

Study on the antifungal activity of two extracts of *Eugenia aromatica* and *Rosemarinus officinalis*

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ABSTRACT

Extracts of two plant species (*Eugenia aromatica* and *Rosemarinus officinalis*) were evaluated for their antifungal effect on the mycelial growth of *Candida* species (*Candida tropicalis*, *C. parapsilosis*, *C. albicans* and *C. krusei*) under laboratory conditions. Results showed that the extract of the two plant species exhibited a variable degree of effect against *Candida* species. Also, variable susceptibility of the tested fungal species to the two plant species was noticed. The extracts of the tested plants were found to be effective against mycelial growth of *Candida* standard species when compared to the control. High levels of the tested extracts were most toxic against of the four *Candida* species. Also, results obtained with the domestic fungal species, and their isolates were almost the same as in all introduced fungi. When compared between the four domestic isolates or extract of the two plant species, no significant differences were observed by any of them.

INTRODUCTION

The fungi i.e. *Candida tropicalis*, *C. parapsilosis*, *C. albicans* and *C. krusei* are causing acute vaginal inflammations. Therefore, females used the herbal therapy to treat the symptoms caused by these fungi infection (Ramadan *et al.*, 1994; Bara and Vanetti, 1995 and Shobha *et al.*, 1996). Usually the antibiotics are used to treat diseases caused from *Candida* species, such as: Fluconazole with dose, 50-100 mg for 1-4 weeks, Itrazol with dose, 100-200 mg for weeks to 7 months.

Medicinal plants and herbs have clear clinical effect and high cure rate from certain diseases. Huge number of such plants has been reported (Al-Rawi, 1964). Crude extract, essential oils and volatile components from

these plants have been tested for that matter. Clove (*Eugenia caryophyllus* L., *Syzygium aromaticum* L.) and rosemary (*Rosmarinus officinalis* L.) were among the plants that were investigated for this purpose. Many studies on crude extracts from clove and rosemary have been tested for their antifungal effects (Ramadan *et al.*, 1994; Tombe *et al.*, 1995 and Khalil, 2001). They reported their effect may be attributed to the antifungal activity of the natural components or its chemical compounds.

Different methods have been used for separation and identification of compounds from clove (Myint *et al.*, 1995; Fuh *et al.*, 1996 and Medvedeff *et al.*, 1997), and rosemary (Al-Hader *et al.*, 1994 and Okamura *et al.*, 1994). The compounds isolated from clove included eugenol, alpha, and beta coryophyllene, eugenol acetate (Fuh *et al.*, 1996), and carvacrol (Martini *et al.*, 1996). The compounds isolated from rosemary included diterpenoids, triterpenoids, verbenone, camphor, benzyl acetate, limonine, alpha-pinene, cineole (eucalyptole) flavenoids (antioxidants), berneol, P-cymene (Al-Hader *et al.*, 1994; Haraguchi *et al.*, 1995 and Temes and Schwarz, 1995).

Among these compounds were ones which proved to be antifungal (Soliman *et al.*, 1994; Tombe *et al.*, 1995; Martini *et al.*, 1996 and Medvedeff *et al.*, 1997). The objective of this research was to determine the effects of extracts of two plant species (*E. aromatica* and *R. officinalis*) on four of the standard fungal species which causing female genital tract inflammation and also to determine their effect on the isolates of four tested fungi which were isolated from three patients at one of Riyadh Hospitals.

MATERIALS AND METHODS

Four fungal species, namely *Candida tropicalis*, *C. krusei*, *C. parapsilosis*, and *C. albicans* were used in this study. These fungi were obtained from Diffco Laboratories, Mi., U.S.A and the three clinical isolates of each fungus mentioned above were obtained from three patients at one of Riyadh Hospitals. Each fungal species was propagated on Czapek's agar medium for further studies. Extracts of two medicinal plants; *Eugenia aromatica* and *Rosmarinus officinalis* were tested for their antifungal activity. Samples of 20 and 40 g of each of the tested medical plant species (all parts) were separately extracted by boiling in 100 ml sterilized distilled water for 30 min. The extracts were then filtered through two layers of

sterilized cheese-cloth. The filtrate was centrifuged at 4000 rpm for 20 min. The yielded supernatants were sterilized through centered glass (G4) to be used in further studies.

The concentration 2 and 4% of *E. aromatica* and *R. officinalis* extracts were prepared by adding suitable amount of sterilized distilled water to the crude extracts of either *E. aromatica* or *R. officinalis* (V/V). Two or four ml of each tested concentration was added to Czapek's broth medium in conical flasks before solidification. Inoculation was done with fungal discs 5 mm in diameter obtained from four standard species of *Candida* and three clinical isolates of *Candida tropicalis*, *C. krusei*, *C. parapsilosis* and *C. albicans*, which obtained from three patients at one of Riyadh Hospital, 7 days old culture. Four replicates were used for each tested concentration.

Another group of Czapek's broth medium conical flasks free from plant extracts and inoculated with the tested fungi species was used as check treatment. All conical flasks were incubated at $25\pm 2^{\circ}\text{C}$ for 7 days. Dry and fresh weights were determined. The obtained data were statistically analyzed, according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The antifungal properties of the two aqueous plant extracts (*E. aromatica* and *R. officinalis*) against *Candida* species were evaluated *in vitro*. Data in Table (1) showed that clove or rosemary extracts exhibited a variable degree of antifungal activity against *Candida* species and their antifungal effect decreased with dilution. All the four tested fungi species (*Candida tropicalis*, *C. albicans*, *C. parapsilosis* and *C. krusei*) were significantly affected in terms of reduced the mycelial growth (fresh and dry weight) of four *Candida* species when compared to control. Data also showed that rosemary extract was more effective against *Candida albicans* than clove extract. All the tested concentrations significantly reduced mycelial growth of the four tested *Candida* species as compared to the control. Also, clove extract gave the highest mycelial growth (fresh weight) of *C. albicans* followed by other fungi. However, rosemary with *C. albicans* or clove with *C. tropicalis* gave the lowest mycelial growth of dry weight.

Table (1). Effect of extract of *Eugenia aromatica* and *Rosemarinus officinalis* on the *Candida* standard species.

Plant extracts	Rate g/l	Tested fungi							
		<i>Candida tropicalis</i>		<i>C. parapsilosis</i>		<i>C. albicans</i>		<i>C. krusei</i>	
		Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
<i>Eugenia aromatica</i>	20	46.34	29.34	44.30	30.78	46.50	30.70	44.00	30.00
$\bar{X}$	40	40.11	24.11	42.41	26.40	43.50	25.00	40.00	26.33
<i>Rosemarinus officinalis</i>	20	43.22	26.72	43.35	28.59	45.00	27.85	42.00	28.16
$\bar{X}$	40	44.46	32.12	41.33	34.66	40.30	29.40	42.50	34.20
$\bar{X}$	40	40.25	26.23	37.55	26.53	37.30	24.40	41.22	28.00
$\bar{X}$	40	42.35	29.17	39.44	30.59	38.80	26.90	41.86	31.10
control	0	50.23	33.24	51.50	34.30	50.00	35.00	50.00	35.00

L.S.D. at 5% for: Extract (a): 1.46; Rate (b): 2.16; Fungi (c): 1.98; a x b x c: 3.0

Results presented in Table (2) indicate that extracts of *E. aromatica* or *R. officinalis* affected three *C. tropicalis* isolated from patients at a domestic hospital at Riyadh, when compared to control. No different responses were observed among three isolates of *C. tropicalis* or both tested plant extracts. High level from both extracts gave the highly effect on the fresh and dry weights of the three *C. tropicalis* isolates. The trend of results obtained for the three *C. tropicalis* isolates and extract of both plants was almost the same as in the isolates of *C. albicans*, *C. kruesi*, and *C. parapsilosis*.

The higher effects which formed by *E. aromatica* and *R. officinalis* extract against most the fungi species (Tables 1 and 2), suggest the greater concentration or the stronger effect of the antifungal agents in the 40 g extract of each of the two plant species. Effect of the antifungal activity demonstrated in this study for extracts of *E. aromatica* and *R. officinalis* may be due to its chemical compounds or natural components (Cowan, 1999). Earlier studies indicated antimicrobial activity of such plant extracts. As a fact, antifungal activity of clove oil has been reported against several pathogenic fungi (Medvedeff *et al.*, 1997) and that of eugenol has been reported against other fungi (Tombe *et al.*, 1995). Antimicrobial activity against human pathogenic fungi has been reported both in garlic as well as in clove extracts (Arora *et al.*, 1999). Antifungal activity of clove extract (eugenol) and *Rosmarinus officinalis* L. have been investigated in several reports (Soliman *et al.*, 1994; Tombe *et al.*, 1995 and Medvedeff *et al.*, 1997). Our study agreed with the above reported investigation in the antifungal activity of clove or rosmariny extract.

Therefore, it could be concluded that as far as natural extracts of antifungal activity was concerned, both tested extracts at a high level were play an important role in reducing the growth of *Candida* species, which caused female genital tract inflammation diseases.

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Table (2). Effect of *Eugenia aromatica* and *Rosemarinus officinalis* extracts on the three clinical isolates of *Candida* from patients at one of Riyadh Hospitals.

Plant extract	Rate g/l	Fungus isolates											
		Isolates of <i>C. tropicalis</i>						Isolates of <i>C. parapsilosis</i>					
		Isolate (11)		Isolate (12)		Isolate (13)		Isolate (11)		Isolate (12)		Isolate (13)	
		Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
<i>Eugenia aromatica</i>	2	45.00	29.00	45.00	28.10	46.10	29.11	44.30	30.78	44.00	30.12	43.99	31.00
	4	40.11	24.11	39.22	23.10	41.00	24.25	42.41	26.40	41.11	26.00	40.98	25.77
$\bar{X}$		43.05	26.50	42.11	25.60	43.55	26.68	43.35	28.59	42.55	28.06	42.48	28.38
<i>Rosemarinus officinalis</i>	2	44.45	32.12	44.00	31.12	43.99	31.90	41.33	34.66	41.00	34.00	40.98	33.99
	4	40.25	26.23	40.00	25.23	39.98	26.00	37.33	28.53	37.00	28.00	36.87	27.77
$\bar{X}$		42.35	29.17	42.00	28.17	37.48	28.95	39.33	31.59	39.00	31.00	38.92	30.88
Control	0	50.00	33.00	50.11	33.22	49.99	32.00	50.50	33.30	50.00	33.00	50.70	32.65
L.S.D. at 5% for													
Extract (a)	:												
Rate (b)	:												
Isolate (c)	:												
a x b x c	:												
		1.94						1.33					
		3.01						2.51					
		2.04						1.98					
		5.20						3.99					

Continued.

Plant extract	Rate g/l	Fungus isolates											
		Isolates of <i>C. albicans</i>						Isolates of <i>C. krusei</i>					
		Isolate (11)		Isolate (12)		Isolate (13)		Isolate (11)		Isolate (12)		Isolate (13)	
		Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
<i>Eugenia aromatica</i>	20	46.50	28.70	46.00	27.55	45.99	28.00	44.00	30.00	44.10	30.11	43.98	29.98
	40	43.50	23.00	42.98	23.34	43.00	22.99	40.00	26.33	40.20	26.00	44.22	25.78
$\bar{X}$		45.00	25.85	44.49	25.44	44.49	25.99	42.00	28.16	42.15	28.00	44.10	27.88
<i>Rosemarinus officinalis</i>	20	40.30	29.40	40.00	28.99	41.00	29.00	42.50	34.20	42.44	34.00	42.00	43.10
	40	37.30	24.40	36.78	24.00	37.00	23.00	41.22	28.00	40.99	27.87	41.00	28.10
$\bar{X}$		38.80	26.90	38.39	26.49	39.00	26.00	41.86	31.10	41.71	30.93	41.50	30.60
Control	0	51.00	35.20	51.10	35.00	50.76	34.89	50.20	35.00	50.00	35.12	50.11	35.20

L.S.D. at 5% for

Extract (a)	:	2.04	1.99
Rate (b)	:	2.33	2.01
Isolate (c)	:	1.09	1.08
a x b x c	:	4.21	3.33

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دراسة علي النشاط المضاد لفطر الكنديدا بواسطة مستخلصين نباتيين
Eugenia aromatica & Rosemarinus officinalis

جهان بنت سعود بن راشد البراهيم
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تمت دراسة تأثير مستخلصين من نبات الأيوحينا آروماتيكا والروسماريتس أوفيثينالس على النمو الميسليومي لأربع أنواع من فطر الكنديدا في المعمل ومن النتائج المتحصل عليها :

- أظهرت المستخلصات النباتية لكل من النوعين النباتيين درجة من التغير في النشاط المضاد على أنواع فطر الكنديدا .
- جميع الأنواع الفطرية تأثرت بالمستخلصات النباتية ، ولكن تأثيرها كان أكثر وضوحاً على النمو الميسليومي للفطريات المستوردة من الكنديدا (عند المقارنة بالكنترول) .
- التركيز العالي في كلا من المستخلصين النباتيين كان أكثر تأثيراً على الفطريات محل الدراسة.
- جميع الأنواع الفطرية وعزلاتها المحلية أعطت تقريباً نفس النتائج المتحصل عليها مع العزلات الفطرية المستوردة .
- فروق معنوية بين المستخلصين النباتيين أو بين عزلات الأربع فطريات المحلية أو بين أى منهم