quantitatively assessed by the presence or absence of inhibition zones and the minimal inhibitory concentration (MIC) values. This essential oil and extract displayed varied antibacterial activities across the studied pathogens. As can be seen from table 3, the least MIC value of alcoholic extract of *Marrubium vulgare* was 2.5 mg/mL and the highest MIC and MBC value of essential oil of *Marrubium vulgare* were 2.5 mg/mL and 5mg/ml respectively (Table 3, 4). The in vitro antibacterial activity of essential oil of *Marrubium vulgare's* leave showed a significant activity against microorganisms, especially Gram positive bacteria with inhibition zones and MIC values in the range of 6.6-25.2 mm and 1120-2600 µg/mL, respectively, whereas Gram negative bacteria exhibited a higher resistance [26]. In conclusion, the screening of extracts and essential oil made by tested *M. vulgare* has demonstrated that most of the screened plats are potential rich sources of antibacterial agents.

Acknowledgements

This study was supported by Institute of Plant Biotechnology University of Zabol, Zabol, Iran.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Science and ResearcherBranch, Islamic Azad University, Kerman.

References

1. Wink M. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry. 2003; 64(1): 3-19.
2. Semnani KM, Saeedi M, Babanezhad E. The essential oil composition of Marrubium vulgare L from Iran. J Essent oil Res. 2006; 20: 488-490.
3. Chopra RN, Nayer SL, Chopra IC. Glossary of Indian medicinal plants. 5th ed. New Delhi: Council of Scientific and Industrial Research; 1956: 157.
4. Roman Ramos R, Alarcon-Aguilar F, Lara-Lemus A and Flores-Saenz JL. Hypoglycemic effect of plants used in Mexico as antidiabetics. Arch Med Res. 1992; 23(1): 59-64.
5. El-Bardai S, Lyoussi B, Wibo M and Morel N. Comparative study of the antihypertensive activity of Marrubium vulgare and of the dihydropyridine calcium antagonist amlodipine in spontaneously hypertensive rat. Clin Exp Hyprtens. 2004; 26(6): 465-474.
6. de Souza MM, de Jesus RAP, Cechinel-Filho V and Schlemper V. Analgesic profile of hydroalcoholic extract obtained from Marrubium vulgare. Phytomedicine. 1998; 5(2): 103-107.
7. Schlemper V, Ribas A, Nicolau M and Cechinel-Filho V. Antispasmodic effects of hydroalcoolic extract of Marrubium vulgare on isolated tissues. Phytomedicine. 1996; 3(2): 211-216.
8. Meyre-Silva C, Cechinel-Filho V. A Review of the Chemical and Pharmacological Aspects of the Genus Marrubium. Curr Pharm Des. 2010; 16(31): 3503-3518.
9. Kahlouche-Riachi F, Mansour-Djalab H, Serakta-Delmi M, et al. Evalution of antibacterial activity of some wild plant of Algeria. Int J Med Arom Plant. 2012; 2(2): 275-280.
10. Nolte O, Haag H, Zimmerman A and Geiss HK. Staphylococcus aureus positive for Panton-Valentine Leukocidin genes but susceptible to methicillin in patients with furuncles. Eur J Clin Microbiol Infect Dis. 2005; 24(7): 477-479.
11. Miklasevics E, Haeggman S, Balode A, et al. Report on the first PVL-Positive community acquired MRSA strain in Latvia. Euro Surveill. 2004; 9(11): 29-30.
12. Havaei SA, Ohadian S, Pourmand MR and Faghri J. Prevalence of Genes Encoding bi-component leukocidins among clinical isolates of methicillinresistant Staphylococcus aureus. Iran J Public Health. 2010; 39(1): 8-14.
13. Boubaker K, Diebold P, Blanc DS. Panton- Valentine Leukocidin and staphylococcal skin infections in school children. Emerg Infect Dis. 2004; 10(1): 121-124.
14. Rim JY, Bacon AE 3rd. Prevalence of community-acquired methicillin-resistant Staphylococcus aureus colonization in a random sample of healthy individuals. Infect Control Hosp Epidemiol. 2007; 28(9): 1044-1046.
15. Hanafy MS, Hatem ME. Studies on the antimicrobial activity of Nigella sativa seed (black cumin). J Ethanopharmacol. 1991; 34(2-3): 275-278.
16. Turkyilmaz S, Kaya O. Determination of some virulence factors in Staphylococcus Spp isolated from various clinical samples. Turk J Vet Anim Sci. 2006; 30: 127-132.

17. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing (2002), 16th International supplement. CLSI document M100-S12 Davis, H.R (1997).
18. Yu JQ, Lei JC, Yu H, et al. Chemical composition and antimicrobial activity of the essential oil of Scutellaria barbata. Phytochemistry. 2004; 65(7): 881-884.
19. Ekrami A, Kalantar E. Bacterial infections in burn patients at a burn hospital in Iran. Indian J Med Res. 2007; 126(6): 541-544.
20. Cai XD, Cao Y, Chen C, et al. [Investigation of nosocomial infection in the neonatal intensive care unit] Chinese [Abstract]. Zhongguo Dang Dai Er Ke Za Zhi. 2010; 12(2): 81-4.
21. Wildemauee C, Godard C, Vershragen G, et al. Ten years phage typing of Belgian clinical methicillin-resistant S. aureus isolates. J Hosp Infect. 2004; 56(1): 16-21.
22. Teissedre PL, Waterhouse AL. Inhibition of oxidation of human lowdensity lipoproteins by phenolic substances in different essential oils varieties. J Agric Food Chem. 2000; 48(9): 3801-3805.
23. Morteza-Semnani K, Saeedi M. The essential oil composition of Marrubium astracanicum Jacq. From Iran. J Essent Oil Bearing Plants. 2004; 7(3): 239-242.
24. Khanavi M, Ghasemian L, Hosseiny Motlagh E, et al. Chemical composition of the essential oils of Marrubium parviflorum Fisch. & C.A. Mey and Marrubium vulgare L. from Iran. Flav Fragr J. 2005; 20(3): 324-326.
25. Asadipour A, Mehrabani M, Nazeri V and Tabarraii M. [Composition of the essential oil of Marrubium vulgare L.] Persian. J Pharm Sci Tabriz Univ Med Sci. 2005; 2: 77-82.
26. Zarai Z, Kadri A, Ben-Chobba I, et al. The in-vitro evaluation of antibacterial, antifungal and cytotoxic properties of Marrubium vulgare L. essential oil grown in Tunisia. Lipids Health Dis. 2011; 10: 161.

***Please cite this article as*:** Bokaeian M, Saboori E, Saeidi S, Niazi A, Amini N, Khaje H, Bazi S. Phytochemical analysis, antibacterial activity of Marrubium vulgare L against Staphylococcus aureus in vitro. Zahedan J Res Med Sci. 2014; 16(10): 60-64.