Caspian J Reprod Med, 2015, 1(1): 26-30

Caspian Journal of Reproductive Medicine

Journal homepage: www.caspjrm.ir

Original article

A comparative study of antifungal activity of Lavender brew, Lavender essential Oil, and Clotrimazole: an in vitro study

Fereshteh Behmanesh [1], Hajar Pasha [2,*], Seyyed Ali Asghar Sefidgar [3], Aliakbar Moghadamnia[4], Zahra Basirat [5]

[1]Department of Midwifery, Babol University of Medical Sciences, Babol, Iran

[2] Infertility and Reproductive Health Research Center, Health Research Institute & Department of Midwifery, Babol University of Medical Sciences, Babol, Iran

[3] Department of Mycology and Parasitology, Babol University of Medical Sciences, Babol, Iran

[4] Department of Physiology and Pharmacology, Babol University of Medical Sciences, Babol, Iran;

[5] Infertility and Reproductive Health Research Center, Department of Obstetrics and Gynecology, Babol University of Medical Sciences, Babol, Iran

Received: 6 Sep 2014 Accepted: 9 Feb 2015

Abstract

Background: Vaginal yeast infections (candidiasis) are among the most common gynecologic infections in women. Due to the high prevalence of vaginal candidiasis and its recurrence, followed by such chemical treatment as Clotrimazole, this study was performed with the broad aim of comparing the in vitro effects of Lavender brew, Lavender essential oil, and Clotrimazole on the growth of the standard strains of Candida albicans.

Methods: The fungus cell count was done through Thoma counting chambers and Hemocytometer slide. Having prepared the dilution (6 × 106 of standard Candida albicans, S.C.a-PTCC-2657) in the Sabouraud Agar liquid medium, the Lavender essential oil, brew and Clotrimazole were added to different dilutions (½ , ¼ , ⅛) (in 4 stages) before the fungus cell count was done. Having obtained the necessary information, the data were analyzed through Statistical Package for the Social Sciences (SPSS), and a general linear model was used for the analysis of the data. The test results were then compared.

Results: The number of fungi cells in Lavender brew (14 × 106) and Lavender essential oil (35× 106) decreased significantly compared with those of Clotrimazole (93 × 106) and fungus control (188 × 106) ($p<0.01$). Also, the fungus cell count in dilutions of ½ and ¼ of the drugs in the brew, and in dilutions of ⅛ in essential oil were less than those of other proportions. The highest antifungal efficacies of the drugs were observed in higher dilution ($p< 0.01$), and Clotrimazole had the least antifungal effect.

Conclusion: Lavender brew and Lavender essential oil had more antifungal effect on the standard Candida albicans when compared with Clotrimazole.

Keywords: Antifungal activity, Clotrimazole, Lavender

Introduction

Gynecologic infections are among the most common reasons for which women refer to physicians. One of the diseases caused by underlying conditions of endogenous vaginal flora is candidacies (1). Candida species, notably Candida albicans, is the major fungal pathogen in humans. Despite all the breakthroughs in medical science as well as the advent of new medical methods, the severity and the incidence of these

***Corresponding author:** Hajar Pasha, Department of Midwifery, Babol University of Medical Sciences, Ganjafroz, Babol, Iran, Tel: +98-11-32199593, Email: pashahajar@yahoo.com

infections have dramatically experienced a rise in recent years, which could mainly be due to the increased number of such immune-suppressed cases as HIV-Aids, cancer, diabetes, the use of broad spectrum antibiotics, cytotoxic chemotherapy, and organ transplantation (2, 3). It is also worth mentioning that such an increase in the incidence of opportunistic severe fungal infections has greatly motivated the interest not only for in vitro antifungal susceptibility testing, but also for the diagnosis and the treatment of fungal infections, resistant to common antifungal agents (4). On the other hand, there is a new tendency to use supplementary medicine, like herbal medicine, for medical care, which has recently gained popularity (5). It is evident that most of the natural agents, especially those extracted from plants, are used against different diseases, and are more effective with fewer side effects (6). Although there is a dearth of adequate research on herbal medicine, the history and the use of such medicinal plants during the past few centuries, as well as the naturalness of such products can, to a great extent, prove their relative safety. Therefore, it is highly recommended to use them in the case of chronic diseases, or for the prevention of such diseases due to their limited side effects and low costs (7). Lavender, as a kind of medicinal herb, can be effectively used in the treatment of vaginal discharges, and has a wide number of applications in supplementary medicine. Investigations conducted on lavender in both Iran and Italy have proved the fact that this medicinal herb has antifungal effects on different fungal species (8-9). Clotrimazole is one of the commonly known chemical drugs, used for the treatment of vulvovaginitis candidasis, but the use of this chemical drug can bring about such side effects as elevated liver enzymes, hepatotoxic effects, painful urination, depression (due to systemic drug absorption effects), and irritation and burning sensation, or contact dermatitis. The increasing resistance to commonly used antifungal agents necessitates the need for more investigations for new formations (4, 10). To this end, this study was conducted with the broad aim of comparing the in vitro effects of Lavender brew, Lavender essential oil, and Clotrimazole on the growth of the standard strains of Candida albicans.

Materials and Methods

This quasi-experimental study, performed based on the fungus cell count in liquid medium through Thoma hemocytometer slide (micro dilution method), was an attempt to determine the antifungal effects of lavender. The micro dilution method, one of the antifungal sensitivity standard tests, is used as a preferred method for determining antifungal sensitivity in vitro (11).

This method was done in 4 consecutive stages. In the first stage, with regard to the sterile environment, the researchers took just a needle-point-size amount (fildoplatin) of fungus from the Candida albicans pure medium (S. C. a-PTCC-2657), cultured it in three sabouraud slant, solid medium as deep and superficial, and then let it incubate at 37°C for 48 hours (the purpose for the use of pure medium in this tube was due to its numerous fungus pure culture, needed in the subsequent phases of the study). In the second stage, candida, with dilution of (6×10^6), was prepared in sabouraud liquid culture medium. To do so, candida was first taken from fungus sample by a sterile circular loop, and then it was added to intosabouraud liquid medium. After shaking the mixture, the sample was taken by a sterile syringe, and the cells were counted by Thoma slide. If the amount of fungi in liquid medium was more than the considered dilution range, the diluting process would continue until the desired dilution was reached (6×10^6) (Cells were counted by Thoma hemocytometer slide, and the sabouraud liquid medium was used to dilute the medium). The produced dilution was used as fungus control in all stages of observing the effects of lavender brew, lavender essential oil, and Clotrimazole drop. The preparation of the considered drug was the aim of the third stage. It should be mentioned here that all the ready-used drugs, except for the lavender brew, were taken from the pharmaceutical company. The local Clotrimazole drop (1%) was made in Behvazan Pharmaceutical Company, and the lavender essential oil was prepared in Golkaran Company. Also, 15 gr of dried lavender flowering petals was added to 500 cc of boiling distilled water, then after letting it cool for10- 15 minutes, some of the lavender brew was poured into some sterile test tubes using a filter under sterile conditions. In the fourth stage, the following 10 sterile tubes were prepared: Candida albicans pure medium (S. C. a-PTCC-2657), Control fungus with dilution of (188×10^6) ml, and Control drug, control fungus+ sabouraud.

In this study, the researchers made an attempt to prepare a total number of six test tubes in three pairs, which are as follows: two test tubes, containing drug with dilution of ½ + fungus with dilution of (6×10^6) + sabouraud, two other test tubes. Containing drug with dilution of ¼+ fungus with dilution of (6×10^6) + sabouraud, and still two other ones, containing drug with dilution of⅛+ fungus with dilution of (6×10^6) + sabouraud. All the tubes were kept in an incubator at 37°C for 48 hours, and after that the fungal cells were counted by Thoma hemocytometer slide. The tests for each drug were repeated at least twice for each dilution. The sample specifications and the experimental results were regularly recorded in the observation sheet, and were subsequently analyzed by descriptive statistics and frequency distribution tables. Having obtained the necessary information, the data were analyzed by SPSS through descriptive-analytical statistics, and GLM univariate test.

Table 1. Medium of fungi cells in different drugs

Drugs	Medium of fungi cells
Lavender brew	$14\times10^{6\,a}$
Lavender essential oil	$35\times10^{6\,b}$
Clotrimazol	$93\times10^{6\,c}$
Fungi Control	$188\times10^{6\,d}$

*Different letters represent statistical difference between different groups (p< 0.001)

Results

The findings of this study showed that the number of fungus cells in lavender brew (14×10^6) and lavender essential oil (35×10^6) dropped significantly in comparison with those of fungi control (188×10^6) and Clotrimazole (93×10^6) (p= 0.001). The results also demonstrated that the fungi cell counted by Thoma hemocytometer slide in drugs with dilution of ½ was 23×10^6 in lavender brew, and that it had the least fungi cell counts when compared with lavender essential oil (50×10^6) and Clotrimazole (120×10^6). It is also worth mentioning that in dilution of ¼, the lavender brew, and in dilution of ⅛, the lavender essential oil had the least fungi cell counts and the highest antifungal efficacy when they were compared with other proportions (Figure 1). The evaluation of the mentioned drugs in different dilutions of (½, ¼, ⅛) illustrated that the highest antifungal efficacy (less fungi cell counts) were in higher dilutions of drugs, for which the statistical difference (p=0.005) was significant. Hence, the fungi cells in lavender essential brew in dilutions of (½, ¼, ⅛) were 23×10^6, 16×10^6, and 3×10^6, respectively. Therefore, it can be claimed that the fungi cells were the least in dilution of ⅛. Also, fungi cell counts in lavender oil, in different dilutions of ½ , ¼ , and ⅛, were 50×10^6 , 56×10^6 , and 0.25×10^6 respectively, which was similar to the results of lavender essential brew. This result can further suggest that the least fungi cell counts were in the highest dilution of the drug (⅛) (Table 1).

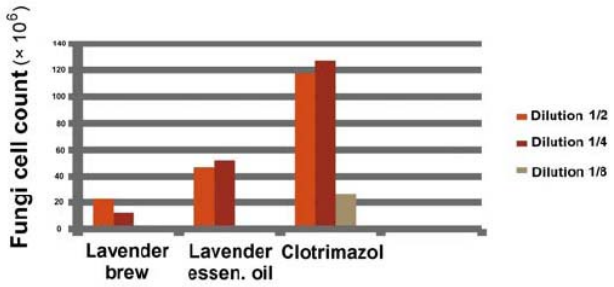

Fig 1: The effect of various dilution of Brew lavender, Essential oil lavender, Clotrimazol , and control fungus on the number of fungi cells in liquid medium through Thoma hemocytometer slide (Microdilution Method) (p<.005).

Discussion

The results of this study showed that lavender has antifungal effects. There are many research studies, reporting mixed results, about the antifungal effects of lavender. One study, for instance, demonstrated that lavender oil had considerable antifungal effects on candida albicans; whereas, another one reported that lavender oil had very weak inhibitory effects (12-13). In the same line, another similar study indicated that lavender essential oil had in vitro antimicrobial activity against bacteria, fungi and some insects, and that it delayed the production of spore in filamentous fungi, and completely inhibited the growth of T-mentagraphytes as a result (12). Another study also proved that lavender essential oil had antimicrobial activity against bacteria and fungus, was an antidepressant agent, and had a great role in the creation of positive mood and relaxation (14-15). Yet another study revealed that lavender oil reduced the fungi growth, and that the speed of infections spread in tissues was consequently diminished (17).

Generally speaking, it can be claimed that lavender oil has a long history of medicinal use. It was used in traditional Chinese medicine (TCM) to treat several conditions including infection, infertility and anxiety (16). The results of the present study showed that lavender had the highest antifungal efficacy when compared with Clotrimazole. According to the results, fungi cell counts in lavender brew and essential oil were less when compared with those of fungi control and Clotrimazole. It is worth mentioning that Clotrimazol had the highest fungi cell count and the least antifungal efficacy. There was also a significant statistical relationship between the efficacy of drugs on the fungi cell counts (p= 0.001). In a study conducted by Shin and Lim, it was shown that the majority of the evaluated essential oil had significant inhibiting activities against six species of trichophyton fungi. The essential lavender oil and other plants strongly inhibited the fungi. Also, the antifungal effects of azoles increased when they were combined with them, but the effective dose and the side effects decreased as a result. It was suggested that medical use of herbal essence might be a solution to inhibit the rapid growth of resistant fungi and to minimize the side effects caused by antifungal drugs. Nevertheless, more clinical experimentations are required for the better and more accurate evaluation of their medical applications (10).

The findings of this study also showed that the number of fungi cells in liquid medium using Thoma hemocytometer slide in ½ and ¼ dilutions of drugs had the lowest fungi cells in lavender brew when compared with that of lavender essential oil. Also, most antifungal effects in ⅛ dilution were related to lavender essential oil. The antifungal effects of the drugs in different dilutions of (½, ¼ and ⅛) showed that the drugs could be more effective with more dilutions (⅛) (p= 0.005). Similar studies also showed that the different densities of lavender had numerous antibiotic effects (29), and that lavender oil acted differently in different concentrations (5). In the same vein, a similar research study by D'Auria et al. in Rome also demonstrated that lavender essential oil and its main components, linalool and linalyl acetate, had fungus killing ability and inhibiting effects, and that at lower concentration (with higher dilutions), it inhibited germ tube formation and hyphal elongation. That could suggest the notion that lavender essential oil is effective against Candia albicans and may thus reduce fungal progression and the spread of infection in tissues (17). According to another similar study by Devkatte et al., who examined the effects of the different essential oils like lavender on growth of Candida albicanse, most of the used essential oil had antifungal effects with minimum concentration, and could be used as antifungal agents against azole-resistant drugs (6). In fact, the majority of the oils used in the study were fungicidal at low concentrations. All in all, it goes without saying that there is an urgent need for further investigations in order to achieve combinations that possess better and more effective antifungal agents with fewer side effects.

Conclusion

With regard to the achieved results from fungus cell count method in liquid medium, done by Thoma hemocytometer slide, and with attention to the significant antifungal activity of lavender plant, it can be suggested that lavender could serve as a source of compounds with therapeutic potential, and can be used against Candida-related infections. Also, with regard to its fewer fungus cells and higher efficacy on Candida when compared with Clotrimazole, lavender may be a suitable substitute for chemical drugs, which can be used for the treatment of vaginal candidiasis, and can also be applied as a supplementary drug. The authenticity of these claims necessitates further laboratory experimentations and clinical studies with more samples.

Acknowledgements

We would like to extend our sincere gratitude to the vice-chancellor of Research and Technology of Babol University of Medical Sciences for his financial support and also to Dr Hajahmadi for his valuable comments.

Conflicts of interest

None declared.

References

1. Berek, Jonathan S. Berek and Novak's Gynecology. 15th ed, Philadelphia Wolters Kluwer Health /

Lippincott Williams & Wilkins, United States. 2011. Pp; 600-615.
2. Zuzarte M1, Gonçalves MJ, Cruz MT, Cavaleiro C, Canhoto J, Vaz S, Pinto E, Salgueiro L. Lavandula luisieri essential oil as a source of antifungal drugs. Food Chem. 2012;135(3):1505-1510.
3. Liu ZY1, Sheng RY, Li XL, Li TS, Wang AX. Zhonghua Yi Xue Za Zhi. [Nosocomial fungal infections, analysis of 149 cases]. 2003;83(5):399-402 (Chinese).
4. Agarwal V, LalP, Pruthi V. Effect of plant oils on candida albicans. J Microbiol Immunol Infect. 2010;43(5):447-451.
5. Hanamanthagouda MS, Kakkalameli SB, Naik PM, Nagella P, Seetharamareddy HR, Murthy HN. Essential oils of Lavandula bipinnata and their antimicrobial activities. Food Chem. 2010;118(3):836–839.
6. Devkatte AN, Zore GB, Karuppayil SM. Potential of plant oils as inhibitors of Candida albicans growth. FEMS Yeast Res. 2005;5(9):867-873.
7. Angioni A1, Barra A, Coroneo V, Dessi S, Cabras P. Chemical composition, seasonal variability, and antifungal activity of Lavandula stoechas L. ssp. stoechas essential oils from stem/leaves and flowers. J Agric Food Chem. 2006;54(12):4364-4370.
8. Mahboubi M, Feizabadi MM, Mahin Safara M. Antifungal activity of essential oils from Zataria multiflora, Rosmarinus officinalis, Lavandula stoechas, Artemisia sieberi Besser and Pelargonium graveolens against clinical isolates of Candida albicans. Pharmacognosy Magazine. 2008; 4(15):15-18 (Persian).
9. Shin S1, Lim S. Antifungal effects of herbal essential oils alone and in combination with ketoconazole against Trichophyton spp. J Appl Microbiol. 2004;97(6):1289-1296.
10. Abdel-Aal AM ,Taha MM, El-Mashad N, El-Shabrawy W. Antifungal Susceptibility testing: New trends. gyptian Dermatology Online Journal, http://www.edoj.org.eg. 2007; 3 (1): 1.
11. Larrondo JV1, Agut M, Calvo-Torras MA. Antimicrobial activity of essences from labiates.
Microbios. 1995;82(332):171-172.
12. Larrondo JV ,Agut M, Calvo MA. Effect of essential oils on candida albicans: a scanning electron microscope study. Biomed Lett 1991;48:262-272.
13. Cavanagh HM, Wilkinson JM. Biological activity of lavender essential oil. Phytoter Res. 2002; 16(4):301-308.
14. Morris N. The effects of lavender (Lavendula angustifolium) baths on psychological well-being: two exploratory randomised control trials. Complement Ther Med. 2002;10(4):223-228.
15. Kemper KJ. Lavender (Lavandula spp). The Longwood Herbal Task Force. http://www.mcp.edu/herbal (August 9, 2001).
16. D'Auria FD1, Tecca M, Strippoli V, Salvatore G, Battinelli L, Mazzanti G. Antifungal activity of Lavandula angustifolia essential oil against Candida albicans yeast and mycelial form. Med Mycol. 2005;43(5):391-396.
17. Bouzouita N, Kachouri F, Hamdi M, Chaabouni M M. Volatile constituents and antimicrobial activity if Lavandula stoechase L. Oil from Tunisia. J Essential Oil Res. JEOR, 2005;17(5):584-586.