Brazilian Journal of Microbiology (2009) 40: 439-445
ISSN 1517-8382

# COMPARATIVE EFFICACIES OF *ZATARIA MULTIFLORA* ESSENTIAL OIL AND ITRACONAZOLE AGAINST DISSEMINATED *CANDIDA ALBICANS* INFECTION IN BALB/C MICE

**Khosravi A.R. [1*], Shokri H. [1], Tootian Z. [2], Alizadeh M. [1], Yahyaraeyat R.[1]**

[1]Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran;[2] Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Submitted: July 01, 2008; Returned to authors for corrections: August 06, 2008; Approved: May 03, 2009.

## ABSTRACT

Disseminated candidiasis is a serious problem in public health that results from the invasion of *Candida* species, in particular *Candida albicans*. The aim of this study was to compare the efficacies of *Zataria multiflora* essential oil and itraconazole in clearing *C. albicans* from the visceral organs of BALB/c mice suffered from disseminated candidiasis. *Zataria multiflora* essential oil was extracted using Clevenger-type apparatus and analyzed by gas chromatography mass spectrometry (GC–MS). For clearance experiment, mice (20-25 g, N=8 per group) received essential oil at doses of 30, 48 and 64 mg/kg and itraconazole at dose of 200 mg/kg intraperitoneally (IP) 2 days before and after intravenous inoculation of $0.5 \times 10^6$ *C. albicans* blastospores. The treated animals were sacrificed on day 20, and 0.1 g of the tissue homogenates was plated onto specific media. In GC-Mass, the main components of the essential oil were carvacrol (61.29%) and thymol (25.18%). The results demonstrated that IP administration of 64 mg/kg of the essential oil had the highest efficacy in reducing *C. albicans* and produced 39.5, 21.8, 141.5, 174 and 501-fold reductions in mean CFUs per 0.1 gram in *Candida* infections of the liver, spleen, lungs, brain and kidneys, respectively, compared to positive control. Itraconazole showed significantly more responsiveness than the essential oil at dose of 30 mg/kg in clearing *C. albicans* from the kidneys ($P<0.02$), brain ($P<0.02$) and spleen ($P<0.04$), and less responsiveness than that of 64 mg/kg in clearing the organism from the brain ($P<0.01$), lungs ($P<0.0005$) and kidneys ($P<0.0005$), whereas no significant difference was observed between this drug and *Z. multiflora* at dose of 48 mg/kg. These data explain the increased rate of yeast clearance and reduced dissemination to the viscera of *Z. multiflora* treated mice.

**Key words**: *Zataria multiflora* essential oil, *Candida albicans*, Disseminated candidiasis, Carvacrol.

***Corresponding Author. Mailing address:** Mycology Research Center, Faculty of Veterinary Medicine, Azadi street, Tehran, Iran. P.O.Box 14155-6453.; Phone: 0098 21 61117151 Fax: 0098 21 66933222.; E-mail: khosravi@ut.ac.ir

## INTRODUCTION

With the increasing number of immunocompromised patients and the greater use of broad-spectrum antifungal agents, broad-spectrum antibiotics, abdominal surgery, indwelling central venous lines, parenteral nutrition and cytotoxic chemotherapy, disseminated candidiasis has become a significant cause of mortality and morbidity (19, 21). Despite recent advances in antifungal therapy, the cure rate of disseminated candidiasis remains unsatisfactory because of a lack of nontoxic effective antifungal agents with favorable pharmacokinetic properties (26). The advent of azoles provides new options for the treatment and prevention of invasive candidiasis (24). However, the emergence of resistance to antifungal azoles poses a new challenge to our limited therapeutic strategies (4, 8). New agents with potent antifungal efficacy, improved safety and high levels of tissue penetration are clearly needed.

Plant essential oils have been known to show inhibition of proliferation or killing activity against a wide variety of micro-organisms (11, 17). *Zataria multiflora* is a plant belonging to the Laminaceae family that geographically grows only in Iran, Pakistan and Afghanistan (1). This plant has the local name of Avishan Shirazi (in Iran) and traditional uses such as antiseptic, anesthetic and antispasmodic (14). The main constituents of the essential oil of this plant are phenolic compounds such as carvacrol, thymol and eugenol (22). The essential oil has excellent in vitro activity against *Candida* species (7, 9). Up to now, little is known, however, about the in vivo activity of *Z. multiflora* in the treatment of cutaneous and mucosal fungal infections (15), but there is no study on disseminated candidiasis. The present study was undertaken to evaluate the efficacies of different doses of *Z. multiflora* essential oil in mice model of disseminated candidiasis and to compare its effect with itraconazole.

## MATERIALS AND METHODS

### Test organism

The strain of *C. albicans* (ATCC 10261) was isolated from the blood of a patient with disseminated candidiasis and stored in sterile distilled water at room temperature. To prepare the inoculum, fresh cultures were grown on Sabouraud glucose agar (*Merck Co., Darmstadt, Germany*) for 48 h at 30°C. The yeast cells were harvested, washed with normal saline and adjusted by hemocytometer approximately $1 \times 10^8$ /ml.

### Laboratory animals

Forty female BALB/c mice, weighing 20 to 25 g, were used for all experiments. The mice were housed and maintained at the standard conditions. The animals were divided into 5 groups and 8 mice per group were used for each experiment. All experiments were repeated 3 times.

### Extraction of the essential oil

Samples of *Z. multiflora* were collected from Shiraz, Iran on April 2008. Air-dried aerial parts of the plant were subjected to steam distillation for 2 h using Clevenger-type apparatus. The essential oil obtained of the air-dried material was analyzed by gas chromatography (GC) (*Thermoquest 2000, UK*). The chromatograph was equipped with DB5 capillary column (30 × 0.25 mm ID × 0.25 µm film thicknesses) and the data were acquired under the following conditions: initial temperature 50 °C; program rate 2.5 °C; final temperature 265 °C and injector temperature 250 °C. The carrier gas was helium and the split ratio was 120. The essential oil was also analyzed by gas chromatography mass spectrometry (GC–MS) (*Termoques Finningan, UK*) and the same capillary column and analytical conditions indicated above. The MS was run in the electron ionization mode, using ionization energy of 70 eV.

**Itraconazole**

Standard antifungal powder of itraconazole (*Janssen, Beerse, Belgium*) was obtained from their respective manufacturers. Itraconazole stock solution was prepared in polyethylene glycol (*Sigma Chemical Co., St. Louis, Mo*.) and sterilized through a 0.45 µm sterile, disposal and non-pyrogenic syringe filter (*Nalgene, USA*).

**Animal infection**

Normal mice were infected by intravenous injection of $0.5 \times 10^6$ saline-washed blastospores of *C. albicans*. Positive control received only IP injection of the organism. Three treatment groups received the essential oil of *Z. multiflora* (homogenized in distilled water by vortexing in high speed for 2 h) at doses of 30, 48 and 64 mg/kg by IP injection. The first dose was given 2 days before inoculation of *C. albicans* and the next dose was given 2 days after infection. The positive controls did not receive any drug regimen. For *Z. multiflora* groups, mice were sacrificed 20 days following the administration of different doses of the essential oils. Mice in the last group received itraconazole (200 mg/kg) via IP injection and sacrificed on day 20. The kidneys, spleen, liver, lungs and brain of each animal were weighed and examined by qualitative and quantitative cultures. The visceral organs from animals were ground with a pestle and mortar and 0.1 gram of tissue was plated on Sabouraud glucose agar. The plates were incubated at 35°C for 48 h, and the number of CFU per 0.1 gram of each tissue was determined.

**Statistical analyses**

The results were expressed as the mean CFU±standard deviation (SD) from 3 experiments. Mann-whiteny and Kruskal-wallis tests were used to compare the differences among the groups by SPSS software (Version 15). A *P* value less than 0.05 was statistically considered significant.

## RESULTS

**Chemical composition of *Z. multiflora* essential oil**

The yield of the essential oil of air-dried aerial parts of the representative sample of *Z. multiflora* was 1.66% (v/w). Percentages of components of the essential oil (as determined by GC and GC–MS) were summarized in table 1. As shown in table 1, the main components of the essential oil were carvacrol (61.29%) and thymol (25.18%).

**Table 1.** Essential oil composition of *Z. multiflora* identified by gas chromatography and gas chromatography–mass spectrometry

| **Component** | component (%) |
|---|---|
| Carvacrol | 61.29 |
| Thymol | 25.18 |
| Linalool | 1.96 |
| ρ-Cymene | 1.90 |
| β- Caryophyllene | 1.82 |
| α- Pinene | 0.34 |
| α- Thujene | 0.10 |
| Myrcene | 0.27 |
| α - Terpinene | 0.33 |
| γ- Terpinene | 0.76 |
| Thymul methyl ether | 0.95 |
| Carvacrol methyl ether | 0.95 |
| Camphene | 0.01 |
| 1-Octen-3-ol | 0.02 |
| 3-Octanone | 0.05 |
| β- Penene | 0.09 |
| α – Phellandrene | 0.02 |
| Limonene | 0.21 |
| Linalool oxide trans | 0.07 |
| Linalool oxide cis | 0.06 |
| 4-Terpineol | 0.30 |
| α- Terpineol | 0.54 |
| Bornyl acetate | 0.01 |
| Carvacerol acetate | 0.02 |
| β – Phellandrene | 1.82 |
| Aromadendrene | 0.23 |
| Alloaromadendrene | 0.11 |
| α- Humulene | 0.02 |
| Valencene | 0.14 |
| Spathulenol | 0.37 |
| Widdrol | 0.64 |
| **Total** | **99.09** |

**Clearance of *C. albicans* from the kidneys**: The whole untreated mice died on days 9-10, whereas the different treated groups were sacrificed on day 20. The clearance of *C. albicans* from untreated mice and mice treated with different doses of *Z. multiflora* and itraconazole was summarized in table 2. In the untreated mice receiving $0.5 \times 10^6$ *C. albicans*, the number of yeast cells in the kidneys of mice was ranged from 80 to 429 (mean, 350.8) on 10 days postinfection. In the mice receiving the different doses of *Z. multiflora*, the mean counts were 200 CFUs at dose of 30 mg/kg, 53.8 CFUs at 48 mg/kg and 0.7 CFUs at 64 mg/kg on 20 days postinfection. In mice receiving itraconazole, the mean counts were determined 65.9 CFUs at dose of 200 mg/kg on 20 days postinfection.

**Clearance of *C. albicans* from the liver**: In the untreated animals, the number of *C. albicans* from the liver was ranged from 0 to 75.6 (mean, 51.3). In the mice receiving the different doses of *Z. multiflora*, the organisms decreased at dose of 30 mg/kg from 51.3 to 36.3 CFUs, to 2.9 CFUs at dose of 48 mg/kg and to 1.3 CFUs at dose of 64 mg/kg. Itraconazole had close efficacy with the essential oil at dose of 48 mg/kg, which resulted in decreasing the CFUs from 51.3 to 3.3 CFUs.

**Clearance of *C. albicans* from the spleen**: The mean counts of *C. albicans* from the spleen were 8.7 CFUs in untreated animals, 6.7, 1.8 and 0.4 CFUs at doses of 30, 48 and 64 mg/kg of the essential oil, respectively, and 1.9 CFUs at itraconazole group.

**Clearance of *C. albicans* from the brain**: As shown in table 2, there were no significant differences in the number of *C. albicans* from the brains of untreated and treated animals except for mice receiving 64 mg/kg of *Z. multiflora* ($P<0.05$).

**Clearance of *C. albicans* from the lungs**: The highest and lowest numbers of *C. albicans* from the lungs were demonstrated in untreated mice (mean; 28.3) and mice receiving 64 mg/kg (mean; 0.2) of the essential oils, respectively.

In this study, *Z. multiflora* essential oil demonstrated a significant dose dependent antifungal effect in clearing *C. albicans* from the organs. In comparison to itraconazole, essential oil had less active at dose of 30 mg/kg in clearing *C. albicans* from the kidneys ($P<0.02$), brain ($P<0.02$) and spleen ($P<0.04$), similarly clearances at dose of 48 mg/kg from all tissues and higher active at dose of 64 mg/kg in clearing the organism from the brain ($P<0.01$), lungs ($P<0.0005$) and kidneys ($P<0.0005$).

**Table 2.** Comparative efficacies of *Zataria multiflora* essential oil and itraconazole on disseminated candidiasis in normal mice (Mean±SD of CFU/0.1 gram)

| Organ / Material | Brain | Lungs | Liver | Spleen | Kidney |
|---|---|---|---|---|---|
| **Control** | 69.7± 0.10 | 28.3± 0.06 | 51.3 ± 0.03 | 8.7±0.01 | 350.8±0.41 |
| ***Z. multiflora* (30mg/kg)** | 44.9±0.12 | 13±0.09 | 36.3±0.10 | 6.7 ±0.06 | 200 ±0.44 |
| ***Z. multiflora* (48mg/kg)** | 51.8±0.20 | 4.7 ±0.05 | 2.9±0.01 | 1.8 ±0.01 | 3.8±0.21 |
| ***Z. multiflora* (64mg/kg)** | 0.4±0.01 | 0.2±0.01 | 1.3±0.09 | 0.4± 0.01 | 0.7±0.03 |
| **Itraconazole (200mg/kg)** | 27.2±0.10 | 10.7±0.08 | 3.3±0.03 | 1.9±0.01 | 65.9±0.27 |

## DISCUSSION

Recently new antifungal agents were used for treatment of disseminated candidiasis, including imidazoles and aminoacids (4). In some cases, these drugs showed failure to treatment, side effects and high relapse of disease (5, 12, 20, 25). For these reasons, the widespread efforts were made to identify natural agents to combat these opportunistic infections. *Zataria multiflora* is a valuable medicinal plant grown extensively in Iran, and the chemical compositions of essential oil have been extensively characterized (18, 19). The results of the clearance experiments using infecting doses of $0.5 \times 10^6$ in normal mice demonstrated the reduction of *C. albicans* from the kidneys, spleen, liver, brain and lungs of mice treated with 48 and 64 mg of *Z. multiflora* essential oil per kg twice with a 4-day interval. Control mice were infected with the same dose of *C. albicans* and *Candida* invasion was appeared in this group on day 9 and the whole animals died on day 10. In treatment groups, mice were sacrificed 20 days after administrating the last dose of the essential oil and itraconazole. In the present study, high-dose IP administration (64 mg/kg) of the essential oil demonstrated the highest efficacy in reducing *C. albicans* and produced 39.5, 21.8, 141.5, 174 and 501-fold reductions in mean CFUs per 0.1 gram in candidiasis of the liver, spleen, lungs, brain and kidneys in the mice model, respectively. The higher essential oil concentration resulted in higher inhibitory effect. No any major side effects have been shown in using *Z. multiflora* components in human and animals in this study and other investigations (15, 16). In our previous studies, the essential oil significantly increased innate and cellular immune responses in animal models. It was demonstrated that this essence has multiple biological effects on in vivo condition such as stimulation of immune system and antimicrobial activity (16, 23). Overall, the antifungal activity of the essential oils is related to the respective composition of the plant essential oils, the structural configuration of the constituent components and their functional groups and possible synergistic interactions between components (6). It was demonstrated that the main components with phenolic structures in *Z. multiflora*, such as carvacrol and thymol, have high activity against tested *Candida* species (21). In Iran, Fataneh (7) and Mahmoudabadi *et al*. (17) demonstrated anti-*Candida* activity of carvacrol and thymol in 2 in-vitro studies. In the present study, these compounds (carvacrol and thymol) were found as 2 main components, which was in agreement with published data (10, 13, 18). Itraconazole is a triazole anti-fungal agent with broad spectrum activity against a wide range of systemic fungal. It has found particular application in the treatment of immuno-compromised patients with fungal infections, especially fungal infections in patients undergoing chemotherapy, or afflicted with AIDS and AIDS-related conditions, or organ transplant recipients (2). Comparing to itraconazole with the essential oil, this drug showed significantly more responsiveness than the essential oil at dose of 30 mg/kg in clearing *C. albicans* from the kidneys ($P<0.02$), brain ($P<0.02$) and spleen ($P<0.04$), and less responsiveness than that of 64 mg/kg in clearing the organism from the brain ($P<0.01$), lungs ($P<0.0005$) and kidneys ($P<0.0005$), whereas no significant difference was observed between itraconazole and *Z. multiflora* at dose of 48 mg/kg in all tissues.

In summary, different concentrations of the essential oil exhibited considerable inhibitory effects against disseminated candidiasis. Further studies are needed to evaluate the toxic effect of *Z. multiflora* essential oil, via injection route, it could be used to treat human with disseminated candidiasis and this treatment would be lower

cost when compared with itraconazole.

**ACKNOWLEDGMENT**

This work was supported by the Research Council of University of Tehran.

**RESUMO**

**Eficiência comparada do óleo essencial de *Zataria multiflora* e itraconazol contra infecção disseminada de *Candida albicans* em camundongos BALB/c**

A candidíase disseminada é um problema sério de saúde publica decorrente da invasão por espécies de *Candida*, e *Candida albicans* em particular. O objetivo deste estudo foi comparar a eficiência do óleo essencial de *Zataria multiflora* e itraconazol na remoção de *C. albicans* das vísceras de camundongos BALB/c com candidíase disseminada. O óleo essencial de *Zataria multiflora* foi extraído empregando um aparelho do tipo Clevenger e analisado por cromatografia a gás e espectrometria de massa (GC-MS). Para os experimentos de remoção, camundongos (20-25g, n=8 por grupo) receberam óleo essencial nas doses de 30, 48 e 64 mg/kg e itraconazol na dose de 200 mg/kg via intraperitoneal (IP) por dois dias antes e após a inoculação intravenosa de 0,5 x $10^6$ blastósporos de *C. albicans.* Os animais tratados foram sacrificados no vigésimo dia e 0,1g dos tecidos homogeneizados foram semeados em meios específicos. De acordo com o GC-MS, os principais componentes do óleo essencial foram carvacrol (61,29%) e timol (25,28%). Os resultados mostraram que a administração IP de 64 mg/kg de óleo essencial apresentou a eficiência mais alta na redução de *C. albicans* e resultou na redução de 39,5, 21,8, 141,5, 174 e 501 vezes na contagem média de *C. albicans* por 0,1g do fígado, baço, pulmões, cérebro e rins, respectivamente, quando comparado ao controle positivo. O itraconazol apresentou redução de *C. albicans* maior do que o óleo essencial na dose de 30mg/kg nos rins (P<0,02), cérebro (P<0,02) e baço (P<0,04) e menor no cérebro (P<0,01), pulmões (P<0,0005) e rins (P<0,0005) na dose de 64 mg/kg, enquanto não houve diferença entre esse droga e óleo essencial na dose de 48mg/kg. Estes resultados explicam a remoção aumentada de leveduras e a disseminação reduzida para as vísceras de camundongos tratados com *Z. multiflora.*

**Palavras-chave:** óleo essencial de *Zataria multiflora, Candida albicans,* candidíase disseminada, carvacrol

**REFERENCES**

1. Ali, M.S.; Saleem, M.; Ali, Z.; Ahmad, V.U. (2000). Chemistry of *Zataria multiflora* (Lamiaceae). *Phytochem,* 55 (8), 933-936.
2. Anaisse, E.J.; McGinnis, M.R.; Pfaller, M.A. (2003). *Clinical Mycology*. Churchill Livingstone, London, UK.
3. Charai, M.; Mosaddak, M.; Faid, M. (1996). Chemical composition and antimicrobial activities of two aromatic plants: *Oreganum majorana* L. and *O. compactum* Benth. *J. Essen. Oil. Res.* 8, 657-664.
4. Coste, A.; Selmecki, A.; Forche, A.; Diogo, D.; Bougnoux, M.E.; d'Enfert, C.; Berman, J.; Sanglard, D. (2007). Genotypic evolution of azole resistance mechanisms in sequential *Candida albicans* isolates. *Eukaryot Cell*. 6, 1889-1904.
5. De Silva Barros, M.E.; Hamdan, J.S. (2005). Determination of susceptibility/resistance to antifungal drugs of *Trichophyton mentagrophytes* isolates by a macrodilution method. *Can. J. Microbiol*. 51, 983-987.
6. Dorman, H.J.D.; Deans, S.G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol*. 88 (5), 308-316.
7. Fataneh, F. (1991). *Anti-fungal activity of Zataria multiflora extract in vitro*. Thesis, School of Pharmacy, Esfahan University of Medical Sciences, Iran.

8. Frosco, M.B.; Barret, J.F. (1998). Importance of antifungal drug-resistance: clinical significance and need for novel therapy. *Exp. Opin. Invest. Drugs*. 7, 175-197.
9. Javidnia, K.; Tabatabai, M.; Shafiee, A. (1999). Volatile constituents and antimicrobial activity of *Zataria multiflora*, population Iran. *Ir. J. Chem. Chemical Eng*.18, 1-5.
10. Jeongmok, K.; Marshall, M.R.; Wei, C. (1995). Antibacterial activity of some essential oil components against five food-borne pathogens. *J. Agri. Food Chem*. 43, 2839-2845.
11. Jollois, R. (1996). *Laromatherapie exactement, Nouvelles editions Roger Jollois*. Paris, France.
12. Gustavo Giusiano, G.; Mangiaterra, M.; Saito, V.G.; Rojas, F.; Gómez, V.; María Cristina Díaz, M.C. (2006). Fluconazole and itraconazole tesistance of yeasts isolated from the bloodstream and catheters of hospitalized pediatric patients. *Int. J. Exp. Clin. Chemother*. 52(5), 254-259.
13. Hili, P.; Evans, C.S.; Veness, R.G. (1997). Antimicrobial action of essential oils: the effect of dimethyl sulphoxide on the activity of cinnamon oil. *Let. Appl. Microbiol*. 24, 269-275.
14. Hosseinzadeh, H.; Ramezani, M.; Salmani, G. (2000). Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria multifora* Boiss extracts in mice and rats. *J. Ethnopharmacol*. 73, 379-385.
15. Khosravi, A.R.; Eslami, A.R.; Shokri, H.; Kashanian, M. (2008). *Zataria multiflora* cream for the treatment of acute vaginal candidiasis. *Int. J. Gynaecol. Obstet*. 101(2):201-202.
16. Khosravi, A.R.; Franco, M.; Shokri, H.; Yahyaraeyat, R. (2007). Evaluation of the effects of *Zataria multiflora*, *Geranium pelargonium*, *Myrth* and *Lemon* essences on immune system function in experimental animals. *J.Vet.Res*. 62 (4), 119-123.
17. Mahmoudabadi, A.Z.; Dabbagh, M.A.; Fouladi, Z. (2006). In-vitro anti-*Candida* activity of *Zataria multiflora* Boiss. *Evid. Based Complement Alternat. Med.* 4 (3), 351-353.
18. Mohagheghzadeh, A.; Shams-Ardakani, M.; Ghannadi, A.; Minaeian, M. (2004). Rosmarinic acid from *Zataria multiflora* tops and in vitro cultures. *Fitoterapia*. 75 (3-4), 315-321.
19. Ostrosky-Zeichner, L.; Rex, J.H.; Bennett, J.; Kullberg, B.J. (2002). Deeply invasive candidiasis. *Infect. Dis. Clin. North Am.* 16, 821-835.
20. Pai, M.P.; Turpin, R.S.; Garey, K.W. (2007). Association of fluconazole area under the concentration-time curve/MIC and dose/MIC ratios with mortality in nonneutropenic patients with candidemia. *Antimicrob. Agents Chemother*. 51(1), 35-39.
21. Pelczar, M.J.; Chan, E.C.S.; Krieg, N.R. (1988). Control of microorganisms, the control of microorganisms by physical agents. *In*: *Microbiology*. McGraw-Hill International, New York, USA, p. 469-509.
22. Shaffiee, A.; Javidnia, K. (1997). Composition of essential oil of *Zataria multiflora*. *Planta Med.* 63, 371-372.
23. Shokri, H.; Asadi, F.; Bahonar, A.R.; Khosravi, A.R. (2006). The role of *Zataria multiflora* essence (Iranian herb) on innate immunity of animal model. *Iran J.Immunol*. 3(4), 164-168.
24. Slavin, M.A.; Osborne, B.; Adams, R.; Levenstein, M.J.; Schoch, H.G.; Feldman, A.R.; Meyers, J.D.; Bowden, R.A. (1995). Efficacy and safety of fluconazole prophylaxis for fungal infections after marrow transplantation-a prospective, randomized, double-blind study. *J. Infect. Dis*. 171, 1545-1552.
25. Smith, J.; Andes, D. (2008). Therapeutic drug monitoring of antifungals: Pharmacokinetic and pharmacodynamic considerations. *Ther. Drug Monit.* 30(2),167-172.
26. Walsh, T.J.; Pizzo, P. (1989). Treatment of systemic fungal infections: recent progress and current problems. *Eur. J. Clin. Microbiol*. 7, 460-475.