EVALUATION OF ANTIFUNGAL ACTIVITY OF SATUREJA MONTANA ESSENTIAL OIL BEFORE AND AFTER INCLUSION IN BETA-CYCDODEXTRINE

ENTELA HALOCI, VILMA TOSKA, ANNA BALDISSEROTTO, ENKELEJDA GOCI, SILVIA VERTUANI, STEFANO MANFREDINI

Entela Haloci, PhD student Ferrara University Italy, Pharmacy Department, Vilma Toska Pharmaceutical Department, Fakulteti i Farmacise, Tirane Albania, Department of Life Sciences and Biotechnology, Ferrara University, Italy, Enkelejda Goci Pharmaceutical Department, Aldent University, Tirana Albania, Stefano Manfredini Pharmaceutical Chemistry, President of the School of Pharmacy Head of Pharmaceutical Department, Ferrara University, Italy. Email: entelahaloci@yahoo.com

Received: 27 Feb 2014 Revised and Accepted: 29 Mar 2014

ABSTRACT

Objective: The aim of this study is the evaluation of the antifungal activity of Satureja montana essential oil collected in north of Albania, and how it is affected by microencapsulation in β-cyclodextrin (β-CD).

Methods: GC/FID spectrometry analysis of the isolated oil was used. The antifungal activities of essential oil before and after microencapsulation were individually evaluated against following dermatophytes M.gypseum, M.canis, A.cajetani, T.violaceum, T.mentagrophytes, E.floccosum, T. Rubrum, T. tonsurans and phytopatogens B.cinerea and P.oryzae using a disc diffusion method.

Results: GC/FID analyses resulted in the identification of twentyone compounds in the oil of Satureja Montana essential oil. Carvacrol was the major constituent of the S. montana oil (around 60 %). Other important compounds were the monoterpenic hydrocarbons p-cymene, y-terpinene and the oxygenated compounds borneol. Maximum activity of Satureja montana oil was observed against Tviolaceum, T. Rubrum, T. tonsurans, T.mentagrophytes and P.oryzae (inhibition zone > 70 %).

Conclusion: From the results is evident that microencapsulation does not change the antifungal activity of essential oil, this should consent to achieve the optimal antifungal activity with minimum side effects of essential oil, and improved stability upon storage due to benefits of microencapsulation in β-cyclodextrine. Moreover, after encapsulation improved activity were obtained (inhibition zone > 70).

Keywords: Satureja montana, Essential oil, Gas/Fid, Antifungal, Sustainability

INTRODUCTION

Fungal infection is very often occurring on these days. They are getting more and more resistant to antifungal agents which are very expensive ones and associated with by a several side effects. On the other hand traditional medicine is cheaper and more effective than modern medicine. Patients have a reduced risk to use herbal drugs than antifungal agents. is often less expensive and equally effective as compared to several drugs and sustainable, because it consent to preserve traditional recipes and folk medicine.

We choose Satureja montana as it is well-known for its essential oil contents and dermatological benefits. To expand its dermatological application we investigate the preparation of essential oil from samples collected in different zones of Albania, their antifungal activity and their inclusion in cyclodextrine complexes, in order to achieve better stability and better compatibility with skin application. The present study was conducted to compare the antifungal properties before and after encapsulation of the essential oil. The ratios of oil: beta-cyclodextrine used is 20:80 and 10:90 (w/w).

MATERIALS AND METHODS

Plant Material

Herbal plants of Satureja montana were collected from different zones of Albania and were identified from our botanist at the Botanic Department, University of Tirana, Albania. Sample of drug is recorded in Herbarium Deposit in University of Tirana.

Isolation of the essential oil

The hydrodistillation was carried out with a Cleveger-type apparatus according to the Hungarian Pharmacopoeia VII. (1986). A Drug quantity of 20 g was used, it was distilled with 500 ml of water for 3 hours. The resulting essential oil was dried over anhydrous sodium sulphate and stored at 4°C.

Reagents

All reagents and solvents used were obtained from Sigma Aldrich Company. Gas/Fid Tip Varian CP3800, Stationary Phase Capilar VF: 1ms; Film thickness 0.25 m (L) 25 mm (ID) 0.25 mmx (OD) 0.39 mm. Mobile Phase is helium. Standards of carvacrol, p-cymen, y-terpinen, borneol, thymol were obtained from Sigma Aldrich company. beta-Cyclodextrin was purchased from Titolchimica (Italy).

Fungal Strains

Fungal strains were developed by Dipartimento di Scienze della Vita Bietecnologie, University of Ferrara Dermatophytes: The dermatophytes used were Arthroderma cajetanum (Netherlands), CBS 495.70 strain; Epidermophyton floccosum var. floccosum (Netherlands) CBS 35893 strain; Trichophyton violaceum (Africa) CBS 459.61 strain; Trichophyton tonsurans (Netherlands) CBS 483.76 strain; Trichophyton mentagrophytes (Netherlands) CBS 160.66 strain; Microsporum canis (Iran) CBS 131110 strain; Trichophyton rubrum (Turkey) CBS 132252 strain; Microsporum gypseum (Iran) CBS 130948 strain; the phytopatogens used were Botrytis cinerea (Netherlands) CBS 1798.71 strain; Pyricularia oryzae (unknown) CBS 43370 strain; obtained from CBS-KNAW Fungal Biodiversity Centre, An institute of the Royal Netherlands Academy of Arts and Sciences, Utrecht, Netherlands. The cultures were maintained in the laboratory as agar slants on a suitable culture medium, that is, on Sabouraud dextrose agar (SDA; Difco), for the dermatophytes and on Potato dextrose agar (PDA) for phytopatogens.

Gas/Fid method

Gas/Fid conditions GC analysis of the essential oil was performed using a Varian CP-3800 instrument equipped with a capillary column. Helium was used as the carrier gas at the constant flow of 1.2 ml/min and split ratio 1:30. The oven temperature was held at 50 °C for 1 min, then programmed to 280°C at a rate of 5°C /min.
Helim flux is 30ml/min and air flux is 300ml/min. The injector temperature is 280°C and detector (FID) temperature is 300°C. Injection volume is 1μl.

**Antifungal Activity**

The essential oil samples before and after encapsulation were tested for antifungal activity by the disc diffusion method using 100μL of suspension of the tested fungal strains containing 2x10^5 spore mL⁻¹ colony Mueller–Hinton agar and dextrose agar were distributed to sterilized Petri dishes with a diameter of 9 cm. Fig 2. The filter paper discs (6 mm in diameter) were individually impregnated with 20μl and 100μl of the essential oils dissolved in dimethylsulfoxide (DMSO). The Petri dishes were kept at 4°C for 2 h.

The plates inoculated with dermatophytes were incubated at 30°C for 7 days and plates incubated with phytopatogens were incubated at 30°C for 5 days. The diameters of the inhibition zones were measured in millimeters.

Controls were set up with equivalent quantities of DMSO.

Studies were performed in triplicate. In addition, as negative controls is used DMSO.

**DISCUSSION**

Satureja montana essential oil had considerable inhibitory effects on following fungi Microsporum gypseum, Microsporum canis, Nannizzia cajetani, Tricholosporum violaceum, Trichophyton mentagrophytes, Epidermophyton floccosum, Trichophyton rubrum, Trichophyton tonsurans and Phytopatogens, Botrytis cinerea and Pyricularia oryzae especially M.gypseum 76.29 %, T. mentagrophytes 77.17%, T.tonsurans (83.33%), T.rubrum (93.55%), and T.violaceum (100 %). The inhibition zones of essential oil after encapsulation are considerably higher to those encapsulated in β-cyclodextrine. This means that the encapsulation process does not impair the antifungal activity of Satureja montana essential oil. On the other hand the slow releasing of essential oils from β-cyclodextrine complex causes higher inhibition zone some time the highest one (100 %). Table 1 shows that sample M3 and M4 (Table 2) having high levels of carvacrol (66.22, 67.68) and thymol (18.2, 25.78) also display higher inhibition zone than other samples. On the other hand the inhibition zone of essential oil after encapsulation are very closed to those before encapsulation as stated above, this is an important finding to enlarge potential application of the antifungal properties of Satureja montana essential oil.
Microencapsulation of essential oil in β-cyclodextrine has shown higher inhibition zone than the ratio 20:80. The solubility of essential oil at these ratios, maybe at the base of the observed activity, thus we suggest as optimal ratio 10:90. However, the improvements on the antifungal activity and physico-chemical stability of microencapsulation of essential oil in β-cyclodextrine have been clearly demonstrated in this study. Another important consideration relates to range of concentrations: concentrations below 20 μg/ml are devoid of antifungal activity and on the other hand there is no need to use concentrations over 100 μg/ml.

CONCLUSION

Satureja montana essential oils with high concentration of carvacrol and thymol are the most effective against fungi taken into consideration in this study. Microencapsulation of essential oil in beta-cyclodextrine rather than decreasing, improves the antifungal properties of Satureja montana essential oil. As a final consideration emerging from the study, antifungal activity ranges are easily achieved between 20 and 100 μg/ml and encapsulation should infere better tolerability to Satureja montana oil thus extending ranges of application and possible industrial application to the pharmaceutical/nutritional/cosmetic fields. Further study are currently ongoing to assess formulation in finished product and tolerability studies in humans and in vitro.

CONFLICT OF INTERESTS

Declared None.

ACKNOWLEDGEMENTS

Authors are thankful to Tirana University, Ferrara University, National Drug Control Laboratory of Albania, S.M. whish to thank, Ministry of Education, University and Research (MIUR Italy) (PRIN, Grant 20105YY2HL_006) the University of Ferrara (grants FAR 2011, 2012, 2013) and Ambrosialab srl, Ferrara for the financial support.

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