Ann Mil Health Sci Res. 2018 June; 16(2):e82841. doi: 10.5812/amh.82841.

Published online 2018 September 23. **Research Article**

Anti-Microbial Effect of *Aloe vera* Extract on Clotrimazole-Resistant *Malassezia Furfur* Strains Isolated From Patients with Seborrheic Dermatitis in the City of Sari

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Received 2018 August 01; **Revised** 2018 September 04; **Accepted** 2018 September 07.

Abstract

Background: *Malassezia* is a dimorphic and lipophilic fungus that causes skin diseases in opportunistic conditions.
Objectives: The aim of the present study was to investigate the antimicrobial activity of *Aloe vera* extract on growth of clotrimazole-resistant *Malassezia furfur* isolated from patients with seborrheic dermatitis.
Methods: In this descriptive cross-sectional study, 102 samples were selected from skin lesions of suspected cases of seborrheic dermatitis. *Malassezia* isolates were identified by biochemical tests. To determine drug sensitivity, the broth microdilution method was used. After preparation of *Aloe vera* extract, the antifungal effect of different concentrations of this extract on clotrimazole-resistant isolates was determined by uing the agar well diffusion method, and its minimum inhibitory concentration (MIC) was evaluated by the broth microdilution method.
Results: In this study, 72 strains of *Malassezia* were isolated, out of which the highest frequency belonged to *Malassezia furfur* with a frequency of 56 (77.8%) cases, while 50% of them were resistant to clotrimazole. Upon evaluation of antifungal effects of *Aloe vera* extract, the maximum inhibition zone diameter was seen for the extract in the concentration of 250 mg/mL. This parameter was observed for yeast isolates resistant to clotrimazole at the mean of 25 mm. Changes to the MIC of *Aloe vera* extract in the range of 1024 - 2 μL/mL showed that 89% of *Malassezia furfur* isolates resistant to clotrimazole did not grow in concentrations with MIC $\geq$ 512 μL/mL ($P < 0.001$).
Conclusions: According to our results, *Aloe vera* extract showed proper inhibitory effects against clinical clotrimazole-resistant *Malassezia furfur* isolates at high concentrations. Therefore, we could hope to treat fungal diseases by producing an appropriate herbal medicine.

Keywords: Seborrheic Dermatitis, *Malassezia*, Clotrimazole, *Aloe vera*, Traditional Iranian Medicine

1. Background

Malassezia species are the natural flora of the skin in humans and warm-blooded animals (1). They are susceptible to heat, and most of them need long chain fatty acids to grow. This fungus can be seen in two forms of yeast and mycelium (dimorphic) in 90% of adults (2).

These yeasts are mostly seen in regions such as the neck, back, arms, and abdomen due to sebaceous glands accumulation and in individuals aged 10 to 19 years due to increased activity of sebaceous glands that provide the necessary lipids for the development of *Malassezia* species. Conditions such as AIDS, taking antibiotics, pregnancy, and taking steroids can cause different skin problems, which can be intensified by *Malassezia* species; however, the role of these yeast species in some skin diseases is ambiguous. For many years, all the damage caused by *Malassezia furfur*, especially skin disorders such as dandruff, seborrheic dermatitis, and pityriasis versicolor, were attributed to a single species (3).

Seborrheic dermatitis is a chronic dermatitis observed in both infants and adults. In adults, rash tends to appear on the central parts of the face, eyebrows, and head and often causes the skin to scale. Seborrheic dermatitis can also occur under the armpits, on groin, or in middle of the chest. In men, this condition sometimes occurs in the beard area. The cause of seborrheic dermatitis is unclear, but it is sometimes associated with an overgrowth of *Malassezia*, which naturally exists on the skin surface (4). Although seborrheic dermatitis could be treated with antifungal antibiotics, unfortunately, drug resistance cases are reported frequently. Therefore, the use of different antimi-

crobial compounds or agents to prevent or control such resistances is recommended. Among these compounds are medicinal herbs that are not only economically justifiable, but their availability, extraction, and application in pharmacy and medicine are confirmable.

Nowadays, scientists pay particular attention to using herbal and traditional medicine. In countries like Iran, there is a long history of applying herbal medicine for treating diseases. For instance, the great Iranian scientist Abu Ali Sina (Avicenna) had considered using herbs. *Aloe vera* belongs to the *Asphodelaceae* family. This plant is similar to broad-leaved cactus and is a permanent, meaty, and juicy plant, which reaches a maximum height of two meters. Surprisingly, water constitutes 96% of *Aloe vera* gel. The compounds found in *Aloe vera* gel contain polysaccharides that are capable of reducing and restoring inflammation. It also has antimicrobial properties. This plant contains hydroxyl anthracene derivatives and aloe resin A, B and C. Also, glucose, mannose, and cellulose sugars, oxidase, amidases, and catalase enzymes, vitamins B1, B2, B6, C, and E, folic acid, and minerals such as calcium, sodium, magnesium, zinc, copper and chromium form its other nutrients (in low quantities) (5).

2. Objectives

In this study, considering the community approach to traditional medicine and treatment with natural substances and with respect to the fewer side effects of herbal remedies, we determined the effect of *Aloe vera* extract on the growth of clotrimazole-resistant *Malassezia furfur* species isolated from patients with seborrheic dermatitis *in vitro*.

3. Methods

3.1. Sample Collection and Mycologic Identification

Sampling was performed on 102 suspected cases of seborrheic dermatitis (with symptoms such as scaling and some itching) from three specialized skin clinics in the city of Sari, Mazandaran province, north of Iran. After completeing a written informed consent, the participants filled a checklist that contained items on age, gender, occupation, and nightly sleep time. The participnts were selected using the randomized smpling method. Those who had taken steroids or antibiotics were excluded from the study. The specimens were collected from the skin of the neck, face, arm, abdomen, shoulder, and scapula using a sterile surgical knife. The scales were stained with methylene blue and 10% KOH and were then cultured on dixon agar (Merck, Germany), which was modified with cyclohexamide and chloramphenicol in the form of transplantation. The samples were incubated for 7 to 10 days at 32°C.

The yeast cells were first examined directly by microscopy based on their colony forms and then adjacent to Calcofluor-white. In the present study, 1 - 2 single spores were identified as positive 1 (+1) and 2 - 6 integrated spores or 3 - 12 single spores were considered as positive 2 (+2) (6).

Finally, *Malassezia* species were identified by catalase and esculin hydrolysis tests after growing on sabouraud dextrose agar (Merck, Germany).

3.2. Antifungal Susceptibility Test

Drug susceptibility test to clotrimazole was performed using the broth microdilution method according to CLSI-M27-A3 instruction (7). After preparing the stock from clotrimazole powder (Gibco, Germany) in 25% ethanol and diluting it at the range of 16 - 0.03 μg/mL, dilutions were poured in 96-well ELISA microplates containing dixon broth (Merck, Germany) adjacent to yeast suspension (1×10^3 cells per mL) counted by neubauer chamber. Then, they were placed in a shaking incubator at 32°C for 72 hours. In reporting the minimum inhibitory concentration (MIC), ELISA Reader was used in addition to determination of turbidity. To determine the minimum fungicidal concentration (MFC), 100 μL of the wells' content with a concentration higher than the MIC, together with 100 μL of the positive control were separately transferred to dixon agar medium and were incubated for 3 - 5 days at 32°C. The concentration of the well with no growth on the mentioned medium was reported as MFC. In this study, *Candida krusei* ATCC6258 and Candida parapsilosis ATCC 22019 were used as controls.

3.3. Preparation of Aloe vera Extract

To prepare the plant extract, *Aloe vera* was procured freshly from a greenhouse. After extraction of gel from the *Aloe vera* leaf, it was mixed and soaked with 70% ethanol at a ratio of 1 to 10 (each 100 cc of gel was soaked with 1000 cc of ethanol), and then the mixture was placed in a shaker for 24 hours. After 24 hours, the mixture was filtered and placed in a rotary container to evaporate the solvent (ethanol). Afterwards, the extract was placed in a water bath for 24 hours to form a honey-like liquid.

3.4. Evaluating the Inhibitory Effect of Aloe vera Extract

The antifungal effect of different concentrations of *Aloe vera* extract on all the clotrimazole-resistant isolates was investigated using the agar well diffusion method. In this method, first a microbial suspension of 0.5 McFarland was prepared and cultured in the form of spreadsheet on

a dixon agar medium. Wells with a diameter of 7 mm were created on the medium and filled with 100 μL of the extract at the concentrations of 250, 125, 62.5, 31.25, and 15.6 mg/mL. One well containing distilled water was considered as the negative control. After adding dilutions to wells, the plates were incubated at 32°C for 5 days. After that, the mean diameter of the inhibition zones was reported in milimeters, and inhibition zones with a diameter of more than 12 mm were considered as sensitive and the ones with a diameter of less than 10 mm were considered as resistant to the extract.

The MIC of the honey-like *Aloe vera* extract, which had shown a good antifungal effect in the well diffusion method, was determined by the broth microdilution method at a dilution of 1024 - 2 μL/mL.

3.5. Statistical Analysis

Comparison of mean treatments was performed using Duncan's multiple range test and one-way analysis of variance (ANOVA) at a confidence level of 0.001. All statistical analyses were performed using SPSS, and all figures were drawn with MS excel (all measurements were repeated three times).

4. Results

After conducting biochemical tests to identify *Malassezia* species from the 102 collected samples, 72 samples were reported to be positive. In investigating the frequency of *Malassezia* strains among patients suspected of seborrheic dermatitis, *Malassezia furfur* showed the highest frequency of 77.8% (56 cases), which was more frequently seen in female patients (86%), followed by *Malassezia* globosa and *Malassezia* restricta with 12 (16.7%) and 4 (5.5%) cases, respectively.

The highest and lowest frequencies of *Malassezia furfur* isolates were observed in students (52%) and nurses (2%), respectively, which means that students are more exposed to this yeast, thus, they are suggested as a high-risk group in this study. When investigating the relationship between sleep time and the prevalence of the disease, it was found that the highest frequency of *Malassezia furfur* was observed in people who went to sleep between 12 and 1 AM and the lowest frequency was found in the ones who slept between 9 and 10 PM (Figure 1). This indictes that the number of patients with *Malassezia furfur* is higher among people who go to sleep at midnight. In determining the drug sensitivity test by broth microdilution test, the MIC was measured in the range of 4 - 16 μg/mL and the MFC level was also in the range of 8 - 16 μg/mL. This means that 50% of *Malassezia furfur* isolates were resistant to clotrimazole, 32% were susceptible and 18% were semisusceptible (Table 1).

The results of the well diffusion method showed that the highest and lowest mean diameters of the inhibition zone were observed in the concentrations of 250 mg/mL (average = 25.35 mm) and 15.6 mg/mL (average = 11.52 mm), respectively (Table 2). In fact, all the concentrations of the extract showed good antimicrobial effect (Figure 2), but with an increase in the concentration of the extract, the diameter of the inhibition zone also increased.

In this study, the MIC of *Aloe vera* extract gainst clotrimazole-resistant *Malassezia furfur* isolates was determined in the range of 1024 - 2 μL/mL by the broth microdilution method. The MIC of *Aloe vera* extract was obtained in 89% of clotrimazole-resistant *Malassezia* isolates at a concentration of 512 μL/mL and higher. In fact, only 11% of clotrimazole-resistant strains showed resistance to the extract, which indicates the satisfactory antimicrobial effect of *Aloe vera* extract. MFC values of *Aloe vera* extract for 86% of clotrimazole-resistant *Malassezia* isolates was determined at the concentration of 512 μg/mL and for 3% of clotrimazole-resistant isolates at the concentration of 1024 μL/mL, indicating the fungicidal properties of this extract at concentrations of 512 to 1024 μL/mL.

5. Discussion

One of the most important diseases associated with *Malassezia* is seborrheic dermatitis, which is of paramount importance in terms of appearance. The causative agents of these diseases do not involve the host's immune system but the superficial skin areas, and are thus seen as a chronic problem in most cases (8).

In this study, 70.6% of the total samples collected from suspected cases of seborrheic dermatitis were reported to be positive for *Malassezia* species. This is while in a study, the frequency of yeast cells in all samples of patients with seborrheic dermatitis was reported to be 54% (9), which is less than the results of our study in this regard.

The results of this study showed that the frequency of *Malassezia* strains isolated from suspected cases of seborrheic dermatitis was higher in women (86%) than in men (14%), which could be due to differences in the secretion of sebum, and the type and amount of sex hormones. In a study, the highest frequency of *Malassezia* was observed in women (60%) (10), which is similar to the result of our study in this regard. However, the results of some reports indicated the higher risk of diseases associated with *Malassezia* in men than women (11).

The highest frequency of *Malassezia* was observed between the ages of 12 and 16 years old (41%). Also, the number of patients was higher among the ones who went to sleep

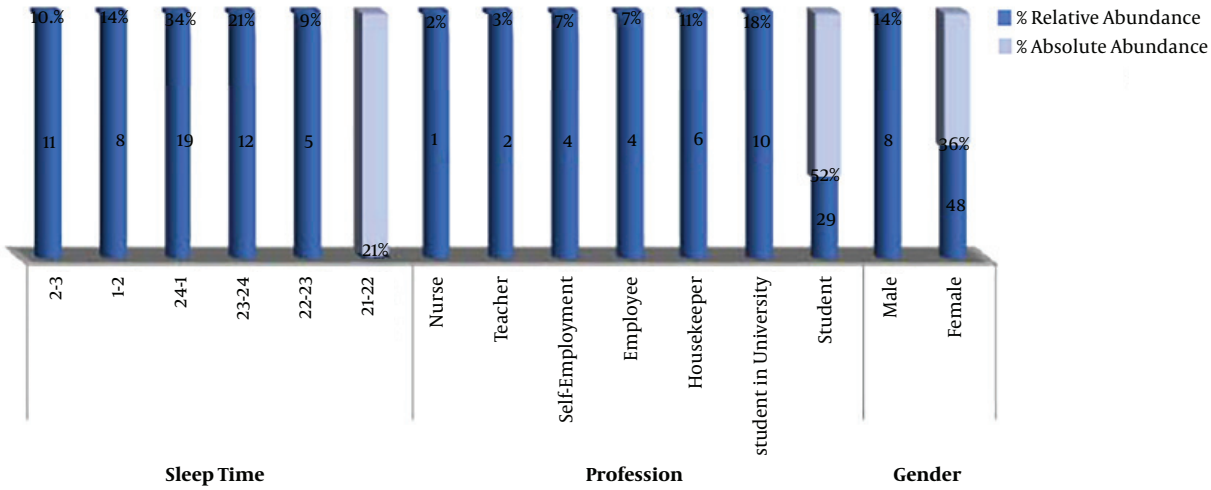

Figure 1. Frequency of *Malassezia* isolates in terms of gender, profession, and sleep time

Table 1. The Frequency of Clotrimazole Resistance in *Malassezia* Isolates From Patients

Characteristics	Resistant	Semi-Sensetive	Sensetive	Total
Gender				56 (100)
Female	23 (41.07)	9 (16.07)	16 (28.57)	
Male	5 (8.93)	1 (1.79)	2 (3.57)	
Profession				56 (100)
Student	14 (25)	6 (10.71)	9 (16.07)	
Non-student	14 (25)	4 (7.15)	9 (16.07)	
Sleep time				56 (100)
Late time	19 (33.93)	7 (12.5)	12 (21.4)	
On time	9 (16.07)	3 (5.4)	6 (10.7)	

Table 2. Diameter of Inhibition Zone Due to the Effect of *Aloe vera* Extrac on Growth of *Malassezia* Isolates[a]

Aloe vera Extrac Concentrations (mg/mL)	Inhibition Zone Diameter(mm)[b]
250	25.4 ± 0.3
125	22.5 ± 00.01
62.5	14.65 ± 00.02
31.25	13.59 ± 00.00
15.6	11.52 ± 00.00

[a] Variance analysis test (P value < 0.001).
[b] Values are expressed as mean ± SD.

between 12 and 1 AM (34%) than those who went to sleep between 9 and 10 PM (2%).

In another study, *Malassezia* species isolated from patients with seborrhoic dermatitis were more common in the age group of 20 - 29 years old (10), while in our study, *Malassezia* had the lowest frequency in the age group of 20 - 24 years old.

In the present study, *Malassezia furfur* was found to be

Figure 2. The antimicrobial effects of *Aloe vera* extract concentrations on the growth of *Malassezia furfur*

the most prevalent in *Malassezia* strains by species, compared to *Malassezia globosa* and *Malassezia restricta*. However, in a study by Hedayati et al. (10), *Malassezia globosa* was found as the dominant species of *Malassezia*, followed by *Malassezia furfur* and *Malassezia restricta*. Nakabayashi et al. (11) reported that in patients with seborrheic dermatitis, *Malassezia globosa* and *Malassezia furfur* were the most frequent ones. In the present study, *Malassezia furfur* had the highest frequency in seborrheic dermatitis patients.

In a study by Klarostaghi et al. (12) that was conducted to see if *Malassezia* was present in acne lesions, only *Malassezia furfur* was found (2.5%). The geographical and climatic conditions of the area, the low prevalence of other species in the geographic region under study, and even the type of sampling technique could be the reasons for this finding.

In numerous studies, an increase in the number of *Malassezia* yeasts in dandruff and the association between the number of *Malassezia* yeasts and the severity of seborrheic dermatitis have been reported (4). In addition, it has been shown that treatment with antifungal agents such as clotrimazole heals lesions and reduces the number of *Malassezia* yeasts on the skin of patients with seborrheic dermatitis (13).

In a study by Yurayart et al. (14) more than 95% of *Malassezia* strains isolated from dogs with seborrhoic dermatitis were reported to be susceptible to clotrimazole. In another study by Khazaiyan et al. (15), 12.5% of *Malassezia* isolates were resistant to clotrimazole. This resistance was less than the one reported in our study. Resistance to antibiotics in different regions of Iran and the world can be as a result of genetic changes in the causative strains, differences in the amount of antibiotics used, and changes in the availability of new antibiotics.

Resistance to conventional antibiotics by bacteria is a global phenomenon that is encompassing all human pathogens and all antibiotic groups (16). Therefore, it is essential to carry out further studies to obtain antimicrobial agents from other sources such as plants. One of these plants is *Aloe vera*, whose extract is well known to have beneficial effects, but limited research has so far been conducted on its microbial effects. In this study, the antimicrobial effects of *Aloe vera* extract were shown to be more optimal than those of clotrimazole.

The results of the present study revealed a direct relationship between the diameter of the inhibition zone and *Aloe vera* extract concentration. This means that in all cases the diameter of the inhibition zone grows upon the increase in concentration, which is consistent with the study conducted by Mohebi et al. (17).

In the study of Sadrnia et al. (5) no inhibition zone was observed in all studied concentrations. However, in the present study, *Aloe vera* extract had inhibitory effect in all the studied concentrations. The difference in reported concentrations can be due to the difference in the applied extraction methods and the strains used in experiments.

In this study, the MIC and MFC values of the *Aloe vera* extract against the clotrimazole-resistant *Malassezia* isolates were determined in the ranges of 256 - 1024 μL/mL and 512 - 1024 μL/mL, respectively. The results indicated that 89% of clotrimazole-resistant strains showed susceptibility to the extract, which proves adequate antimicrobial effect of *Aloe vera* extract.

In a study by Kasim et al. (18), the results showed that 70% of *Acalypha fimbriata* aqueous and ethanolic extracts had the highest activity against pathogenic fungi, including *Malassezia furfur*. They also reported the MIC levels to be 50 mg/mL (18). Other studies carried out in Iran and other parts of the world also show that *Aloe vera* extract has antimicrobial activity (19).

4.1. Conclusions

The results of the present study showed that *Aloe vera* had satisfactory antimicrobial activity against clotrimazole-resistant *Malassezia furfur* isolates *in vitro* and that its antimicrobial activity increased upon increasing its concentration. Nevertheless, further studies seem to be needed *in vivo* to determine the effective dosage on fungi and ultimately to use *Aloe vera* to control chronic and inflammatory diseases. These results, along with

further research in this field, can lead to the emergence of proper antifungal herbal remedies and reduce the use of synthetic antifungal drugs due to their toxicity and the side effects of these drugs.

Acknowledgments

The authors are grateful to all those who helped in conducting this study, as well as the staff of the microbiology Lab at the Islamic Azad University, Gorgan Branch, Gorgan, Iran. We wish to thank the specialized skin clinics in the city of Sari.

Footnotes

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Ethical Considerations: We didn't need this code.

References

1. Faergemann J. Atopic dermatitis and fungi. *Clin Microbiol Rev*. 2002;**15**(4):545–63. [PubMed: 12364369]. [PubMed Central: PMC126862].
2. Taheri Sarvtin M, Shokohi T, Hedayati MT. Malassezia species in skin diseases. *4 th Annual Students-Research Congress*. 2013.
3. Guillot J, Gueho E. The diversity of Malassezia yeasts confirmed by rRNA sequence and nuclear DNA comparisons. *Antonie Van Leeuwenhoek*. 1995;**67**(3):297–314. [PubMed: 7778898].
4. Gupta AK, Batra R, Bluhm R, Boekhout T, Dawson TL. Skin diseases associated with Malassezia species. *J Am Acad Dermatol*. 2004;**51**(5):785–98. doi: 10.1016/j.jaad.2003.12.034. [PubMed: 15523360].
5. Sadrnia M, Arjomandzadegan M. [Comparative study on the effects of aloe vera extract in clinical strains of Staphylococcus aureus, Klebsiella, Staphylococcus epidermidis and Escherichia coli compared to antibiotics of choice]. *Arak Med Univ J*. 2014;**17**(6):39–46. Persian.
6. Back O, Faergemann J, Hornqvist R. Pityrosporum folliculitis: A common disease of the young and middle-aged. *J Am Acad Dermatol*. 1985;**12**(1 Pt 1):56–61. [PubMed: 3980804].
7. Rex JH; Clinical Laboratory Standards Institute. *Reference method for broth dilution antifungal susceptibility testing of filamentous fungi: Approved standard*. 3rd ed. Pennsylvania: Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
8. Rincon S, Celis A, Sopo L, Motta A, Cepero de Garcia MC. Malassezia yeast species isolated from patients with dermatologic lesions. *Biomedica*. 2005;**25**(2):189–95. [PubMed: 16022373].
9. Serlak M, Civic M, Zia M. [The frequency of Malassezia yeast in healthy people and patients with seborrheic dermatitis]. *Res Anal J Aligudarz Nurs Fact*. 2012;**3**((4 and 5)):105–11. Persian.
10. Hedayati M, Haji Esmail H, Ehsani A, Haj Heidari Z, Shokoohi T, Mohammadpour RA, et al. [The effect of ketoconazole 2% solution in comparison with ketoconazole 2% shampoo on clinicalsigns and Malassezia yeasts in seborrhoeic dermatitis patients]. *J Mazandaran Univ Med Sci*. 2008;**18**(67):7–16. Persian.
11. Nakabayashi A, Sei Y, Guillot J. Identification of Malassezia species isolated from patients with seborrhoeic dermatitis, atopic dermatitis, pityriasis versicolor and normal subjects. *Med Mycol*. 2000;**38**(5):337–41. [PubMed: 11092380].
12. Klarostaghi A, Hajheydari Z, Hedayati M, Shokohi T. [Investigating the presence of malassezia in acne lesions referring to the dermatology clinic of Bouali Sari Hospital and assessing the level of drug sensitivity of isolated species to ketoconazole, miconazole and clothrimazole]. *J Mazandaran Univ Med Sci*. 2011;**21**(81):11–9. Persian.
13. Shemer A, Kaplan B, Nathansohn N, Grunwald MH, Amichai B, Trau H. Treatment of moderate to severe facial seborrheic dermatitis with itraconazole: An open non-comparative study. *Isr Med Assoc J*. 2008;**10**(6):417–8. [PubMed: 18669136].
14. Yurayart C, Nuchnoul N, Moolkum P, Jirasuksiri S, Niyomtham W, Chindamporn A, et al. Antifungal agent susceptibilities and interpretation of Malassezia pachydermatis and Candida parapsilosis isolated from dogs with and without seborrheic dermatitis skin. *Med Mycol*. 2013;**51**(7):721–30. doi: 10.3109/13693786.2013.777165. [PubMed: 23547880].
15. Khazaiyan S, Navidian A, Navvabi Rigi S. [The effect of oral Aloe vera gel on the intensity of primary dysmenorrhea]. *Med Surg Nurs J*. 2012;**1**(1):49–54. Persian.
16. Jalal Pour SH, Kasra Kermanshahi R, Nouhi AS, Zarkesh Isfahani H. [Comparing the frequency of â-lactamase enzyme in isolated nosocomial infectious bacteria]. *Zahedan J Res Med Sci*. 2010;**12**(4):3–10. Persian.
17. Mohebi M, Alizadeh Behbahani B, Ansari Far E, Nowshad M. [Antimicrobial effect of aloe vera and chitosan in vitro]. *Iran J Infect Dis Trop Med (IJIDTM)*. 2014;**67**:21–9. Persian.
18. Kasim LS, Olatunde A, Effedua HI, Adejumo OE, Ekor M, Fajemirokun TO. Antimicrobial activity of six selected plants against some strains fof pathogenic organisms. *J Microbiol Antimicrob*. 2012;**4**(3). doi: 10.5897/jma11.013.
19. Nejatzadeh-Barandozi F. Antibacterial activities and antioxidant capacity of Aloe vera. *Org Med Chem Lett*. 2013;**3**(1):5. doi: 10.1186/2191-2858-3-5. [PubMed: 23870710]. [PubMed Central: PMC3729540].