International Journal of Research in Pharmacy and Science

Research Article

Evaluation of Antibacterial properties of essential oil of *Pistacia khinjuk*

Tahvilian R[1], Moradi R[1], Hajialiani M[1], Zangeneh MM[1,2], Zangeneh A[1,2], Yazdani H[1,3], Zhaleh H[1]

[1]Research pharmaceutical center, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2]Microbiology Section, Pathobiology & Basic Sciences Department, Veterinary faculty, Razi University, Kermanshah, Iran.
[3]Department of Chemistry, Shahid Beheshti University, Tehran, Iran.

Address for Correspondence:
Mohammad Mahdi Zangeneh
E mail: m.mehdizangeneh@yahoo.com

Access this article online	
QR Code	Website: www.ijrpsonline.com

ABSTRACT

Pistacia khinjuk (PK) is a native plant in Iran, which the plant has been used as an antiflammatory, antioxidant, antifungal, and antiviral agent in Iran. The goal of the study was to evaluate the in vitro antibacterial properties of the essential oil of PK (Combined with the Dominance γ-terpinene) against *Pseudomonasaeruginosa* (PA). As a screen test to find antibacterial activities of the PK, agar well and agar disk diffusion methods were employed. Macrobroth tube test was performed to determinate MIC. According to results of GC-MS analysis, γ-terpinene (81.14%) (w/w) was the abundant component of the essential oil. The inhabitation zone in many of samples have been increased when the essential oil amount has increased. The MIC and MBC values were 0.015g/ml for PK in the bacterium. Thus, the present research indicates the antibacterial effects of the PK on PA, offering to use as antibacterial supplement in the developing countries towards the development of novel therapeutic agent.

Key words: *Pistacia khinjuk*, Essential oil, Antibacterial properties, Macro-dilution method, Agar disk diffusion method, Agar well diffusion

INTRODUCTION

A plant essential oil is a substance or an active with eligible properties that is removed from the tissue of a plant, to be used for a special aim[1, 2]. In fact, essential oils are made from volatile compounds that are produced by the secondary metabolism of aromatic and ethnomedicinal herbs and can be obtained by different methods[3, 4]. Herbal essential oils have antibacterial activities on a wide number of Gram positive and Gram negative bacteria[5, 6]. The compounds of plant essential oils contain multiplex health-related activities such as anti-inflammatory, antioxidant, anti-parasitic, antiviral, antifungal, and antibacterial activities[7, 8]. In Iranian ethnomedicine, plant essential oils are consumed by the population for the treatment of diseases including bacterial diseases[9-11].

PK commonly known as *Khenjuk* or *Kelkhong* in Persian, is a wild bush that is widely distributed in the Mediterranean and Middle East areas, such as Iran[12, 13]. PK is one of the eatable plants which have produced a lot of interest throughout human history as a medicinal panacea. PK have been used in folk medicine as anti-inflammatory, antipyretic, antibacterial, anti-fugal, antiviral, in treatment diarrhea and throat infection[14-16]. Essential oils of some *Pistacia*species consist of components such as γ-Terpinene, cymene, β-caryophyllene, sabinene, α-phellandrene, cineol. The terpinenes are a group of isomeric hydrocarbons that are classified as terpenes. They have the same molecular formula and carbon body, but they differ in the position of carbon-carbon double bonds. γ- Terpinene is a monoterpene and a main component of essential oils made from plants fruit and shows strong antioxidant effects in different assay systems[17, 18].

Based on knowledge of authors, there is a very little data about antibacterial effects of the essential oil collected from Kermanshah province, west of Iran, and there is no study on antibacterial properties of essential oil of PK (Combined with the Dominance γ-Terpinene) in all over the world. Therefore, the purpose of the recent study was consideration of antibacterial activities of the PK on PA with broth macro-dilution and agar well and disk diffusion methods.

EXPERIMENTAL SECTION

Source of microorganisms

Bacterium specie namely PA (PTCC No. 1707) was procured from Iranian Research Organization for Science and Technology as lyophilized. Bacterial strain was activated on Tryptic Soy broth, constant at 37°C for 18 h. Then 60 µl of the broth was transferred to Nutrient agar and incubated at 37°C for another

24 h; cell concentration was then adjusted to obtain final concentration of 10^8 CFU/ml using Muller Hinton broth.

Culture media

Mueller-Hinton Agar (Müller-Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing) was prepared according to the manufacturer's instruction (Oxoid, UK), autoclaved and distributed at 20 ml per plate in 12 x 12 cm Petri dishes. Set plates were incubated overnight to ensure sterility before use. Then, Mueller-Hinton broth containing different concentrations of the essential oil and of the final bacterium inoculums (1×10^8 CFU/ml) were added in to each well.

Plant sample collection

In the empirical-experimental study, medicine plant collected from Kermanshah. The sample was cleaned from any strange, plants, dust, or any other contaminants.

Essential oil extraction

Essential oil from fresh, clean, weighed aerial part PK extracted by hydro-steam distillation using the Clevenger apparatus were collected and stored in sterile vials. Briefly, 100 to 150 g of plant was introduced in the distillation flask (1L), which was conjuncted to a steam generator via a glass tube and to a condenser to retrieve the oil. This was recovered in a funnel tube. Aromatic molecules of the essential oil was liberated from the plant material and vaporized into hot steam in 98°C. The hot steam forced the plant material to release the essential oil without burning the plant material itself. Then, steam containing the essential oil was passed through a cooling system in order to compress the steam. The steam was applied for 3h. After settling the recovered mixture, essential oil was withdrawn. The supernatant essential oil was purged through anhydrous Na_2SO_4 to dry the yielded essential oil. Then, the essential oil was collected in tightened vials and stored in a refrigerator. For the antimicrobial activity test, several dilutions of the essential oil were done using dimethyl sulfoxide (DMSO).

Evaluation of antimicrobial activities

Agar well diffusion and agar disk diffusion were used as screen tests to evaluate antibacterial property of essential oil of PK based on standard protocol. The solution of the compound was yielded in 1g/ml from which six fold serial dilutions (v/v) were prepared. 60 µl of each dilution was poured on each well and disk in order. After a period of 24 hours incubation, the diameters of growth inhibition zones around the disks and wells were measured. DMSO was used as negative control whereas Gentamicin was used as positive control in case of PA. Minimum inhibitory concentration (MIC) means the lowest concentration of the probable antimicrobial agent which prevents growing of bacteria (regardless of killing the bacteria or stopping the growth of them). The lowest dilution which no gross microbial growth has been seen indicates MIC. Minimum bactericidal concentration (MBC) means the lowest concentration of the agent which causes death to test bacteria. The last can be revealed by pouring 60 µl of MIC tube and three dilutions before contents on agar plate. In this case, give incubation period, the lowest concentration which makes no growth indicates MBC. For determination of MIC value, macrobroth dilution method was applied. Interpretation of the results was done due to national accepted letter[19].

Statistical Analysis

Antibacterial effect was determined by One way variance analysis (ANOVA), using the SPSS 18 software package. Data were considered statistically significant at $p\leq0.01$.

RESULTS

Chemical composition

22 compounds such as γ-Terpinene (81.14 %), β-Pinene (3.93 %), α-TERPINOLENE (2.38 %), Camphene (1.6 %), dl-Limonene (1.45 %), 3-Cyclohexene-1-carboxaldehyde (1.25 %), β-Myrcene (1.1 %), sabinene (1.09 %), l-Bornyl acetate (0.81 %), DELTA.3-Carene (0.72 %), Terpan (0.43 %), α-Campholenal (0.4 %), m-Cymene (0.39 %), Tricyclene (0.35 %), α-TERPINEOL (0.34 %), trans-Pinocarveol (0.33 %), α-Pinene epoxide (0.3 %), p-Cymen-8-ol (0.2 %), Linalol (0.2 %), α-Terpinene (0.18 %), Myrtenol (0.16 %), 1-Phellandrene (0.11 %) representing 99.92% of the total alcoholic extract composition of PK were identified using mass gas-chromatograph. The most substance found in essential oil of PK was γ-Terpinene. In contrast, *1-Phellandrene* was the least constituents discovered in the essential oil[14].

Agar well diffusion test

In regard to, the widest zones were seen in 0.062 and 0.031 g/ml concentrations (10 mm). It was no growth inhibition in negative control. The data are discoverable in figure 1.

Agar disk diffusion test

The widest zone was formed due to positive controls (34 mm) and after it, the widest zone was formed due to 0.062 g/ml concentration (14 mm) and it was no halo in negative control. The data are discoverable in figure 2.

MIC determination

In the essential oil, MIC was 0.015 g/ml for PA.

MBC ascertaining

MBC was 0.015 g/ml for the bacterium.

DISCUSION

The use of plant to treat infections is an old practice in a large part of the world[20-25]. Interest in plants with antimicrobial properties has revived as a result of new obstacles associated with the use of antibiotics [26-30]. Essential oil of plants have strong antibacterial activities and these effects have been evaluated and reviewed[1, 3].PK is an endemic and resistance

species in dry and sub-dry forests in mountainous regions of Western Iran. Some species of *Pistacia* have been used in folk medicine in eczema treatment ,throat infections, renal stones, asthma and stomach ache, and as a astringent, anti-inflammatory, antipyretic, antibacterial, antiviral, pectoral and stimulant[14, 16].

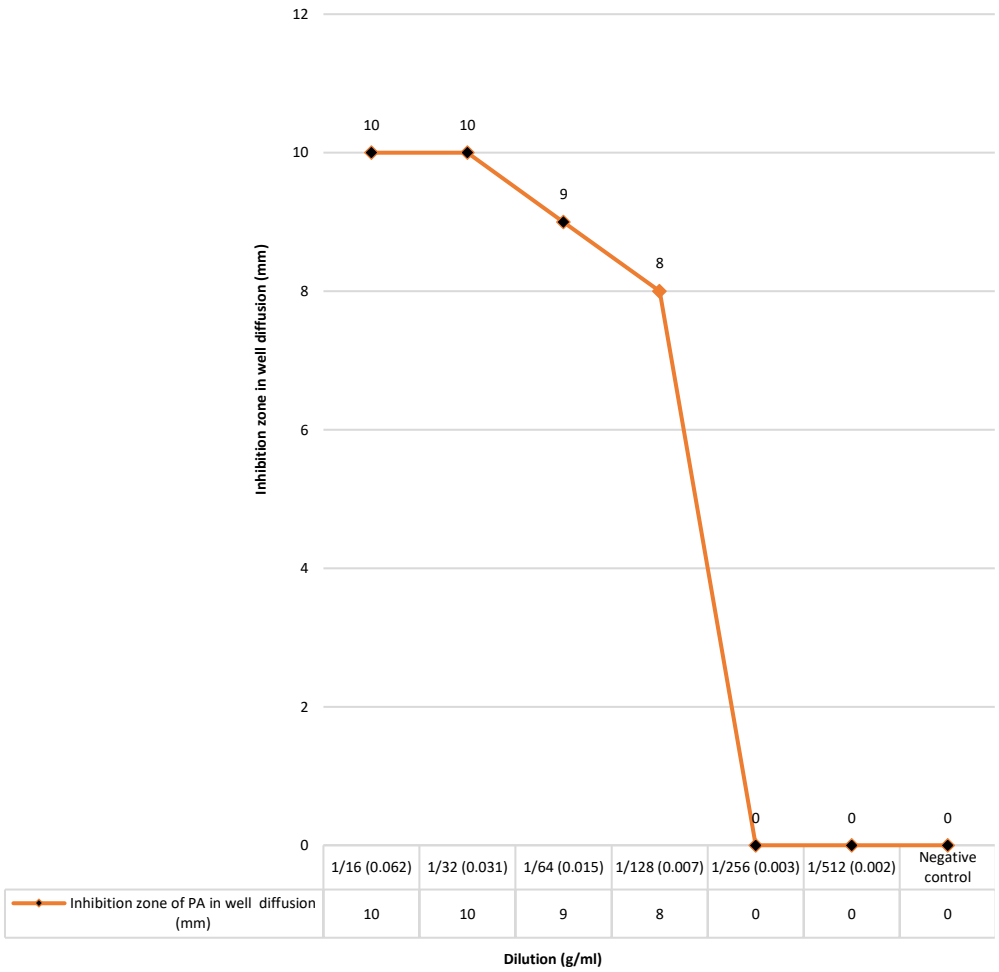

Figure 1. The diameters of growth inhibition zones of PA in agar well diffusion test in different dilutions of PK.

In the essential oil of PK, 22 compounds were identified. The major compounds were found to be γ-terpinene (81.14%) (w/w), β-Pinene (3.93%) (w/w), α-Terpinolene (2.38%) (w/w), Camphene (1.6%) (w/w), dl-Limonene (1.45%) (w/w), 3-Cyclohexene-1-carboxaldehyde (1.25%) (w/w), β-Myrcene (1.1%) (w/w), and sabinene (1.09%) (w/w)[14]. Concerning the method of essential oil, extraction and inhibiting from using high temperature to reduce the rate of demolition of efficacious herbal compound. Its bioactive components may be γ-terpinene and other components that we do not know. Our results agree with the past antibacterial studies related to these species[31, 32]. In the essential oil, the main constituent was found to be γ-terpinene. γ-terpinene was assessed for its ability to persuade cellular protein leakage in *Proteus vulgaris* and *Escherichia coli* (Gram negatives) as well as *Listeriamonocytogenes* and *Streptococcuspyogenes* (Gram positives). Both the Gram negative and Gram positive test bacteria showed a similar trend of protein permeation when treated with γ-terpinene. Protein permeation could be used as an index of the membrane damage caused by this compound. γ-terpinene was assessed for its capability to induce cellular lipid permeation in *P. vulgaris* and *E. coli* as well as *L. monocytogenes* and *S.pyogenes*[33]. The effect of γ-terpinene might be the result of its phenolic structure which intervenes with the lipid bilayer of the outer membranes[34].

As the figures showed, the inhabitation zone in many of samples has been increased when the essential oil amount has increased. The results defined that in tested bacterium, there wasn't a substantial difference in terms of sensitivity to PK. In agar disk diffusion test, the widest inhibition zone was seen in 0.062 g/ml concentration (The value of growth inhibition zone was 14 mm in this dilution, and the value of growth inhibition zone of Gentamicin against PA was 34 mm) and no inhibitory activities of essential oil of the plant in 0.002 and 0.003 g/ml

concentrations. In agar well diffusion test, the widest zone was seen in 0.062and 0.031 g/ml concentrations (10 mm) and no inhibitory effect of PK in 0.002 and 0.003 g/ml concentrations. PK with 0.015 g/ml concentration has inhibited PA and has killed it. Thus, the research represents the antibacterial properties of the ethnomedical plant on PA.

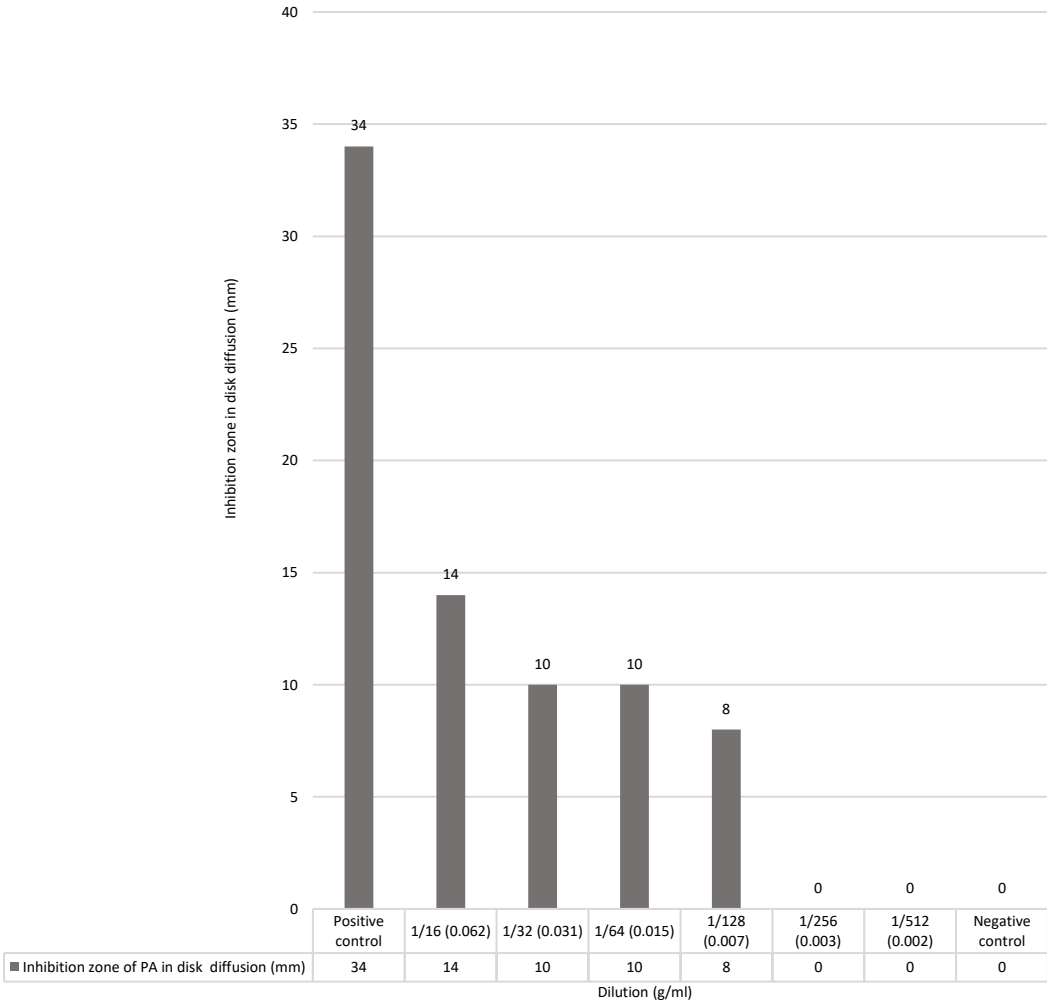

Figure 2. The diameters of growth inhibition zones of PA in agar disk diffusion test in different dilutions of PK.

CONCLUSION

From the research it can be deduced that the essential oil of PK (Combined with the Dominance γ-terpinene) possess antibacterial properties, and the antibacterial effects of PK was due to the presence of different active compounds. Therefore, the phytochemical compounds responsible for the antibacterial activities of bacterium can be subjected to isolation of the therapeutic antibacterial. Our results indicate the use of the plant in traditional ethnomedicine and suggest that PK possess compounds with good antibacterial effects. Fractionation and characterization of active molecules will be the future work to survey. Additional clinical trials would be needed to affirm and better assessment the potential of the plant as an antibacterial agent in topical or oral utilizations.

ACKNOWLEDGMENT (FUNDING / SUPPORT)

We, the authors wish to thank Medical Sciences University of Kermanshah, Iran for the financial support of this work.

REFERENCES

1. Najafi F, Tahvilian R, Zangeneh MM, et al. Screening of essential oil of Allium sativum for antibacterial effects against Bacillus subtilis. *International Journal of Recent Scientific Research.* 2016; 7(11): 14172-14176.
2. Foroughi A, Pournaghi P, Najafi F, et al. Evaluation of antibacterial activity and phytochemical screening of Pimpinellaanisem's essential oil. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(11): 1886-1890.
3. Zangeneh MM, Najafi F, Tahvilian R, et al. Ethnomedicinal Plant: Antibacterial effects of essential oil of Allium

sativum against Pseudomonas aeruginosa (PTCC No. 1707) in west of Iran. *International Journal of Recent Scientific Research*.2016; 7(11): 14243-14247.

4. Foroughi A, Pournaghi P, Tahvilian R, et al. Evaluation of the composition and antibacterial effects of the *Viola odoratalin* oils. *International Journal of Current Medical and Pharmaceutical Research*. 2016; 2(12): 1093-1097.
5. Najafi F, Zangeneh MM, Tahvilian R, et al. In vitro antibacterial efficacy of essential oil of *Allium sativum* against *Staphylococcus aureus*. International Journal of Pharmacognosy and Phytochemical Research 2016; 8(12): 2039-2043.
6. Foroughi A, Pournaghi P, Tahvilian R, et al. Ethnomedicinal plants: Study on the chemical composition and antibacterial activity of the *Nigella sativa* (Black seed) oil's. *International Journal of Pharmaceutical and Clinical Research*. 2016; 8(11): 1528-1532.
7. Najafi F, Tahvilian R, Zangeneh MM, et al. Medicinal plant: Assessment of the chemical composition and in vitro antibacterial activities of the *Viola odorata Linnoil's* against *Bacillussubtilis* (ATCC No. 21332) in west of Iran. *International Journal of Scientific & Engineering Research*. 2016; 7 (11): 1330-1339.
8. Foroughi A, Pournaghi P, Zhaleh M, et al. Antibacterial activity and phytochemical screening of essential oil of *Foeniculumvulgare*. *International Journal of Pharmaceutical and Clinical Research*. 2016; 8(11): 1505-1509.
9. Foroughi A, Pournaghi P, Najafi F, et al. Chemical composition and antibacterial properties of Chenopodiumbotrys L. essential oil. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(11); 1881-1885.
10. Zangeneh MM, Najafi F, Tahvilian R, et al. Effect of *Allium sativum*oil on *Escherichia coli O157:H7*. *Online Journal of Veterinary Research*. 2017; 21(1): 19-24.
11. Foroughi A, Pournaghi P, Najafi F, et al. Antibacterial effect and phytochemical screening of essential oil *of Pimpinellaanisum* against *Escherichia coli O157:H7* and *Staphylococcus aureus. International Journal of Current Pharmaceutical Review and Research*; 2016; 7(6): 367-371.
12. Bailey LH. *Manual of Cultivated Plants*, 4th ed, *Macmillan, New York*. 1958; 2648.
13. Ghasemi Pirbalouti A. Medicinal plants used in Chaharmahal and Bakhtyari districts, *Iran. Herba Polon*, 2009; 55: 69-75.
14. Tahvilian R, Moradi R, Zhale H, et al. Ethnomedicinal Plants: Study on Antifungal Activity of Essential oil of *Pistaciakhinjuk* (Combined with the Dominance γ-Terpinene) Against *Candida albicans*. *International Journal of Pharmaceutical and Clinical Research*.2016; 8(10): 1369-1373.
15. Tahvilian R, Moradi R, Zhale H, et al. Ethnomedicinal Plants: In vitro antibacterial effect of essential oil of Pistaciakhinjuk. *International Journal of Scientific & Engineering Research*. 2016; 7(10): 437-447.
16. Tahvilian R, Moradi R, Hajialiani M, et al. Chemical composition and screening of antibacterial activities of essential oil of *Pistaciakhinjuk* against *Bacillus subtilis* (ATCC No. 21332). *International Journal of Current Medical and Pharmaceutical Research*. 2016; 2(12): 1098-1102.
17. Delazar A, Reid RG, Sarker SD. GC-MS analysis of the essential oil from the oleoresin of Pistacia atlantica var. Mutica. *Chemistry of Natural Compounds*, 2004; 40: 24-27.
18. Monaco P, Previtera L, Mangoni L. Terpenes in Pistacia plants: A possible defence role for monoterpenes against gall-forming aphids. *Phytochemistry*, 1982; 21: 2408-2410.
19. Clinical and laboratory standards institute (CLSI), M7-A7, 2006; 26 (2).
20. Foroughi A, Pournaghi P, Tahvilian R, et al. Assessment of chemical composition and antibacterial effects of Anethole-rich hydroalcoholic extract of Pimpinellaanisum. *International Journal of Pharmaceutical and Clinical Research.* 2016; 8(11): 1459-1463.
21. Foroughi A, Zangeneh MM, Zangeneh A, et al. A survey on antibacterial activities of Allium eriophyllum alcoholic extract: An ethnomedicinal plant. *Iranian J Publ Health*, 2016; 45 (2): 32.
22. Zangeneh MM, Tahvilian R, Najafi F, et al. Effect of hydroalcoholic extract of *Scrophulariastriata* on *Escherichia coli O157:H7* (ATCC No. 25922). Online Journal of Veterinary Research. 2016; 20(12): 761-767.
23. Zangeneh MM, Najafi F, Moradi R, et al. Evaluation of the in vitro antibacterial activities of alcoholic extract of Stevia rebaudiana against Escheriachia coli O157: H7 (ATCC No. 25922). Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry. 2016; 4(3): 131- 136.
24. Zangeneh MM, Najafi F, Tahvilian R, et al. Ethnomedicinal Plants: *In vitro* Antibacterial Effects of Ethanolic Extract of *Stevia rebaudiana. Int J Ayu Pharm Chem*. 2017; 6(1): 251-259.
25. Zangeneh MM, Poyanmehr M, Najafi F, et al. In vitro antibacterial activities of ethanolic extract of *Stevia rebaudiana* against *Bacillus subtilis* (ATCC No. 21332). *International Journal of Research in Pharmaceutical and Nano Sciences.* 2016; 5(6): 320-325.
26. Zangeneh MM, Najafi F, Tahvilian R, et al. Assessment of in vitro antibacterial properties of the hydroalcoholic extract of *Scrophulariastriata* Against *Staphylococcus aureus* (ATCC No. 25923). *International Journal of Pharmacognosy and Phytochemical Research* 2017; 9(1): 40-44.
27. Zangeneh MM, Najafi F, Tahvilian R, et al. Effect of *Scrophulariastriata* hydro-alcoholic extract on growth of *Bacillus subtilis* ATCC No. 21332. *Online Journal of Veterinary Research*. 2017; 21(2): 51-57.
28. Foroughi A, Zangeneh MM, Kazemi N, et al. An in vitro study on antimicrobial properties of Allium noeanumreut ex regel: An ethnomedicinal plant. *Iranian J Publ Health.* 2016; 45 (2): 32.
29. Zangeneh MM, Tahvilian R, Najafi F, et al. Evaluation of the in vitro antibacterial effect of the hydroalcoholic extract

of Scrophulariastriata. *International Journal of Scientific & Engineering Research*.2016; 7(10): 1693-1702.

30. Zangeneh MM, Najafi F, Tahvilian R, et al. Study on the in vitro antibacterial properties of alcoholic extract of *Stevia rebaudiana*in west of Iran. *International Journal of Scientific & Engineering Research*. 2016; 7 (11): 1352-1359.
31. Moraghebi F, Tymori M, Khoshnevis M, et al. Antimicrobial activity the leaves of *Pistaciaatlantica* on gram-positive bacteria, 1999; 5: 84-91.
32. Alma MH, Nitz S, Kollmannsberger H, et al. Chemical Composition and Antibacterial Activity of the Essential Oils from Gum of Turkish Pistachio (*Pistaciavera L*.). *J. Agric. Food Chem*., 2004; 52: 3911-3914.
33. Oyedemi SO, Okoh AI, Mabinya LV, et al. The proposed mechanism of bactericidal action of eugenol, α-terpineol and γ-terpinene against Listeria monocytogenes, Streptococcus pyogenes, Proteus vulgaris and Escherichia coli. *African Journal of Biotechnology*. 2009; 8 (7); 1280-1286, 2009.
34. Janssen AM, Scheffer JJC, Svendsen A. Antimicrobial activity of essential oils. *Planta-Med*. 1985; 5: 365-395.