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Original Article

EVALUATION OF ANTIFUNGAL ACTIVITY OF *ORIGANUM VULGARE AND ROSMARINUS OFFICINALIS* ESSENTIAL OIL BEFORE AND AFTER INCLUSION IN β-CYCLODEXTRINE

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ABSTRACT

Objective: The aim of this study is to evaluate and compare the antifungal activity of essential oil of *Origanum vulgare* and *Rosmarinus officinalis* collected in north region of Albania, and how is it modified by microencapsulation with β-cyclodextrin (β-CD).

Methods: Chemical composition of both isolated essential oils was determined by gas chromatography/mass spectrometry. The disc diffusion method was used to screen the antifungal activities of essential oils, before and after microencapsulation, 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.*

Results: The major identified compounds for Rosmarinus officinalis and Origanum vulgare essential oils, by GC/MS analyses, were respectively: 1, 8cineol, camphor, verbenone, borneol and carvacrol, thymol for O. vulgare essential oil. Maximum antifungal activity of essential oil of O. vulgare was observed against T. tonsurans, T. violaceum, T. floccosum,T. mentagrophytes. Meanwhile the essential oil of R. officinalis exhibits a moderate antifungal activity against T. violaceum. The essential oils demonstrated higher inhibition zones after microencapsulation in β-cylcodextrine.

Conclusion: From the results obtained we can conclude as follows: 1. Antifungal activity of Origanum vulgare essential oil is higher compare to the antifungal activity of Rosmarinus officinalis ones due to high content of carvacrol in Origanum vulgare essential oil. 2. Microencapsulation does not change the antifungal activity of both essential oils; 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 observed.

Keywords: *Origanum vulgare*, *Rosmarinus officinalis,* Essential oil, GC/MS, Antifungal activity, Microencapsulation, β-cyclodextrine.

INTRODUCTION

Aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of fungal disease. In this study we choose *Origanum vulgare* and *Rosmarinus officinalis* for investigation of the antifungal activities of their essential oils,in addition to our ongoing studies about antifungal activity of *Satureja montana* essential oil [4-7, 10-15, 17-18]. Origanum vulgare and Rosmarinus officinalis essential oil, due to their chemical composition, have many traditional uses based on their antibacterial, antifungal, carminative, spasmolytic, antioxidant activity. To explore their dermatological benefits we investigate the antifungal activity of essential oil from samples collected in north zones of Albania, before and after inclusion in β-cyclodextrine complexes. β-cyclodextrine is a polymer that can include in its structure different essential oils improving the stability in emulsions and compatibility with skin application. The ratios of oil: β-cyclodextrine used is 20:80 [27-29].

MATERIALS AND METHODS

Plant material

Herbal plants of *O. vulgare* and *R. officinalis* were collected from north zone of Albania. The plants were dried at room temperature and a voucher specimen of each plant sample was deposited at the Faculty of Pharmacy, University of Medicine,Tirana.

Isolation of the essential oil

The air-dried plant samples were subjected to hydrodistillation using a Clevenger-type apparatus following this procedure: 20 g of the plant material was stored in 500 ml flask where 250 ml of water R was used as distillation liquid. The distillation was performed for 3 h with a rate of 2-3 ml/min. The essential oil obtained was dried over anhydrous sodium sulphate and stored at 4 °C [1-3, 19, 23-24].

Reagents

All reagents and solvents used were obtained from Sigma Aldrich Company. β-Cyclodextrine was purchased from Titolchimica (Italy).

Fungal strains

Fungal strains were developed by Department of Science of Vita e Biotecnologie, University of Ferrara. Dermatophytes: Microsporum gypseum, Mycrosporum canis, Arthroderma cajetani, Tricholosporum violaceum, Trichophyton mentagrophytes, Epidermophyton floccosum, Trichophyton rubrum, Trichophyton tonsurans and Phytopatogens: Botrytis cinerea and Pyricuhria oryzae.

The chemical composition analysis of essential oils

The composition of essential oils was determined by gas chromatography-mass spectrometry (GC-MS) that was carried out on a Shimadzu GCMS-QP2010S gas chromatograph fitted with a fused silica HP-5mscapillary column (30 m x 0.25 mm, 0.25 µm film thickness) and with autosampler AOC-20. Column temperature was programmed from 60 °C to 240 °C at 3 °C min-1, and helium was used as carrier gas (1 ml/min). Other operating conditions were as follows: inlet pressure 9.43 psi, injector temperature 250 °C, detector temperature 280 °C, split ratio 1:25, injection volume 1 µl. Ionization of the sample components was performed in the EI mode, (70 eV), with scan range 20-555 amu, and scan time 1.60 s. Qualitative and quantitative analysis: The linear retention indices, RI, for all compounds were determined by injection of the hexane solution containing the homologous series of C8-C26 n-alkanes. The identification of the volatile constituents was accomplished by the visual interpretation, comparing their retention indices and mass spectra with literature data, by computer library search (HP

Chemstation computer library NBS75K. L, NIST/EPA/NIH Mass Spectral Library 2.0) and in the laboratory own database. Compounds concentrations (as % content) were calculated by integrating their corresponding chromatographic peak areas (TIC mode) [1, 3, 14, 25].

Complexation process

Complexes of β-cyclodextrin and essential oils were prepared by co-precipitation method in ratio 20:80 (w/w). A precipitation method was used to prepare the β-cyclodextrin complex (Reineccius 1989). Five grams of β-cyclodextrin was dissolved in 50 ml of an ethanol/water (1:2) mixture at 55 °C (±2). A predetermined quantity of essential oil dissolved in ethanol (10 % w/v) was then slowly added to the warm β-cyclodextrin solution. The mixture was continuously stirred on the magnetic stirrer and the temperature maintained at 55 °C. The mixture was stirred for another 4 h, without heating, while its temperature decreased spontaneously to 25 °C. The final solution was refrigerated overnight at 7 °C. The cold precipitated material was recovered by vacuum filtration. The precipitate was dried in a convection oven at 50 °C for 24 h. The powder was then allowed to air-dry at 25 °C for an additional 24 h in order for the powder to reach its equilibrium moisture content. The obtained complex was stored in airtight glass containers, at room temperature, prior to further analysis [26-30].

Antifungal activity

The essential oil samples before and after encapsulation were tested individually in triplicate for each colony for antifungal activity by the disc diffusion method using 100 µl of suspension of the tested fungal strains containing $2.0x10^5$ spore ml^{-1} colony Mueller--Hinton agar and dextrose agar were distributed to sterilized Petri dishes with a diameter of 9 cm. The filter paper discs (6 mm in diameter) were individually impregnated with 20 µl and 100 µl 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 DMSO [10, 11, 16, 18].

Table 1: It shows chemical compounds of *O. vulgare* essential oil analysed by GC/MS.

Compound	%
α-thujene	0.15
α-pinene	0.90
camphene	0.12
β-pinene	0.20
myrcene	1.37
α-phellandrene	0.65
δ-3-carene	0.02
α-terpinene	1.21
p-cymene	6.74
limonene	0.44
1.8-cineole	0.25
β-ocimene	0.12
γ-terpinene	3.75
terpinolene	0.12
linalool	2.55
borneol	0.35
terpinen-4-ol	0.55
α-terpineol	0.95
methyl eugenol	0.23
bornyl acetate	0.01
geraniol	1.22
thymol	4.76
carvacrol	60.31
eugenol	0.15
β-caryophyllene	1.05
α-humulene	0.25
caryophyllene. oxide	0.30

Table 2: It shows chemical compounds of *R. Officinalis* essential oil analysed by GC/MS

Compound	(%)
α-pinene	11.19
camphene	2.90
β-pinene	0.75
myrcene	1.26
felandrene	0.74
α-terpinene	0.34
p-cymene	0.38
limonene	2.94
1,8-cineole	16.67
terpinene	0.72
terpinolene	1.17
linalool	3.08
camphor	12.92
borneol	8.06
terpinen-4-ol	2.38
α-terpineol	1.94
verbenone	8.29
thymol	1.15
carvacrol	0.98
acetate borneili	3.15

Table 3: It shows the inhibition growth of *R. officinalis and O. vulgare* essential oil to dermatophytes and phytopatogens

% Inhibition of Growth					
		R. officinalis	***R. Officinalis/β-CD 20:80***	***O. vulgare***	***O. vulgare β-CD 20:80***
Dermatophytes					
M. gypseum	20 µg/ml	3.13	4.11	9.26	11.24
	100 µg/ml	14.58	13.91	62.04	67.09
M. canis	20 µg/ml	0.00	0.90	+	11.20
	100 µg/ml	0.00	1.80	53.72	59.06
A. cajetani	20 µg/ml	+	+	+	09.00
	100 µg/ml	+	+	41.94	51.89
T. violaceum	20 µg/ml	14.40	20.15	0.00	02.00
	100 µg/ml	20.45	21.65	100.00	100.00
T. mentagrophytes	20 µg/ml	8.93	9.84	13.28	24.67
	100 µg/ml	10.71	11.20	75.78	89.81
E. floccosum	20 µg/ml	+	+	8.11	18.45
	100 µg/ml	0.00	0.00	94.59	99.56
T. rubrum	20 µg/ml	17.95	18.13	13.95	26.11
	100 µg/ml	38.21	43.14	67.44	74.44
T. tonsurans	20 µg/ml	0.00	0.00	+	09.00
	100 µg/ml	4.26	6.23	100.00	100.0
Phytopatogens					
B. cinerea	20 µg/ml	0.00	0.00	2.82	08.00
	100 µg/ml	0.00	0.00	36.29	45.22
P. oryzae	20 µg/ml	8.25	8.78	15.56	19.67
	100 µg/ml	17.53	18.47	61.11	74.09

Studies were performed in triplicate. In addition, as negative controls is used DMSO. Nystatin was used as positive control 5 µg/ml. The inhibition zone of nystatin were 14.1-22.5 mm.

Table 4: It shows the inhibition zone of positive control used, nystatin.

	M. gypseum	***M. canis***	***A. cajetani***	***T. violaceum***	***T. mentagrophytes***	***E. floccosum***	***T. rubrum***	***T. tonsurans***	***B. cinerea P. oryzae***
Nystatin (5 mg/ml) inhibition zone (mm)	19.0	14.1	19.5	18.2	22.0	16.5	22.5	18.5	16.0

RESULTS AND DISCUSSION

Origanum vulgare essential oil demostrated considerable inhibitory effects (table 3) on following fungis (100 µg/ml): Tricholosporum violaceum (100.0 %),,Trichophyton tonsurans 100.0 %),, Epidermophyton floccosum(99.56%), Trichophyton mentagrophytes (89.81%), Trichophyton rubrum (74.44 %), Pyricuhria oryzae (74.09 %),, Microsporum gypseum (67.09%),, Mycrosporum canis, Arthroderma cajetani (51.89%),, Botrytis cinerea(45.22%).

Rosmarinus officinalis (100 µg/ml): essential oil had considerably effects on following fungis, Trichophyton rubrum(43.14%), Tricholosporum violaceum(21.65%), Pyricuhria oryzae(18.47%), Microsporum gypseum (13.91%), Trichophyton mentagrophytes (11.2%), and showed up a low antifungal activity against Trichophyton tonsurans(6.23%), Mycrosporum canis (1.85%), Epidermophyton floccosum(0%),Arthroderma cajetani (almost 0%) Botrytis cinerea (0%).

The data obtained by GC/MS analyses (table 1,2) showed that the main components of essential oils were: carvacrol (60.31%), p-cymene (6.74%), thymol (4.76%) for O. vulgare essential oil and 1,8 cineol (16.67%), camphor (12.92%), α-pinene (11.19%), verbenone (8.29%), borneol (8.06%) for R. officinalis.

It is evident that O. vulgare essential oil showed higher antifungal activity compare to R. officinalis essential oil and compareble inhibition zone to nystatin used as positive control. Correlating these data with those regarding the chemical composition of O. vulgare and R. officinalis essential oils, we can conclude that the strong antifungal activity demostrated from O. vulgare oil is due to the high concentration of carvacrol. According to the different studies, carvacrol is a powerful antifungal agent, and provides protection against mold and other common bacteria [8, 10-12, 20-22].

In addition in this study are used two different concentrations of essential oils 20 µg/ml and 100 µg/ml to evaluate their antifungal activity and concentration relationship. Concentration below 20 µg/ml is not advised because there is no antifungal activity and on the other hand there is suggested to use concentrations around 100 µg/ml.

The inhibition zones of both essential oils after encapsulation in β-cyclodextrine are considerably increased. This means that the encapsulation process doesn't decrease the antifungal activity of O. vulgare and R. officinalis essential oil, may be due to the slow releasing of oils from β-cyclodextrine, increasing their contact period with fungi strains.

CONCLUSION

Essential oils of O. vulgare and R. officinalis demonstrated antifungal activity. O. vulgare essential oils, due to the high concentration of carvacrol, exhibited higher antifungal activity compare to R. officinalis oil. Microencaspulation of essential oil in β-cyclodextrine doesn't affect negatively in antifungal properties of these essential oils, in contrary, the encapsulation increases the antifungal properties. This study suggests to use these two essential oils in encapsulated form as antifungal agent in dermatological formulations and industry applications for their considerably antifungal activity, extending ranges of application and possible industrial application to the pharmaceutical/nutritional/cosmetic. Further studies are currently ongoing to assess formulation in final product and tolerability in humans and *in vitro* applications.

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CONFLICT OF INTERESTS

Declared None.

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