Зборник Матице српске за природне науке / Proc. Nat. Sci., Matica Srpska Novi Sad, № 113, 249—254, 2007

UDC 599.323.4:616.5-002.828

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# EXPERIMENTALLY INDUCED DERMATOMYCOSES AT RATS AND TREATMENT WITH *LAVANDULA ANGUSTIFOLIA* ESSENTIAL OIL

ABSTRACT: The *in vivo* evaluation of antifungal activity of the *Lavandula angustifolia* essential oil was made on two-month old male Wistar rats. We examined the therapeutic potency against experimentally induced dermatomycoses in rats, using the most frequent dermatomycetes, *Trichophyton mentagrophytes*. The therapeutic efficacy of 1% solution of essential oil as well as commercial preparation-bifonazole, was evaluated. During the 13-day observation period the oil-treated animals were cured completely.

KEY WORDS: essential oil, *Lavandula angustifolia*, Dermatomycetes, antufungal activity

## INTRODUCTION

During the past several years numerous antifungal agents have been formulated and evaluated for use in the management of fungal infections (R y a n, 1994). The emerging resistance of microorganisms to some synthetic antibiotics makes it necessary to continue the search for new antimicrobial substances. With the increasing acceptance of traditional medicine as an alternative form of health care, the searching for active compounds in medicinal plants became very important. Human infection diseases have been markedly increasing during the past ten years, especially in immunocompromised patients. Consequently, as high as 10% percent of hospital acquired systemic infections are caused by fungi.

The increasing resistance of human pathogens to current commercial drugs is a serious medical problem, and has resulted in the need for novel antimicrobial agents.

Natural products derived from plants have traditionally been used in ethnomedicine. In Western medicine, substances derived from higher plants constitute ca. 25% of prescribed medicines and 74% of the 121 bioactive plant-derived compounds currently in worldwide use, which were identified via research based on leads from ethnomedicine (Sokmen et al., 1999). Recent researches showed that higher plants may serve as promising sources of novel antimycotics with no side effects on human and animals (Clark and Hufford, 1993). Essential oils play a great role in these investigations. Studies over the last hundred years have demonstrated the antimicrobial properties of several common spice oils (Bullerman et al., 1977). Maruzzela and Balter (1959) found that 100 essential oils out of 119 spice oils, tested, possessed an antagonist effect an at least one of 12 pathogenic fungi, and 50 of these samples showed a wide spectrum of activities against all fungi tested.

The purpose of this study was to investigate *Lavandula angustifolia* essential oil for potential antifungal activity. The selection of the plant for evaluation was based on traditional use of this plant in treatment of various infection diseases (Jančić et al., 1995; Kovačević, 2000).

## MATERIAL AND METHODS

### *Plant material*

*Lavandula angustifolia* was collected during May in 1999 at the fields of the Institute for Medicinal Plant Research in Pančevo, Belgrade, Serbia. Voucher specimens (No. 04071970 and 25072) were deposited in the Herbarium of Institute of Botany and Botanical Garden "Jevremovac", University of Belgrade.

### *Isolation of essential oils*

Composition of essential oils was investigated using analytical GC/FID and GC/MS techniques. For this purpose, HP 5890 series II gas chromatograph, equipped with split-splitless injector, fused silica capillary column (25 m x 0.32 mm), coated with cross-linked methyl silicone gum (0.5 μm film tickness), and FID was employed. Essential oil solutions in ethanol (1%) were injected in split mode (1:30). Injector was heated at 250°C, FID at 300°C, while column temperature was linearly programmed from 40—280°C (4°C/min).

GC/MS analysis was carried out on a HP-GCD, equipped with split-splitless injector, fused silica capillary column (50 m x 0.2 mm) PONA, coated with cross-linked methyl silicone gum (0.5 μm film tickness) and mass selective detector. The chromatographic conditions were as above. Transfer line (MSD) was heated at 280°C. EMS spectra (70 eV) were acquired in scan mode in m/e range 40—300.

The identification of individual constituents was carried out by comparison of their retention times with those of analytical standards, and by computer searching, matching the mass spectral data with those held in Wiley/NBS library of mass spectra. For quantification purposes area percent reports obtained by FID were used.

### *Bioassays*

*Toxicology*: in order to determine the non toxic concentration of the essential oil investigated, we used male Wistar rats (17—24 g). 0.5 ml of prepared stock solution of essential oil, and a component diluted in ethanol (0.01—1% v/v) were injected intraperitoneal in male Wistar rats (*Pharmacopea Jugoslavica*, 1984). The concentration which was not toxic for the animals investigated, was used for further investigation.

*Animals.* Two-month-old male Wistar rats were maintained at 21°C, and were allowed access to feed and water *ad libitum*.

*In vivo Fungitoxicity Assay.* The *in vivo* investigation of antifungal activity of *Lavandula angustifolia* essential oil was made according to Adam et al., (1998). We used *Trychophyton mentagrophytes* as an infectious agent. The organism was isolated from patients at the Center for Preventive Medicine, MMA, Belgrade, Serbia.

The micromycetes were maintained on Sabouraud Dextrose Agar (SDA), containing 40 g of glucose, 10 g of agar and 10 g of peptone in 1 l of distillate $H_2O$. The cultures were stored at +4°C, and subcultured once a month (Booth, 1971).

On the back of each animal, the areas of 4 $cm^2$ were cleaned and depilated. The infectious inoculum was prepared from a 7-day-old culture of *Trychophyton mentagrophytes*. The inoculum was applied on the animals' back immediately after the depilation and left for 3 days. The establishment of active infection was confirmed on the $4^{th}$ day, by isolation of the pathogens from skin scales cultured from infected loci on SDA plates, containing 100 units/ml of penicillin and streptomycin. Infections were, also, confirmed by visual examination of animals. In the animals in which active infections were confirmed, treatment was initiated on the $5^{th}$ day post inoculation and continued until complete recovery from the infection was achieved. The ointments contained 1% (v/v) of essential oil and component mixed in petroleum jelly. The commercial fungicide, bifonazole, was used as a control. Animals were treated once a day, and the infected areas were scored visually for inflammation and scaling as, well as for the presence of the pathogens by cultivating skin scales from infected loci in SDA plates containing 100 units/ml of penicillin and streptomycin, each day.

## RESULTS AND DISCUSSION

The qualitative and quantitative composition of the essential oils of *Lavandula angustifolia* was presented in Table 1. The essential oil is characteri-

zed with high content of linalool (27.21%) and linalool acetate (27.54%), while limonene is presented with 8.5%.

Tab. 1 — Chemical composition of *Lavandula angustifolia* essential oil

| Components | % | RI |
|---|---|---|
| tricyclene | 0.04 | 301 |
| α-thujene | 0.58 | 307 |
| α-pinene | 0.19 | 319 |
| p-cymene | 0.25 | 471 |
| limonene | 8.50 | 481 |
| 1,8-cineole | 3.34 | 485 |
| cis-linalool oxide | 2.44 | 574 |
| camphenylon | — | 594 |
| fenchon | 0.59 | 605 |
| linalool | 27.21 | 632 |
| endo-fenchol | 0.09 | 664 |
| camphor | 1.07 | 734 |
| borneol | 2.51 | 789 |
| terpine-4-ol | 2.09 | 820 |
| p-cimene-8-ol | — | 837 |
| α-terpineol | 4.30 | 852 |
| myrtenal | — | 864 |
| fenchyl acetate | — | 930 |
| carvon | — | 984 |
| linalool acetate | 27.54 | 1023 |
| bornyl acetate | 0.06 | 1099 |
| lavandulyl acetate | 6.54 | 1111 |
| trans-pinokarvyl acetate | 0.16 | 1135 |
| neryl acetate | 2.02 | 1303 |
| geranyl acetate | 2.95 | 1352 |
| β-selinene | — | 1608 |
| δ-cadinene | — | 1700 |
| viridiphlorol | — | 1859 |
| Total | 97.47 | |

* In elution order on DB-5 column (6)

The essential oil was tested for its potential toxicological activity in 0.1% and 1% (v/v) solutions in ethanol and petroleum jelly, separately. There is no toxicological activity for 0.1% solutions on the rats. However, in this work the animals were treated topically, and according to the literature (A d a m et al., 1998), for further investigation 1% solutions were used.

The therapeutic efficacy of the ointments was evaluated daily by macroscopic examination of lesions, and by screening for the presence of the infections by culturing skin scales from the infected area. The lesions were treated as cured only when the infected area was free of macroscopic lesions, and when the cultures were negative.

First symptoms (small vesicles) at the rats inoculated with *T. mentagrophytes* were observed on the 5$^{th}$ day of the experiment, while, later (8$^{th}$ day), these were exhibiting in bloody wounds, 20 mm in diameter. We started

with the treatment on the 5th day of the experiment. On the 13th day of the treatment with solution of *L. angustifolia* essential oil, the rats were completely cured, there were no visually observed symptoms and the cultures were negative. Animals treated with the commercial drug, bifonazole, were cured after 15 days of treatment.

During the 13-day observation period the treated animals were cured completely. It should be noted that in many cases macroscopic lesions disappeared long before the elimination of the infectious agent, indicating that long treatment periods of application and evaluation are necessary (Adam et al., 1998). It is normal because dermatomycetes infections typically resolve on their own over a variable time period (18 months to 4 years) depending on the immune response. Many of dermatomycetes rarely cause strong inflammatory reactions, making it very difficult for the immune system to recognize and eliminate the fungus.

The animals treated with the commercial drug, bifonazole, were cured after 15 days of treatment.

From the above results it can be concluded that essential oil of *Lavandula angustifolia* has a good therapeutic and antifungal effect *in vivo*, and could represent possible alternative for the treatment of patients infected by dermatomycetes. Even more, because of the side effects of commercial fungicides and possible resistance of pathogens to the synthetic mycotics, the preparation with natural products has an advantage in the treatment of disseases caused by fungi.

## ACKNOWLEDGMENTS

This study was supported by the Ministry of Science and Environmental protection, Grant# 143041.

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## ЕКСПЕРИМЕНТАЛНО ИНДУКОВАНА ДЕРМАТОМИКОЗА КОД ПАЦОВА И ТРЕТМАН ЕТАРСКИМ УЉЕМ *LAVADULA ANGUSTIFOLIA*

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Резиме

Инфекције људи узроковане гљивама су у знатном порасту у последњих 10 година, посебно код имунокомпромитованих пацијената. Чак 10% инфекција код хоспитализованих пацијената изазвано је гљивама. С обзиром да је појава резистенције на комерцијалне фунгициде учестала, јавља се потреба за новим алтернативним антифунгалним агенсима. Природни продукти добијају све већи значај у третману инфективних обољења због свог нетоксичног карактера, високе биодеградибилности и ефикасности. Етарска уља изолована из биљака показују веома добру антимикробну активност.

Експериментална дерматомикоза код Wistar пацова у овом раду изазвана је дерматомицетом *Trichophyton mentagrophytes*. Први симптоми појавили су се након 5 дана од инокулације. Тремтан етарским уљем врсте *Lavandula angustifolia*, 1% раствором почео је одмах након појаве првих симптома. Побољшање симптома примећено је након два дана третмана, а после 13 дана од почетка третмана животиње су у потпуности излечене, на SDA подлогама није било забележено присуство патогена. Бифоназол је довео до излечења након 15 дана третмана. Етарско уље *Lavandula angustifolia* показало је изузетно јак антифунгални потенцијал у третману експериментално индуковане дерматомикозе код пацова, боље од комерцијалног микотика, бифоназола, који је коришћен као позитивна контрола.