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# Ethnomedicinal Plants: Evaluation of in vitro Antibacterial properties of *Verbascum thapsus* against *Bacillus subtilis* ATCC No. 21332

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## Abstract

Ethnomedicinal plants are considered common resources for yielding supplements that could use as alternatives to antibiotics in cure of antibiotic-resistant micro-organism. *Verbascum thapsus* (VT) is a native plant in west of Iran, which the has been applied as an antioxidant, antiflammatory, antiviral, antifungal, and antipyretic in Iran. The aim of the recent study was assessment of antibacterial properties of VT aqueous extract on common pathogen (*Bacillus subtilis* ATCC No. 21332 (BS)). The antibacterial effects of VT was evaluated by macro-dilution method in Mueller-Hinton broth medium and agar well and disk diffusion methods. The results indicated that the aqueous extract of VT have strong levels of antibacterial activities against BS. By augmenting the concentration of the extract, the inhibition zone in many of the samples increased. Partially, in agar disk diffusion the widest inhibition zone of 18 mm was observed at 0.083 g/ml VT with no inhibition with distilled water. In agar well diffusion, the widest inhibition zone of 13 mm occurred at 0.083 g/ml. Minimum inhibitory and bactericidal concentrations of VT were 0.01 g/ml. Our findings revealed that VT aqueous extract had a potential to be used as antibacterial supplement or drug.

## Keywords

*Verbascum thapsus*; *aqueous extract; antibacterial effects; macro-dilution method; agar disk diffusion method; agar well diffusion method*

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## INTRODUCTION

Antibiotics are drugs that used in the treatment of infectious diseases But, in recent times, there have been enhances in antibiotic resistant strains of clinically substantial bacteria, which have caused the emergence of novel bacterial strains that are multi-resistant. The spread of drug resistant bacteria is one of the greatest threats to successful therapy of bacterial diseases[1, 2].

Ethnomedicinal plants have been consumed as alternative remedies for the treatment of different diseases[3-5]. There is growing concern in correlating phytochemical constituents of plant with its pharmacological effects[6, 7]. Out of so many plants most are used to cure bacterial disease[8-10]. The original benefit of natural factors is that they do not enhance the “antibiotic resistance”, an event usually encountered with the long-term use of synthetic antibiotics; because they have a striking role in the defense system to bacterial diseases due to their intrinsic anti-oxidative and anti-bacterial activities[11, 12]. A plant extract is effective substance from the tissue of a plant, to be used for a particular therapeutic purpose. Plant extracts have a major variety of phytochemicals such as phenolic acids, flavonoids, lignin tannins, and other small compounds[13]. In herbal medicines, raw plant extracts in the form of decoction, tincture, and infusion are traditionally used by the population to cure diseases including infectious diseases[14]. The antibacterial effects of extracts have been identified for many years, and their rudiment have found applications as naturally occurring antibacterial supplements in the field of pharmaceutical botany, phytopathology, pharmacology, medical clinical bacteriology, and food maintenance, etc[15]. In Iranian medicine, plant extracts are used in different forms by the population for the prevention, control, and treatment of diseases such as bacterial diseases[19]. In studies presented; Iranian traditional medicine plant extracts have strong antibacterial efficacy against negative and positive bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*[16,17].

VT is the member of family *Scrophulariaceae* and tribe *Scrophularieae*. VT has long been consumed in Iranian traditional medicine as a medicinal plant. In traditional medicine, different extracts of this plant are traditionally used in treating different inflammatory, fungal, viral, parasitic, and bacterial diseases. Likely, the antibacterial effects of the plant are related to its flavonoid, phenolic compounds. These components are drawn to the bacterial outer membrane proteins to deactivate the matrix metalloproteinase and prevent the growth of bacteria or destroyed them[18].

The aim of the recent study was assessment antibacterial properties of aqueous extract of VT against BS in west of Iran (in Kermanshah).

## MATERIALS AND METHODS

### *Plant sample collection*

In the experimental study, medicine plant collected from Kermanshah in west of Iran.

### *Preparation of aqueous extract*

Plant was washed, air dried for 7-8 days, and ground into powder before being placed into a Soxhlet apparatus for extraction with distilled water with increasing polarity to extract phyto-constituents separately at 25ºC for 3-4 h. Whatman filter paper No.1 was usedto filter the extract. Pressure was decreased to evaporate and dry the filtrates (after drying, powder of the extract was obtained).

### *Culture media*

Mueller-Hinton broth and agar (Müller-Hinton agar is a microbiological growth medium that is usually applied for antibiotic susceptibility testing), Tryptic Soy broth and Nutrient agar were accumulated according to the manufacturer's instruction (Oxoid, UK), autoclaved and dispensed at 20 ml per plate in 12 x 12cm Petri dishes. Set plates were incubated overnight to avouch sterility before use.

### *Source of microorganisms*

Lyophilized *Bacillus subtilis* ATCC No. 21332 (BS) provided by The Iranian Research Organization for Science and Technology was activated on Tryptic Soy broth 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 in Muller Hinton broth.

### *Evaluation of antibacterial activities*

Agar disk and agar well diffusion were used as screen tests to assess antibacterial effects of aqueous extract of VT based on standard protocol. The solution of the extract was yielded in 1g/ml from which six fold serial dilutions (v/v) were prepared. 60 μl of each dilution was poured on each disk and well in order. After a period of 24 hours incubation, the diameters of growth inhibition zones around the disks and wells were measured. Distilled water was applied as negative control whereas Amoxicillin was used as positive control. Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) were specified by macrobroth dilution assay based on Clinical Laboratory Standard Institute (CLSI) guidelines[19].

## RESULTS

### *Agar disk diffusion test*

The widest zone was formed due to 0.083 g/ml of the aqueous extract of VT in BS culture. No inhibition zone was observed due to distilled water. Growth inhibition zones due to different dilutions are listed in figure 1.

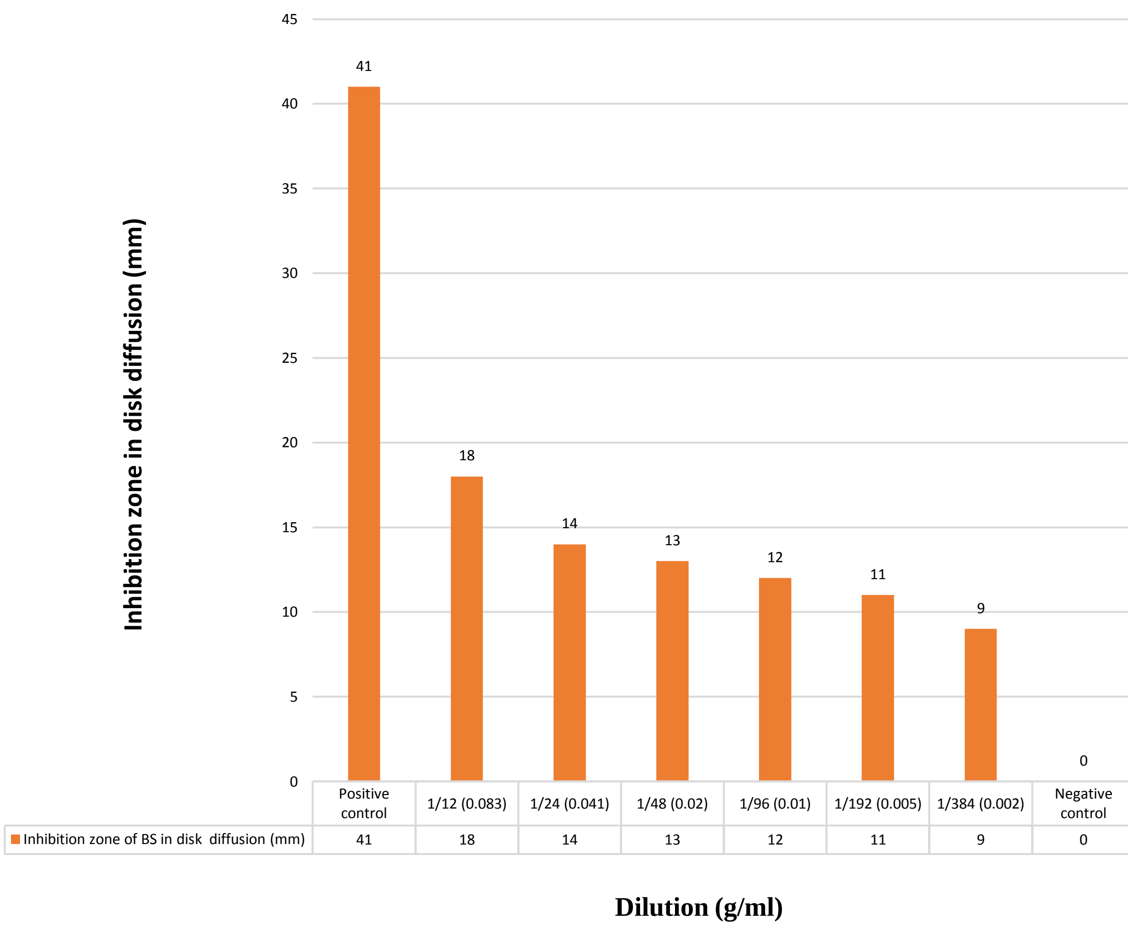


**Figure 1** The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of aqueous extract of VT

### *Agar well diffusion test*

In regard to this plant, the widest zone was seen in 0.083 g/ml, due to SA (13 mm). No inhibition zone was observed due to negative control. The data are discoverable in figure 2.

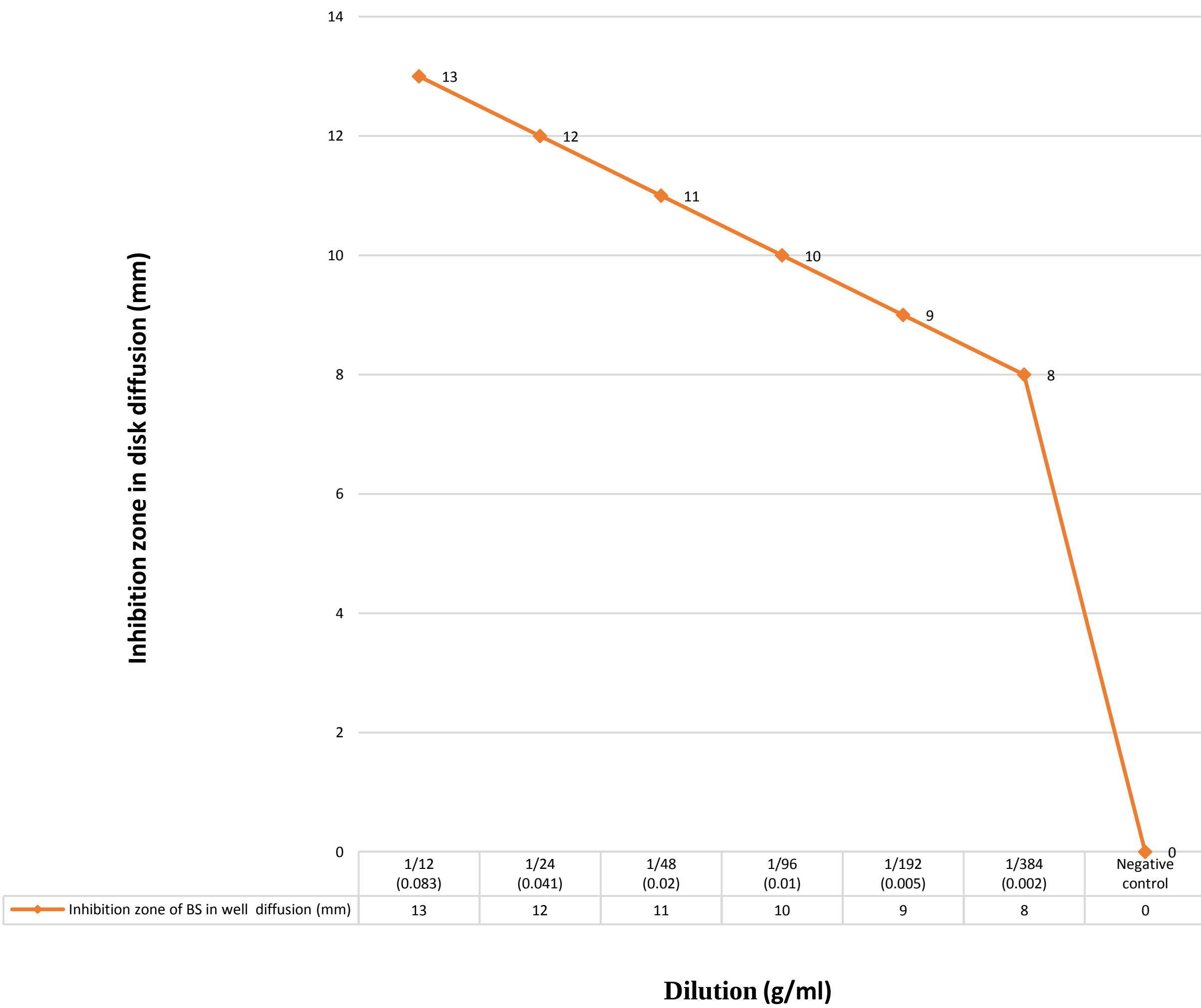


**Figure 2** The diameters of growth inhibition zones in agar well diffusion test in different dilutions of aqueous extract of VT

***MIC determination* and *MBC ascertaining***

In the examined bacterium, MIC and MBC values were 0.01.

## DISCUSSION AND CONCLUSION

Plants are important resources in pharmaceutical market[20-22]. Because of their safety and low cost as well as their effects on a large number of bacteria, ethnomedicinal plants may have the potency to cure bacterial resistance to several types of antibiotics[23-25]. The antimicrobial properties of plant have been assessed and reviewed[26-28], and the mechanisms that enable the natural components of plants and spices to resist bacteria have been discussed[29,30]. Since the antibacterial effectiveness of ethnomedicinal plants destabilized significantly depending on the phytochemical characteristics of plan families and subfamilies, it is not surprising to note the diversity in this efficacy even when using samples taken from the similar plant, but from two different regions[31]. VT has long been used in Asian countries as a medicinal plant for the treatment of diseases; it has been applied for treating several inflammatory and bacterial diseases[19].

As the tables showed, VT aqueous extract have inhibited the growth of BS and eradicated it. Also, by increasing the concentration of VT, the inhibition zone augmented. The results determined that in tested bacterium, there was a substantial difference in terms of sensitivity to aqueous extract of VT. Also, the results indicated that VT with 0.01 g/ml concentration has prevented BS from the growth and in this concentration destroyed it. In agar disk diffusion test, the widest inhibition zone was seen in 0.083 g/ml concentration (The value of growth inhibition zone was 18 mm in this dilution, and the value of growth inhibition zone of Amoxicillin against BS was 41 mm) and no inhibition zone was observed due to distilled water. In agar well diffusion test, the widest zone was seen in 0.083 g/ml concentration (13 mm) and no inhibition zone was observed due to negative control.

In a study, the ethanolic extract of *Verbascum qulebrium* was subjected to phytochemical screening and it was also evaluated against six microorganisms (BS, *Staphylococcus aureus, Salmonella typhi, Saccharomyces pastorianus, Escherichia coli, Pseudomonas aeruginosa)* in nutrient agar using disc agar method and demonstrated this plant have strong antibacterial properties against above bacteria[32]. In similar studies it was indicated that methanolic extract of VT have strong antibacterial effects against *E. coli*, *Yersinia pestis, Bacillus cereus, P. aeruginosa, Listeria monocytogenes* and *S. aureus*[33]. In other studies, it was demonstrated VT has potent antibacterial efficacy against BS, *S. aureus, S. typhi* and *P. aeruginosa* [34]. Our study, presented that aqueous extract of VT have strong antibacterial activities, and its activities could be attributed to qualitative and quantitative discrepancies in the chemical constituents of the individual extract. It can be used as antibacterial supplement.

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## REFERENCES

1. World Health Organization (WHO). Traditional medicine. Fact sheet number 134. Revised May, 2003.

2. Najafi F., Tahvilian R., Zangeneh M.M., Zangeneh A., Moradi R. Medicinal plant: Assessment of the chemical composition and in vitro antibacterial activities of the *Viola odorata Linnoil's* against *Bacillus subtilis* (ATCC No. 21332) in west of Iran. *Int J Sci Eng Res*. 2016: 7 (11): 1330-1339.

3. Tahvilian R., Hajialiani M., Yazdani H., Zangeneh A., Zangeneh M.M., Moradi R., Zhaleh H. Investigate a plant product as an anxiolytic agent. *International Journal of Current Medical And Pharmaceutical Research*. 2017; 3(2): 1374-1377.

4. Hagh-Nazari L., Goodarzi N., Zangeneh M.M., Zamgeneh A., Tahvilin R., Moradi R. Stereological study of kidney in streptozotocin-induced diabetic mice treated with ethanolic extract of *Stevia rebaudiana* (bitter fraction). *Comp Clin Pathol*. 2017; 26: 455–463.

5. Faramarzi E., Zangeneh M.M., Zangeneh A., Moradi R. Effect of *Cinnamomum zelanicum* oil on hyponeophagia anxiety test in Balb C male mice. *Onl J Vet Res*. 2017; 21(2):77-80.

6. Zangeneh M.M., Tahvilian R., Zangeneh A., Moradi R., Najafi F., Haghnazari L. Effect of *Alhagi maurorum* oil on anxiety markers in Balb/C male mice. *Onl J Vet Res*. 2017; 21(3): 115-117.

7. Tahvilian R., Moradi R., Zhale H., Zangeneh M.M., Zangeneh A., Yazdani H., Hajialiani M. Ethnomedicinal Plants: Study on Antifungal Activity of Essential oil of *Pistacia khinjuk* (Combined with the Dominance *γ-Terpinene*) Against *Candida albicans*. *Int J Pharm Clin Res*. 2016; 8(10): 1369-1373.

8. Tahvilian R., Moradi R., Hajialiani M., Zangeneh M.M., Zangeneh A., Yazdani H., Zhale H. Chemical composition and screening of antibacterial activities of essential oil of *Pistacia khinjuk* against *Bacillus subtilis* (ATCC No. 21332). *International Journal of Current Medical and Pharmaceutical Research*. 2016; 2(12): 1098-1102.

9. Najafi F., Tahvilian R., Zangeneh M.M., Zangeneh A., Moradi R. Screening of essential oil of *Allium sativum* for antibacterial effects against *Bacillus subtilis*. *International Journal of Recent Scientific Research*. 2016; 7(11): 14172-14176.

10. Tahvilian R., Moradi R., Zhale H., Zangeneh M.M., Zangeneh A., Yazdani H.,

Hajialiani M. Ethnomedicinal Plants: In vitro antibacterial effect of essential oil of *Pistacia khinjuk*. *Int J Sci Eng Res.* 2016; 7(10): 437-447.

11. Zangeneh M.M., Poyanmehr M., Najafi F., Zangeneh A., Moradi R., Tahvilian R., Haghnazari L. In vitro antibacterial activities of ethanolic extract of *Stevia rebaudiana* against *Bacillus subtilis* (ATCC No. 21332). *Int J Res Pharma NanoSci.* 2016; 5(6): 320-325.

12. Zangeneh M.M., Najafi F., Tahvilian R., Haghnazari L., Zangeneh A., Moradi R., Mahmoudifar A. Effect of *Scrophularia striata* hydro-alcoholic extract on growth of *Bacillus subtilis* ATCC No. 21332. *Onl J Vet Res*. 2017; 21(2): 51-57.

13. Foroughi A., Pournaghi P., Tahvilian R., Zangeneh M.M., Zangeneh A., Moradi R. Assessment of chemical composition and antibacterial effects of Anethole-rich hydroalcoholic extract of *Pimpinella anisum*. *Int J Pharm Clin Res.* 2016; 8(11): 1459-1463.

14. Foroughi A., Zangeneh M.M., Zangeneh A., Kazemi N. A survey on antibacterial activities of *Allium eriophyllum* alcoholic extract: An ethnomedicinal plant. *Iranian J Publ Health*, 2016; 45 (2): 32.

15. Zangeneh M.M., Tahvilian R., Najafi F., Zangeneh A., Souri N., Moeini Arya M., Zhaleh S. Evaluation of the in vitro antibacterial effect of the hydroalcoholic extract of *Scrophularia striata*. *Int J Sci Eng Res.* 2016; 7(10): 1693-1702.

16. Foroughi A., Zangeneh M.M., Kazemi N., Zangeneh A. An in vitro study on antimicrobial properties of *Allium noeanum reut ex regel*: An ethnomedicinal plant. *Iranian J Publ Health.* 2016; 45 (2): 32.

17. Moradi R., Hajialiani M., Zangeneh M.M., Zangeneh A., Faizi S., Zoalfaghari M., Marabi A. Study a plant extract as an antibacterial agent. *International Journal of Current Medical And Pharmaceutical Research*. 2017; 3(2): 1360-1362.

18. Tatli I.I., Akdemir Z.S. Chemical constituents of *Verbascum* L. species, *FABAD J. Pharm. Sci*., 2004; 29: 93-107.

19. Clinical and laboratory standards institute (CLSI), M7-A7, 26(2), 2006.

20. Foroughi A., Pournaghi P., Najafi F., Zangeneh A., Zangeneh M.M., Moradi R. Evaluation of antibacterial activity and phytochemical screening of *Pimpinella anisum's* essential oil. *Int J Pharm Phytochem Res.* 2016; 8(11); 1886-1890.

21. Foroughi A., Pournaghi P., Zhaleh M., Zangeneh A., Zangeneh M.M., Moradi R.

Antibacterial activity and phytochemical screening of essential oil of *Foeniculum vulgare*. *Int J Pharm Clin Res.* 2016; 8(11): 1505-1509.

22. Foroughi A., Pournaghi P., Najafi F., Zangeneh M.M., Zangeneh A., Moradi R. Chemical composition and antibacterial properties of *Chenopodium botrys L.* essential oil. *Int J Pharm Phytochem Res.* 2016; 8(11); 1881-1885.

23. Foroughi A., Pournaghi P., Tahvilian R., Zangeneh M.M., Zangeneh A., Moradi R. Ethnomedicinal plants: Study on the chemical composition and antibacterial activity of the *Nigella sativa* (Black seed) oil's. *Int J Pharm Clin Res*. 2016; 8(11): 1528-1532.

24. Zangeneh M.M., Najafi F., Tahvilian R., Zangeneh A., Souri N., Zarei M.S., Khedri M.R., Bahrami E., Shamohammadi M. 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.

25. Foroughi A., Pournaghi P., Tahvilian R., Zangeneh M.M., Zangeneh A., Moradi R. Evaluation of the composition and antibacterial effects of the *Viola odorata lin* oils. *International Journal of Current Medical and Pharmaceutical Research*. 2016; 2(12): 1093-1097.

26. Najafi F., Zangeneh M.M., Tahvilian R., Zangeneh A., Amiri H., Amiri N., Moradi R. In vitro antibacterial efficacy of essential oil of *Allium sativum* against *Staphylococcus aureus*. *Int J Pharm Phytochem Res*. 2016; 8(12): 2039-2043.

27. Zangeneh M.M., Najafi F., Tahvilian R., Haghnazari L., Zangeneh A., Moradi R., MahmoudiFar A. Effect of *Allium sativum* oil on *Escherichia coli O157:H7*. *Onl J Vet Res*. 2017; 21(1): 19-24.

28. Foroughi A., Pournaghi P., Najafi F., Zangeneh A., Zangeneh M.M., Moradi R. Antibacterial effect and phytochemical screening of essential oil *of Pimpinella anisum* against *Escherichia coli O157:H7* and *Staphylococcus aureus. International Journal of Current Pharmaceutical Review and Research*. 2016; 7(6): 367-371.

29. Poorshamohammad C., Souri N., Amini Z., Kosari F., Jamshidpour R., Zangeneh M.M., Zangeneh A. Cucurbita moschata: A plant with antibacterial properties. *International Journal of Current Medical And Pharmaceutical Research*. 2017; 3(2): 1356-1359.

30. Zangeneh M.M., Zangeneh A., Moradi R., Hajialiani M., Sadeghi S., Khaef S., Nazari M., Malaki H. Effect of *Cucurbita moschata* oil seed on growth of *Staphylococcus aureus* ATCC No. 25923. *Onl J Vet Res*. 2017; 21 (3): 106-109.

31. Zangeneh A., Zangeneh M.M., Hajialiani M., Moradi R., Nazari F., Sanjabi Shirazi F., Ansari N., Lotfihamadani M., Ekhtiari A. Peruse a Plant product as an antimicrobial agent. *International Journal of Current Medical And Pharmaceutical Research*. 2017; 3(2): 1348-1351.

32. Salim M.L., Ammar H.A., Abderrahman S., El-Remawy H.A.. Phytochemical screening and antimicrobial activity of wild Jordanian plants from Al-Balq'a, *Bull. Fac. Pharm. Cairo Univ.*, 1996; 34: 235-238.

33. Prakash V., Rana S., Sagar A. Studies on antibacterial activity of *Verbascum thapsus*. *Journal of Medicinal Plants Studies*. 2016; 4(3): 101-103.

34. Morteza-Semnani K., Saeedi M., Akbarzadeh M. Chemical Composition and Antimicrobial Activity of the Essential Oil of *Verbascum thapsus* L. *Journal of Essential Oil Bearing Plants*. 2012; 15(3): 373-379.