

***In-vitro* inhibitory effect of ethanolic and methanolic extract of *Scrophularia striata* on *Candida* spp.**

Mandana Ahmadi^{1*}, Hossein Mahdavi¹, Mahboobeh Madani², Zohreh Hadadi¹

¹Research and Development Department, Sinafaravar pharmaceutical Company, Najaf abad, Isfahan, Iran

²Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran

Received: Aug 24, 2016, Revised: Nov 2, 2016, Accepted: Jan 14, 2017

Abstract

Candida species are the most common cause of opportunistic fungal infection worldwide. Due to increasing resistance of fungi against conventional drugs, as well as their side effects, alternative natural products have become a renewed interest. In the current study, the anti-*Candida* activities of *Scrophularia striata* extract were examined. In this experimental study, microdilution assay and well diffusion test were used to determine *in vitro* anti-fungal effects of ethanolic and methanolic extract of *Scrophularia striata* on four species of *Candida* including *Candida albicans* ATCC 1167, ATCC 1677, *tropicalis*, and *glabrata*. The results showed that 100 mg/ml methanolic extract has more anticandida activity than ethanolic extract. Diameter of inhibiting growth environment for ethanol extract was 37 mm, and MIC and MFC were 2.1 and 41.5 mm/ml. Results show that ethanolic and methanolic extract of *Scrophularia striata* have anti-fungal activities. Therefore results of this study suggest this extract is a promising anti-fungal agent, and future experiments will interest for performing *in vivo* study.

Keywords: *Scrophularia striata*, *Candida*, ethanolic extract, methanolic extract

Pharm Biomed Res 2016; 2(4): 38-43

Introduction

In the past two decades, the prevalence of candidiasis has been increased. *Candida* species are able to create superficial and systemic infections (1). *Candida albicans* is an opportunistic pathogen, causing mycoses in immunocompromised patients as well as long-term antibiotic users (2-4). Also, other *Candida* species such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* are among the oral mucosal lesions suspected agents in AIDS patients (4-6). Medicinal plants may demonstrate a valuable, original source of recent antifungal drugs and have been shown to contain diverse biochemical and pharmacological actions (7, 8). Their antifungal activity has been showed against some *Candida* species (9-11).

One of the plants proposed to have immunomodulatory and anti-inflammatory effects is *Scrophularia striata* Boiss (Scrophulariaceae). *Scrophularia striata* (in the Scrophulariaceae family) is vernacular to Iran.

Scrophularia striata color is purple (12-14). Many *Scrophularia* species have been investigated and several compounds from different classes of secondary metabolites including iridoids, phenyl propanoids, phenolic acids, flavonoids, quercetin, isorhamnetin 3-Orutinoside and saponins by column chromatography have been isolated (15). These compounds usually are found in root, leaves, or buds of the plant (16, 17). *Scrophularia striata* traditionally has been used for infections, high blood pressure, and stomach disorders (18-20).

previous studies have suggested the inhibitory effect of *S. striata* extract on microorganisms (21, 22). In addition, anti-inflammatory, and immunomodulatory activity of some species of *Scrophularia* have been showed (23, 24). In the present study, we investigated the *in vitro* inhibitory effects of *Scrophularia striata* ethanolic and

*E-mail: mandana.ahmadi84@gmail.com

metanolic extract on including *Candida albicans* ATCC 1167, ATCC 1677, *tropicalis*, and *glabrata*.

Materials and method

This is an experimental study conducted on *Candida albicans* ATCC 1167, ATCC 1677, *tropicalis*, and *glabrata*. *Scrophularia striata* was collected in 2013 May from Zagros mountain in Ilam, Iran and was detected by research center of natural resources in herbarium department in Isfahan.

All part of the plant were washed and dried. Then areal parts of plants such as stalk and leaves were powdered by electric blender. Themaceration method was used to make alcoholic extract. Then, 50 g was macerated with 100 mL of 80% ethanol and methanol on a rotary shaker for 48 and 72 hours, filtered, and then the solution. After, the extracts were taken and filtered by using a millipore filter paper. Then, the extracts were concentrated using a rotary evaporator at 40 °C under reduced pressure. Finally, the extracts were dried and stored at 4 °C till their usage in the different tests. The extracts were dissolved in dimethylsulfoxide (DMSO).

Herbal component can be dissolved in methanol, ethanol, percolation or maceration can be used to extract them. The advantage of alcohol extraction is that different alkaloids or alkaline salts can be suit, in addition to the water-soluble impurities such as polysaccharides, proteins are less extracted but its drawback is more fat-soluble impurities is extracted.

Antifungal activity was studied by the agar well diffusion method. Sabouraud dextrose agar (SDA) was used as the fungal medium. The effect of extract antimicrobial were assessed in the concentration include 3.14, 6.25, 12.5, 25, 50, and 100 (mg/ml) using agar well diffusion method and also by determination minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) using microdilution test method. The extracts were diluted in 1mL of dimethylsulphoxide (DMSO) at the concentrations of 3.14, 6.25, 12.5, 25, 50, and 100 (mg/ml) sabouraud dextrose agar. Petridishes (8 cm diameter) containing 20 ml of SDA were used for antifungal activity assay, performed on solid media by the well diffusion method sterile. Wells were prepared in the seeded agar petridishes. The study compound was introduced in the well (6 mm). The plate were incubated at 37 °C for 24- 48 h. Growth inhabitation of each fungal strains was counted as the percentage of prevent of radial grow relative to the

control, fluconazol. A standard antifungal agent, fluconazol served as a positive control and DMSO was used as a negative control. To identify the minimum inhibitory concentration (MIC) microdilution method was used. In this experiment microplates of 96 cells were used in the first row wells 100 microliter of cultivation medium of SDA were put, then 100 microliter of the extract of the plant was added to the first well. Then 100 microliter of extract was added to the second wells and it was repeated until the stage in which 10 microliter of ferment suspension containing 1000 cells in each milliliter was added to the same well. In each row Alcohol with medium culture and ferment served as negative control and stril physiologic serum with culture medium and ferment served as positive control (24, 25).

Statistical analysis

The experiment was performed three times to minimize the error and the mean values are presented. Data were analyzed using ANOVA test in the $p < 0.05$ (SPSS Software, Chicago, USA)

Results

The growth inhibition value of ethanolic and methanolic extracts of *S.striata* on *Candida albicans* ATCC 1167, ATCC 1677, *tropicalis*, and *glabrata* in tables 1 and 2 are shown.

The strongest activity was seen against *C.glabrata* with a range of 41.3 mm mean inhibition zones and 15.62-21.87 mg/ml MIC values. The MIC and MFC of these extracts are currently being obtained in table 3. The methanolic extract of the tested plant has more anti-candida effects as compared to the ethanolic extract. Moreover, the results showed that 100 mg/ml methanolic extract has more anti-candida activity.

Discussion

Different studies have indicated that the many species of *Scrophularia* contains substances that have antimicrobial activities (26-29). Results obtained in pervious study show that *Scrophularia striata* extracts have selective antimicrobial activity on the basis of the cell-wall differences of bacterial and fungal microorganisms (30, 31). In recent years, tendency of using natural sources as alternative medicine has been raised (32).

Table 1 The antifungal activity of methanolic extract of *S. striata* on *Candida* spp*

Concentration Fungi	3. 12 mg/ml	6. 25 mg/ml	12. 5 mg/ml	25mg/ml	50mg/ml	100 mg/ml
<i>Candida albicans</i> (1167)	3.66 ± 1.52	12.33 ± 1.50	19.8 3 ± 1.25	26.50 ± 1.80	37 ± 2	39.5 ± 0.5
<i>Candida albicans</i> (1677)	4.66 ± 2.08	11.83 ± 1.60	22.16 ± 1.89	25.16 ± 1.60	28.8 ± 0.7	34.6 ± 4
<i>Candida tropicalis</i>	3.00 ± 0.30	12.00 ± 3.40	18.23 ± 23.00	24.50 ± 3.91	28.8 ± 2.0	34.6 ± 2.4
<i>Candida glabrata</i>	3.00 ± 2.00	13.86 ± 0.80	18.50 ± 0.50	28.00 ± 1.00	37.3 ± 2.0	41.3 ± 3.0

* Data present as mm (Mean ± SD)

Table 2 The antifungal activity of ethanolic extract of *S. striata* on *Candida* spp*

Concentration Fungi	3. 12 mg/ml	6. 25 mg/ml	12. 5 mg/ml	25mg/ml	50mg/ml	100mg/ml
<i>Candida albicans</i> (1167)	-	3.16 ± 0.28 mm	7.16 ± 0.28 mm	12.00 ± 0.50 mm	18.23 ± 0.76 mm	25.33 ± 4.33 mm
<i>Candida albicans</i> (1677)	-	4.83 ± 0.73 mm	10.66 ± 1.15 mm	13.20 ± 1.31 mm	29.9 ± 1.8 mm	26.8 ± 1.15 mm
<i>Candida tropicalis</i>	-	7.83 ± 1.04 mm	10.50 ± 0.80 mm	18.66 ± 1.60 mm	33.3 ± 1.5 mm	28.8 ± 3.7 mm
<i>Candida glabrata</i>	1.33 ± 1.52 mm	13.66 ± 3.51 mm	15.16 ± 2.56 mm	24.5 ± 3.77 mm	30.8 ± 1.0 mm	37.3 ± 2.0 mm

* Data present as mm (Mean ± SD)

Table 3 Determination of MIC and MFC of methanolic and ethanolic extract for *Candida* spp*

Extracts	MFC	MIC
Ethanolic	43/75 ± 0.63	21/87 ± 0.59
Methanolic	31.25 ± 0.71	15.62 ± 0.64

* Data present as mg/ml (Mean ± SD)

Plant-based drugs are promising agents for treatment of diseases. (33). *Scrophularia* as a member of Scrophulariaceae has been found to possess antibacterial, antiprotozoal, antitumor, anti-inflammatory, and diuretic activities and have been used in the treatment of mental, nervous and gastrointestinal conditions (34, 35). *S. striata* is traditionally used for treatment of infectious diseases. Alcoholic extracts from aerial parts of *S. striata* have higher antimicrobial activity than that of aqueous extract (36). *S. striata* extract was shown to have antiseptic effects in treating infections caused by gram positive and negative bacteria, fungi and virus (37-39). One of the reasons in treatment of infection due to the effects of flavonol and flavonoid compounds of the *S. striata* extract in combination with cell wall structures or extracellular proteins (40).

A few studies conducted on the essential oils of species of this genus and its family revealed a significant chemical diversity. However, some compounds such as anethole, anisaldehyde, eugenol, benzaldehyde, eugenol acetate are common in the *Scrophularia*. On the other hand, the presence of aromatic compounds in various genera of the *Scrophulariaceae* is one of the characteristics of this family (41, 42). Also they have been known to be rich in iridoid glycosides, mainly aucubin and catalpol. Iridoids represent a large group of cyclopentan-[c]-pyran monoterpenoids occurring as constituents of sympetalous plants including ornamental as well as wild ones (43, 44). In this study *in vitro* inhibitory effect of ethanolic and methanolic extract of *Scrophularia striata* on *Candida* spp. have been studied. As it was expected, ethanolic and methanolic extract of *Scrophularia striata* had inhibitory effects on *Candida* and this effect was increasing by extract concentrations. The increasing public and economic implications caused by fungi means there is a stable fighting to produce safer food crops and to develop new antifungal agents (19). Plant extracts and essential oils are potential sources of novel antimicrobial compounds against bacteria and fungi pathogens. In traditional medicine *Scrophularia striata* has been used for wound disinfection. On the other hand, *Scrophularia* is an antipyretic and it is used for treatment of kidney disorders and pulmonary cancers (21). Plants have severe ability to synthesize phenolic products. These products are secondary

metabolites and serve as plant defense mechanisms by microorganisms (12). Their activity could be due to their ability to complex with extracellular and soluble proteins (15). Thus, previous reports have demonstrated that: saponins, tannins, alkaloids, terpenes, carotenoids, and flavonoids possessed antifungal activity (18). Antimicrobial flavonoids have multiple cellular targets, instead of one specific site of action. Alternatively, these common structural features may simply be necessary for flavonoids to gain presence to or uptake into the microbial cell. It may be that flavonoids are not killing microbial cells but singly inducing the formation of microbial aggregates and thereby reducing the number of CFUs in viable counts (41).

Our study is in agreement with several reports (41). Various investigations demonstrated antifungal effects of *Scrophularia striata* extract. Based on Pirbalouti et al. *Scrophularia striata* water extract in different concentrations (10 to 15 mg) had anti-candida effects on *Candida albicans* (25). Bahraminejad et al. (45) showed that many Iranian plants like *Scrophularia striata* had antifungal role on *Fusarium Oxysporum* and *Rhizoctonia* (23). GhasemiPirbalouti et al. determined anti-candida activity of some of the Iranian medicinal plants. The extracts from different plant species studied showed antifungal activities, with the diameters of inhibition zone ranging from 7 to 46 mm. The most active of the concentration was high concentrations (50-55 µL) inhibiting the growth of yeast. Extracts of *Scrophularia striata* showed antifungal activity against *Candida albicans*. Among the plants tested essential oil the extracts of *Scrophularia striata* and *Ziziphus spinachristi* showed the best anti-*Candida* activity (22).

Bahmani et al. showed MIC of *Scrophularia deserti* ethanolic extract in dose of 480 mg/ml on *Saprolegnia parasitica* was 61% and for Formalin standard control was 57%, which it demonstrates antimicrobial effects of *Scrophularia deserti* (46). Other study showed antimicrobial effects of *Scrophularia striata* ethanolic extract on *E. coli*, in both agar diffusion and microdilution methods. Its ethanolic extract showed MIC and MBC as 90 mg/ml and 100 mg/ml, respectively, but water extract had no anti-microbial activity (47). Moreover, *Scrophularia striata* extract had anti-viral properties (48). Anti-inflammatory and antibiotic properties of *Scrophularia striata* reported

in many studies (49). It is suggested that *Scrophularia striata* extract could be used as an anti-inflammatory agent. Additionally, it could inhibit Helminth in gastro intestinal tract disorder patients (50). Many studies showed *Scrophularia striata* extract have inhibitory effect on various microorganisms and in some studies this extracts have better effects in comparison with antibiotics. However, research on the anti-fungal and anti-bacterial effects of *Scrophularia striata* are in the early stages, and most reports about this plant are based on anti-bacterial, anti-viral and anti-inflammatory traits. This explains the use of this plant in folk medicine for the treatment of various diseases whose symptoms might involve fungal infections. Future phytochemical research is needed to identify the active principles responsible for the antifungal effects of this medicinal plant.

Conclusion

In vitro antifungal activity of the methanolic and ethanolic extract of *Scrophularia striata* on fungi was tested. The methanolic and ethanolic extract of *Scrophularia striata* showed antifungal activity against *Candida albicans* ATCC 1167, ATCC 1677, *tropicalis*, and *glabrata*. Major investigations should be performed on a wider range of fungi in order to determine which component of the extract shows the most potent antifungal activity. The antifungal activity could also be a result of the synergism of its components.

Conflict of Interest

The authors declared no conflict of interest.

References

1. Maryin GS, Mannino DM. The epidemiology of sepsis in the united states from 1979-2000. *N Engl J Med.* 2003;348:1546-54.
2. Eggimann P, Garbino J, Pittet D. Management of candida species infection in critically ill patients. *Lancet Infect Dis* 2003;3:772-85.
3. Patterson TF, Revanker SG, Kirkpatrick WR, Dib O, Fothergill AW, Redding SW, et al. Simple method for detecting Fluconazol-Resistant Yeast whit chromogenic Agar. *J Clin Microbiol* 1996;34:1794-97.
4. Avijigan M, Saadat M, Nilforoosh-zade MA, Hafezi M. Anti-Fungal effect of Echinophoraplatyloba extract on some common dermatophytes *J Med Plan* 2006;2:10-16.
5. Troillet N, Durussel C, Bille J, Glauser MP, Chave JP. Correlation between in vitro susceptibility of candida albicans and fluconazole-resistance oropharyngeal candidiasis in HIV-infected patients. *Eur J Clin Microbiol Infect Dis* 1993;12: 911-5.
6. Khosravi A, Malecan M. Effects of lavandula toechas extracts on staphylococcus aureus and other gram negative bacteria. *J Qazvin Univ Med Sci* 2004;7:3-9.
7. Bannerman RH, Barton J, wen C. Traditional medicine and health care coverage, UK:MAC. Millans/spoottis wood 1993;99-100
8. Appleton SS. Candidiasis: pathogenesis, Clinical Characteristics, and Treatment. *J Calif Dent Assoc* 2000;28:442-8.
9. Borris RP. Natural Products research: perspectives from a major pharmaceutical company. *J Ethnopharmacol* 1996;51:29-28.
10. Zargari A. Medical plants. 7th ed. Tehran: Tehran university pub.1997.
11. Velg J, Stodo L. Medical plants. Tehran: Ghoghnoos 1991.
12. Lersten NR, Curtis JD. Anatomy and distribution of foliar idioblasts in *Scrophularia* and *Verbascum* (scrophulariaceae). *Am J Bot* 1997;84:1638.
13. Ghahreman A. Flora's color of Iran. RIFR1975-1999;1-20.
14. Mozaffarian V. A Dictionary of Iranian Plant Names. Tehran: FarhangMo'aser. 1999.
15. Chalabian F, Norouzi H, Mossavi S. A study of growth inhibitory effect of essential Oils of seven species from different families on some kinds of microbes. *J Med Plan* 2003;3:37-42.
16. Shamsa F, Monsef H, Ghamooshi R, Verdian-rizi M. Spectrophotometric determination of total alkaloid in some Iranian medical plants. *Thai J Pharm Sci* 2008;32:17-20.
17. Tasdemir D, Burn R, Franzblau SG, Sezgin Y, Calis I. Evaluation of antiprotozoal and antimycobacterial activities of the resinglycosides and the other metabolites of *Scrophularia Cryptophila*. *Phytomedicine* 2008;15:209-15.
18. Sherafati-Chaleshtori F, Sherafati-Chaleshtori R, Momeni M. Antimicrobial effect of aqueous and ethanolic extracts of *Scrophularia* (*Scrophularia striata*) on *E. coli* in vitro. *J SKUMS* 1998;32-7.
19. Abbasi N, AziziJalilian F, Abdim M, Saifmanesh M. A Comparative Study of the antimicrobial effect of *Scrophularia striata* Boiss: extract and selective antibiotics against staphylococcus aureus and *Pseudomonas aeruginosa*. *J Med Plan* 2007;1:10-8.
20. Bahmani M, Eftekhari Z . An ethno veterinary study of medicinal plants in treatment of diseases and syndromes of herd dog in southern regions of Ilam province, Iran. *Comp Clin Pathol* 2013;22:403-40
21. Ahmed B, Al-Rehaily AJ, Al-Howirini TA, El- Asyed kA, Ahmad MS. *Scrophularia* D2 and Harpagoside-B: Two new iridoid glycosides and their anti diabetic and anti-inflammatory activity. *Biol Pharm Bull* 2003;462-7.
22. Pirbalouti AG, Bahmani M, Auijigan M. Anti-Candida activity of some of Iranian medical plants cultivated in Iran. *E J Bio* 2009;5:85-8.
23. Bahrminejad S, Abbasi S, Fazeli M. In vitro antifungal activity of 63 Iranian plant species against three different plant species against three different plant pathogenic Fungi. *Afr J Biotechnol* 2011;10:16193-201.

24. Bahmani M, Zamani P, Raeisee M, Bahmani F, MohebnasabM, Alizadeh N. Effect of anti saprolegniaparasitica of wild snapdragon (*Scrophulariadeserti*) in comparison with formalin. Proceedings of the first national congress of economic diseases of rainbow trout. Shahrekord, Iran: Islsmic Azad University of Shahrekord 2009;68.
25. Hajiaghaee R, Monsef-Esfahani HR, Khorramizadeh MR, Saadat F, Shahverdi AR, Attar F. Inhibitory effect of aerial parts of *Scrophularia striata* on matrix metalloproteinases expression. *Phytother Res* 2007;21:1127-9.
26. Vahabi S, Najafi E, Alizadeh S. In vitro antimicrobial effects of some herbal essences against oral pathogens. *J Med Plants Res* 2011;5:4870-78.
27. Stavri M, Mathew KT, Gibbons S. Antimicrobial constituents of *Scrophularia deserti*. *Phytochem* 2006;67:1530-33.
28. Fernandez MA, Garcia MD, Saenz MT. Antibacterial activity of the phenolic acids fractions of *Scrophulariafrutescens* and *Scrophularia asambucifolia*. *J Ethnopharmacol* 1996;53:11-4.
29. Azadmehr A, Afshari A, Baradaran B, Hajiaghaee R, Rezazadeh S, Monsef-Esfahani H. Suppression of nitric oxide production in activated murine peritoneal macrophages in vitro and ex vivo by *Scrophularia striata* ethanolic extract. *J Ethnopharmacol* 2009;124:166-9.
30. Cosentino S, Tuberoso CIG, Pisano B, Satta M, Mascia V, Arzedi E, Palmas F. In vitro antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. *Lett Appl Microbiol* 1999;29:130-135.
31. Karaman I, Sahin F, Gulluce M, Ogutcu H, Sengul M, Adiguzel A. Antimicrobial activity of aqueous and methanol extracts of *Juni- perusocycedrus L.* *J Ethnopharmacol* 2003;85:231-5.
32. Ozaslan M, Didem Karagoz I, Kalender ME, Kilic IH, Sari I, Karagoz A. *In vivo* Antitumoral Effect of *Plantago major L.* Extract on Balb/C Mouse with Ehrlich Ascites Tumor. *Am J Chin Med* 2007;35:841-51.
33. Zamanian-Azodi M, Rezaie-Tavirani M, Heydari-Kashal S, Kalantari S, Dailian S, Zali H. Proteomics analysis of MKN45 cell line before and after treatment with Lavender aqueous extract. *Gastroenterol Hepatol Bed Bench* 2012;5:35-42.
34. Sabzevari O, Hosseini A, Paydar H, Monsef-Esfahani H-R. Hepatoprotective activity of *Scrophularia striata* against acetaminophen-induced liver injury in mice. *Toxicol Lett* 2008;6:634.
35. Mosaddegh M, Naghibi F, Moazzeni H, Pirani A, Esmaili S. Ethnobotanical survey of herbal remedies traditionally used in Kohghiluyehva Boyer Ahmad province of Iran. *J Ethnopharmacol* 2012;141:80-95.
36. Azadmehr A, Afshari A, Baradaran B, Hajiaghaee R, Rezazadeh S, Monsef-Esfahani H. Suppression of nitric oxide production in activated murine peritoneal macrophages *in vitro* and *ex vivo* by *Scrophularia striata* ethanolic extract. *J Ethnopharmacol* 2009;124:166-9.
37. Bahmani M, Qorbani M, Momtaz M, Bahmani E, Rafieian M. The comparison of the in-vitro effects of *Scrophularia deserti* plant and amphotricin B on *Candida albicans*. *Arak Uni Med Sci J.* 2011;13:15–21.
38. Azadmehr A, Afshari A, Baradaran B, Hajiaghaee R, Rezazadeh S, Monsef-Esfahani H. Suppression of nitric oxide production activated murine peritoneal macrophages *in vitro* and *ex vivo* by *Scrophularia striata* ethanolic extract. *J Ethnopharmacol* 2009;124:166-9.
39. Sharafati-Chaleshtori R, Rafieian-Kopaei M. Screening of antibacterial effect of the *Scrophularia striata* against *E. coli* in vitro. *J Herb Med Pharmacol* 2014;3:31-4.
40. Mahboubi M, Kazempour N, Nazar ARB. Total phenolic, total flavonoids, antioxidant and antimicrobial activities of *Scrophularia striata Boiss* extracts. *Jundishapur J Nat Pharm Prod* 2013;8:15-9.
41. Tim Cushnie TP, Andrew J L. Antimicrobial activity of flavonoids. *Int J Antimicrob Agents* 2005; 26:343-56.
42. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants. CSIR 1998;435.
43. Ardeshiryajimi A, Barzegar M, Rezaei-Tavirani M, Hashemi M, Heidari S, Moghadamnia S H, et al. Effects of *Scrophularia striata* extract on human fibroblast cells. *Med Sci J* 2009;19:168-72.
44. Kambizi L, Afolayan AJ. Extracts from *Aloe ferox* and *With aniasomnifera* inhibit *Candida albicans* and *Neisseria gonorrhoea*. *Afr J Biotech* 2008;7:12-5.
45. Baron EJ, Finegold SM. Methods for testing antimicrobial effectiveness, *Bailey & Scott's diagnostic Microbiology.* 8th ed. New York: Mosby Company 1990.
46. NCCLS document M27-A-Reference Method for broth dilution antifungal susceptibility testing of yeasts Approved 1977;17:1-29
47. Bahmani M, Ghorbami M, Momtaz H, Bahmani E, Rafieian M. The comparison of the in- vitro effects of *Scrophularia striata deserti* plant and amphotricin B on *Candida albicans*. *Arak Med Uni J* 2011;13:15-21.
48. Bahrami AM. Pathology of worm infestation in ovine and its treatment with two different plants extraction. *Afr J Biotech* 2011;10:14608-17
49. Bahrami AM, Andvaladi A. Effects of *Scrophularia striata* Ethanolic leaves Extract on *Staphylococcus aureus*. *Int J Pharmacol* 2010;6:431-4.
50. Bahrami AM. the effectiveness of *Scrophularia striata* on Newcastle disease, *AJBAS* 2011;5:2883-88.