Research Article

Study Of The Antifungal Activity Of Six Plants: *Teucrium capitatium L, Thymus cilitatus, Silene vulgaris, Origanum compactum, Anthemis nobilis and Rosmarinus officinalis*

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Abstract

From the ethnobotanical study we found a close relationship between plant species and described the different types of pathologies affecting the population. six plants (*Teucrium capitatium L, Thymus cilitatus, Silene vulgaris, Origanum compactum, Anthemis nobilis, Rosmarinus officinalis*) were chosen to test the study of their antimicrobial activity[1]. The essential oil extraction was performed by the steam distillation method. The antimicrobial activity test was performed on five bacterial strains (*Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Pseudomonas putida, Pseudomonas aureginosa*) [2] and for antifungal activity on two yeasts: *Candida albicans (Ca), and Candida glabrata (Cg)*, which are most frequently identified in clinical samples, a mold: *Aspergillus Niger* and 5 dermatophytes: *Microsporum canis (Mc), gypseum Microsporum, Trichophyton rubrum (Tr)*, T. mentagrophytes and E. floccosum.

The results show that the essential oil of *Teucrium capitatium* L has a great antibacterial activity with inhibition diameters vary between 40mm and 42mm in it reach a 100% inhibition index. Thus it has with the plant *Silene vulgaris* interesting antifungal activity in comparison with the other selected essential oils and Strains tested.

Keywords: *Teucrium capitatium L, Silene vulgaris,* essential oil, antibacterial, antifungal activity inhibition

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Introduction

Fungal infections are diseases caused by microscopic fungi called micromycetes.They are the consequence of the fungal parasite at the skin, mucous membranes, organs, central nervous system, [3] backbone. Fungal infections are divided into three categories: yeast, fungi and dermatophytes. Candidiasis are cosmopolitan illnesses in opportunistic majority caused by Candida yeasts. Their clinical spectrum is diverse, it is superficial damage (especially respiratory, gastrointestinal and genital mucosa) maps the root or scattered. The role of the land and that of the supporting factors are fundamental to the occurrence and development of candidiasis. [4] Ringworm is frequent reasons for consultation. They are due to filamentous fungi, dermatophytes belong to three genera: Epidermophyton, Microsporum, Trichophyton. In this work we will have an interest in the study of the antifungal activity of plants selected from an ethnobotanical study in the area of El Hajeb.

Material and methods:

The plant material is made of sheets of 6 plants; the leaves were dried in sunlight for 2 weeks.

Hydrodistillation:

The extraction of essential oils by steam distillation was conducted in a Clevenger type apparatus, 100g of powdered

plant material with 500ml of water was distilled for 3 hours. The yield was determined for each plant essential oil was stored at 4 ° C in the dark in the presence of anhydrous sodium sulphate

Aqueous extraction:
100 g of powder is extracted from the plant Silene vulgaris by heat at reflux for 2 hours in water, after filtration and evaporation in a Rotavapor at 60°C , the residue obtained is kept until use.

Antifungal activity:

Preparation of Cultures:
The white 6 mm diameter disks were impregnated with 5µl, 10µl, 15µl, 20µl and of the prepared test solution. The disks were placed in petri dishes to which were dried. Fungal strains tested were grown in boxes of kneaded and incubated for 24 h at 37 ° C to obtain a young growing fungal colonies are collected using a pipete pastor and have been in 10 ml of sterile saline.

The selected strains:
The selected fungal isolates are the following two yeast ***Candida albicans (Ca), Candida glabrata (Cg)*** that are most frequently identified in clinical samples, a mold: ***Aspergillus Niger and 5 dermatophytes: Microsporum canis (Mc),Microsporum gypseum (Mg) and Trichophyton rubrum (Tr). T. mentagrophytes, and E. floccosum,*** insulators are obtained after incubation at 28 ° C for 48 hours for yeasts of the genus ***Candida, Aspergillus Niger*** for 72h and 1 week to 1 month for dermatophytes. They are then maintained by regular subculture. The source of stem is the national center for scientific and technical research and Ifrane health delegation.

Disk diffusion method
The antimicrobial activity was determined by the disc diffusion method [5], The method comprises spreading 100 µl of suspension of 106 CFU / ml of microbial cells on petri dishes containing PDA culture media SDA and considering that each box includes disks impregnated with different essential oils and extracts of Silene vulgaris while retaining blank discs asnegative control. The plates were stored at 4 ° C for 1 h. Then, they were incubated for 48 h at 30 ° C. The antimicrobial activity was determined by measuring the growth inhibition zone in millimeters. To develop a comparative study between the antifungal activity of essential oils and ***Silene vulgaris*** extract and antibiotics references, a reference antibiotic disc will be applied to each box as an effective positive control against microbial species.

Determination of the minimum inhibitory concentration:
Is carried out a serial dilution in peptone water microbiennne culture (2 × 106 CFU / ml). After we took 100 ml of each culture were suspended in Sabouraud containing different concentrations ranging from 10µl / ml up to 1000 µl / ml**[6]**. The positive control consisted of Sabouraud broth inoculated only with microbial suspension. The uninoculated tube containing extract was used as negative control. The tubes were incubated for 72 h at 30 ° C. Microbial growth is indicated by the presence of turbidity on the bottom of the tube. The MIC is defined as the lowest concentration of a given extract capable of interrupting, in a liquid medium and for well-defined conditions, any visible growth of a particular microbial strain. The first tube, in ascending order, which has not produced a tube bottom, corresponds to the IJC. The antimicrobial activity of various extracts was studied using the broth method [7].

Determining the minimum fungicidal concentration:
The minimum fungicidal concentration (MFC) was defined as the lowest concentration which completely inhibits microbial growth (≥99, 99%) of the initial inoculum after 48 h incubation at 30 ° C. after confirmation of CMI, CMF is determined using the following procedure: 100 ml of each broth was seeded into petri dishes containing PDA and SDA for the yeast and fungi respectively.

Results and Discussion:
The results of the antifungal activity of the essential oils of various plants and extract Silene vulgaris are summarized in the following tables:

Table 1 Inhibition zone of plants selected against the tested fungi

Plant / Activity / Stem	*Thymus cilitatus*	*Teucrium capitatium L*	*Origanum compactum*	*Silène vulgaris*	*Anthemis nobilis*	*Rosmarinus officinalis*
Candida albicans	**13**± 0,1	**43**± 0,1	**12**±± 0,1	**14**± 0,1	**13**± 0,1	**10,5**± 0,1
Candida tropicalis	**13**± 0,1	**40**± 0,1	**12,5**± 0,1	**<3**± 0,1	**11**± 0,1	**14**± 0,1
Aspergillus niger	**11,5**± 0,1	**43**± 0,1	**11**± 0,1	**12**± 0,1	**12**± 0,1	**13**± 0,1
Microsporum canis	**10,5**± 0,1	**42**± 0,1	**13**± 0,1	**16**± 0,1	**13**± 0,1	**11**± 0,1
Microsporum gypseum	**11**± 0,1	**40,5**± 0,1	**9,5**± 0,1	**10,5**± 0,1	**15**± 0,1	**11**± 0,1
Trichophyton rubrum	**11**± 0,1	**39**± 0,1	**9**± 0,1	**18**± 0,1	**<4**± 0,1	**11,5**± 0,1
T. mentagrophytes	**12**± 0,1	**38,5**± 0,1	**9**± 0,1	**18**± 0,1	**14**± 0,1	**<2**± 0,1
E. floccosum	**11,5**± 0,1	**38**± 0,1	**<2**± 0,1	**14**± 0,1	**14**± 0,1	**10**± 0,1

Table 2 Determination of the minimum inhibitory concentration of essential oil of Teucrium capitatium

Dilutions	**0**	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**	**1/50**	**1/64**	**1/80**	**1/100**
µl/ml (HE/Eau)	**SM**	**1000**	**333.3**	**142.86**	**66.66**	**32.26**	**20.41**	**15.87**	**12.66**	**10.10**
Candida albicans	_	_	_	_	_	_	_	_	_+	++
Candida glabrata	_	_	_	_	_	_	_	_+	+	++
Aspergillus niger	_	_	_	_	_	_	_	_	_+	++
Microsporum canis	_	_	_	_	_	_	_	_	_+	++
Microsporum gypseum	_	_	_	_	_	_	_	_+	+	++
Trichophyton rubrum	_	_	_	_	_	_	_+	+	++	++
T. mentagrophytes	_	_	_	_	_	_	_+	+	++	++
E. floccosum	_	_	_	_	_	_	_+	+	++	++

Table 3 Determination of the minimum inhibitory concentration of Silene vulgaris extrac

Dilutions	**0**	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**	**1/50**	**1/64**	**1/80**	**1/100**
µl/ml (Extrait/Eau)	SM	1000	333.3	142.86	66.66	32.26	20.41	15.87	12.66	10.10
Candida albicans	_	_	_	_	_ +	_ +	_ +	++	++	++
Candida *glabrata*	+	+	+	+	+	+	++	++	++	++
Aspergillus niger	_	_	_	_ +	_ +	_ +	_ +	++	++	++
Microsporum canis	_	_	_	_	_	_	+	++	++	++
Microsporum gypseum	_	_	_ +	_ +	++	++	++	++	++	++
Trichophyton rubrum	_	_	_	_	_	_	+	++	++	++
T. mentagrophytes	_	_	_	_	_	_	_+	++	++	++
E. floccosum	_	_	_	_	_	_ +	_ +	++	++	++

Table 4 Determination of the minimum inhibitory concentration of essential oil of Thymus ciliates

Dilutions	**0**	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**	**1/50**	**1/64**	**1/80**	**1/100**
µl/ml (HE/Eau)	SM	1000	333.3	142.86	66.66	32.26	20.41	15.87	12.66	10.10
Candida albicans	_	_	_	_	_	_ +	_ +	++	++	++
Candida *glabrata*	_	_	_	_	_ +	_ +	+	++	++	++
Aspergillus niger	_	_	_	_	_	_ +	+	++	++	++
Microsporum canis	_	_	_	_	_	_ +	+	++	++	++
Microsporum gypseum	_	_	_	_	_ +	_ +	+	++	++	++
Trichophyton rubrum	_	_	_	_	_	_ +	+	++	++	++
T. mentagrophytes	_	_	_	_	_	_ +	+	++	++	++
E. floccosum	_	_	_	_	_	_ +	_+	++	++	++

Table 5 Determination of the minimum inhibitory concentration of essential oil of origanum compactum

Dilutions	**0**	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**	**1/50**	**1/64**	**1/80**	**1/100**
µl/ml (HE/Eau)	SM	1000	333.3	142.86	66.66	32.26	20.41	15.87	12.66	10.10
Candida albicans	_	_	_	_	_ +	+	++	++	++	++
Candida *glabrata*	_	_	_	_	_	_ +	+	++	++	++
Aspergillus niger	_	_	_	_	_	_ +	_ +	+	++	++
Microsporum canis	_	_	_	_	_	_+	+	++	++	++
Microsporum gypseum	_	_	_	_	_	_ +	_ +	++	++	++
Trichophyton rubrum	_	_	_	_	_ +	_ +	_ +	++	++	++
T. mentagrophytes	_	_	_	_	_ +	_ +	+	++	++	++
E. floccosum	++	++	++	++	++	++	++	++	++	++

Table 6 Determination of the minimum inhibitory concentration of essential oil of *Rosmarinus officinalis*

Dilutions	**0**	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**	**1/50**	**1/64**	**1/80**	**1/100**
µl/ml (HE/Eau)	SM	1000	333.3	142.86	66.66	32.26	20.41	15.87	12.66	10.10
Candida albicans	_	_	_	_ +	_ +	+	++	++	++	++
Candida glabrata	_	_	_	_	_	_ +	_+	+	++	++
Aspergillus niger	_	_	_	_	_	_+	+	++	++	++
Microsporum canis	_	_	_	_	_ +	+	++	++	++	++
Microsporum gypseum	_	_	_	_	_ +	+	++	++	++	++
Trichophyton rubrum	_	_	_	_ +	_ +	+	++	++	++	++
T. mentagrophytes	++	++	++	++	++	++	++	++	++	++
E. floccosum	_	_	_	_ +	+	++	++	++	++	++

Table 7 Determination of the minimum inhibitory concentration of essential oil of *Anthemis nobilis*

Dilutions	0	1/2	1/4	1/8	1/16	1/32	1/50	1/64	1/80	1/100
µl/ml (HE/Eau)	SM	1000	333.3	142.86	66.66	32.26	20.41	15.87	12.66	10.10
Candida albicans	_	_	_	_	_ +	_+	_ +	++	++	++
Candida *glabrata*	_	_	_	_ +	_ +	+	++	++	++	++
Aspergillus niger	_	_	_	_	_+	_ +	+	++	++	++
Microsporum canis	_	_	_	_	_	_ +	_+	++	++	++
Microsporum gypseum	_	_	_	_	_	_ +	_ +	++	++	++
Trichophyton rubrum	++	++	++	++	++	++	++	++	++	++
T. mentagrophytes	_	_	_	_	_+	_+	+	++	++	++
E. floccosum	_	_	_	_	_	_+	++	++	++	++

Table 8 Determination of the nature of the antifungal activity

Nature of Activity / Stem	*Teucrium capitatiumL* MCF/MIC	NA	*Silène vulgaris* MCF/MIC	NA	*Thymus ciliatus* MCF/MIC	NA	*Origanium capactum* MCF/MIC	NA	*Rosamarin officinalis* MCF/MIC	NA	*Anthemis nobilis* MCF/MIC	NA
Candida albicans	1,25	FUNGICIDE	2,14		2,06	FS	2,14	FS	2,33	FS	2,14	FS
Candida tropicalis	1,28		No Inhibition		2,14		2,06		2,06		2,33	
Aspergillus niger	1,25		2,33		2,06		2,06		2,06		2,14	
Microsporum canis	1,25		1,58	FC	2,06		2,06		2 ,14		2,06	
Microsporum gypseum	1,28		3		2,14		2,14		2,14		2,06	
Trichophyton rubrum	1,58		1,58		2,06		2,14		2,33		No Inhibition	
T. mentagrophyte	1,58		1 ,58	FC	2,06		2,14		No Inhibition		2,14	
E. floccosum	1,58		2,06		2,06		No inhibition		2,33		2,06	

FC fungicides FS fungistatic NI No inhibition

NA: Nature Activity; **MIC:** Minimum Inhibitory Concentration; **MCF**: Minimum Concentration Fungicide

Discussion:

In recent years we have witnessed a change in the epidemiology of invasive fungal infection. This coincided with an increase in the survival of a number of increasingly important in immunosuppressed patients [8];[9] and the development of more serious forms of immunosuppression. The species of Candida and Aspergillus remain the leading cause of fungal infectious diseases [10]. Candida albicans is involved in approximately 50% of patients with candidiasis [9], but infections caused by other species, such as Candida glabrata, Candida tropicalis, Candida krusei, Candida parapsilosis and Candida lusitaniae, are up [11]. Over the past two decades there has been an increase in the number of antifungal drugs available for the treatment of invasive fungal infections. They are divided into three classes: azoles, polyenes and echinocandins [12]. All this led us to seek the therapeutic imapacte six plants selected from an ethnobotanical study of the region of El Hajeb against skin diseases through their antibacterial and antifungal activity.

The results listed in Table 1 revealed that all selected plants exert antifungal activity of all strains except for plants: ***Silene vulgaris, Anthemis nobilis, Rosmarinus officinalis, Origanum compactum*** vis-à-vis stem respectively *Candida tropicalis, Trichophyton rubrum, T. mentagrophytes E. floccosum.*

The essential oil of T. capIitatium L has a great antibacterial activity with inhibition diameters vary between 40mm and 43mm in relation to other plants. She has had a significant inhibitory activity with respect to any fungal strains *Trichophyton rubrum, T. mentagrophytes and E. floccosum* were inhibited tottaly from the minimum concentration of 32,26 µl / ml, and the Microsporum strains gypseum Candida glabrata from 20,41 µl/ ml while the concentration of 15,87 µl / ml was sufficient to stop the growth of Candida albicans, Microsporum canis and Aspergillus Niger, the results of each minimum concentration for other plants are presented in the 3,4,5,6,7 tables. The ratio of the minimum inhibitory concentration and minimum fungicidal concentration presented in Table 8 gave the result that the plant ***Teucrium capitatium L*** has a fungicidal activity against other plants that exhibit fungistatic activity, except for ***Silene vulgaris*** extract which is a fungicide for stem ***Microsporium canis, Trichophyton rubrum, T.mentagrophytes;*** hence it can be said that the plant Teucrium capitatium L and Silene vulgaris present a promising approach to identify active molecules of these two plants based on the phytochemical analysis and chromatographic.

Conclusion:

This study, again, the development of the exploitation of essential oils in the fields, pharmaceutical and medicinal and open their doors for the operation of these plants in pharmaceutical market.

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