Innovare
Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 7, 2014

Original Article

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

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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 T.violaceum, 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 Clevenger-type apparatus according to the Hungarian Pharmacopiea 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. [2,16,10]

Reagents

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

Fungal Strains

Fungal strains were developed by Dipartimento di Scienze della Vitae Biotecnologie, University of Ferrara. Dermatophytes: The dermatophytes used were *Arthroderma cajetanum* (Netherlands), CBS 495.70 strain; *Epidermophyton floccosum* var. floccosum (Netherlands) CBS 358.93 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 433.70 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 fluks is 300ml/min. The injector temperature is 280 and detector (FID) temperature is 300°C. Injection volum is 1µl. [3]

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 2.0x105 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 [3,15,18,20].

Tab 1: It shows the inhibition growth of *Satureja montana* essential oil to dermatophytes and phytopatogens

		% Inhibition of Growth		
Dermatophytes		***Satureja montana***	***S.montana-β CD 10:90***	***S.montana-β CD 20:80***
M. gypseum	20 µg/ml	18.56	33.67	+
	100 µg/ml	76.29	81.63	37.25
M. canis	20 µg/ml	1.80	7.69	9.78
	100 µg/ml	36.94	95.60	18.48
A. cajetani	20 µg/ml	4.84	26.23	0.00
	100 µg/ml	12.90	40.98	6.56
T. violaceum	20 µg/ml	28.57	23.33	10.34
	100 µg/ml	100.00	100.00	34.48
T. mentagrophytes	20 µg/ml	4.35	23.46	+
	100 µg/ml	77.17	100.00	9.59
E. floccosum	20 µg/ml	-12.50	17.86	+
	100 µg/ml	37.50	89.29	++
T. rubrum	20 µg/ml	12.90	44.44	+
	100 µg/ml	93.55	100.00	41.67
T. tonsurans	20 µg/ml	4.76	17.86	3.13
	100 µg/ml	83.33	92.86	6.25
Phytopatogens				
B.cinerea	20 µg/ml	5.16	-0.31	0.00
	100 µg/ml	14.68	58.49	3.80
P.oryzae	20 µg/ml	13.68	44.44	3.08
	100 µg/ml	42.11	92.59	21.54

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

Table 2: It shows Gas/Fid results of *Satureja montana* essential oil major components

	Farmaci Popullore (M1)	**Kerrabe (M2)**	**Kruja (M3)**	**Elbasan (M4)**
p-cymen	20.86	5.9	11.04	9.61
y-terpienen	2.05	8.45	3.07	2.86
borneol	0	0.2	0.67	4.76
thymol	6.2	18.2	3.94	25.78
carvacrol	6.11	67.68	46.18	64.22

Fig. 1: It shows inoculation of pregnated disks of *Satureja montana essential* oil.

DISCUSSION

Satureja montana essential oil had considerable inhibitory effects on following fungis Microsporum gypseum, Mycrosporum.canis, Nannizzia cajetani, Tricholosporum violaceum, Trichophyton mentagrophytes, Epidermophyton floccosum, Trichophyton rubrum, Trichophyton tonsurans and Phytopatogens, Botrytis cinerea and Pyricuhria oryzae expecially 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 sometime 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.

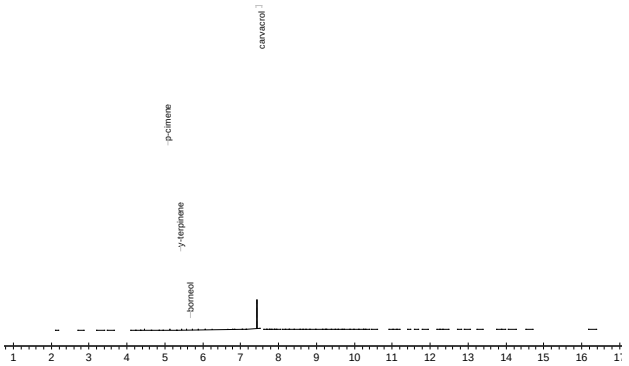

Fig. 2: It shows Gas/Fid chromatogram of Satureja montana (M1)

Furthermore, this occurrence can be explained by longer time of contact with fungis due to the slower release of the oil. Moreover, as shown in Table 1, the ratio oil/β-cyclodextrine10:90 has 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 so we suggest as optimal ratio 10:90. However, the improvements on antifungal activity and physic-chemical stability of microencaspultaion of essential oil in β-cyclodextrine have been clearly demostrated in this study. Another important consideration relates to range of concentrations: concetrations below 20 µg/ml are devoid of antifungal activity and on the other hand there is no need to use concentrations over 100µg/ml.

CONCLUSION

Satureja montana essential oils with high concentration of carvacrol and thymol are the most effective against fungis taken into consideration in this study. Microencaspulation 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 incapsulation 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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