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Research Article

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Evaluation of anti-*Candida albicans* activity of essential oils of six medicinal plants: Synergy concept

Hakim El Alama[1,2*], Aïcha El Aissami[1], Amal Ait Haj Said[3] and Fatima Ezzahra El Alaoui-Faris[1]

[1]*Laboratory of Botany, Mycology and Environment, Faculty of Sciences of Rabat, Mohammed V University - Agdal, 4, Avenue Ibn Battouta, Rabat, Morocco*
[2]*Hospital Pharmacy Ibn Rushd, University Hospital Center Ibn Rushd of Casablanca, 1, Rue des Hôpitaux, Casablanca, Morocco*
[3]*Laboratory of Pharmacognosy, Faculty of Medicine and Pharmacy of Casablanca, Hassan II University, 19, rue Tarik Ibn Ziad, Casablanca, Morocco*

ABSTRACT

This study is to evaluate the anti- Candida albicans activity of essential oils of six medicinal plants and to seek some synergistic associations between essential oils or essential oils and a reference antifungal. Essential oils are extracted from six medicinal plants; their antifungal power is studied alone or in combination. The culture medium is Sabouraud - chloramphenicol (0.5 g / L), the minimum inhibitory concentrations (MIC) are determined by the macrodilution method. The dilution solution is 0.2% agar. Essential oils studied have a strong anti-Candida albicans power; the associations show a synergistic effect between these oils in most cases. Combination of essential oils with amphotericin B reduces the MIC of the antifungal and therefore reflects a synergistic association. This study shows that essential oils studied exert a synergistic effect between them and in combination with amphotericin B, which can reduce the doses of this product while increasing anti- Candida albicans activity.

Key words: *Candida albicans*, essential oils, amphotericin B, synergy.

INTRODUCTION

Essential oils are, according to the National Agency for Medicines and Health Products Safety (ANSM), fragrant product, usually complex composition obtained from a vegetable raw material botanically defined, by steam drive water, by dry distillation or by a suitable mechanical process without heating. The essential oil is usually separated from the aqueous phase by a physical method involving no significant change in its composition [1], it is natural defense products against external aggressions [2], thus they have proven effective against microorganisms [3-6].

Studies evaluating the antifungal activity, especially of the species *Candida albicans*, of essential oils are numerous, however, are rare those who study the synergy between either essential oils or in combination with a reference antifungal.

Anti-*Candida albicans* treatment must be safe, effective and low cost, though these qualities are almost absent in marketed products; because side effects, toxicity, long treatment duration of up to several months, and there resistance for these active principles. Essential oils can be an alternative anti-candida treatment as monotherapy or in combination provided they meet the validation requirements of a valid treatment.

This study is to evaluate the anti- *Candida albicans* activity of essential oils of six medicinal plants and to seek some synergistic associations between essential oils or essential oils and a reference antifungal.

EXPERIMENTAL SECTION

Plant species: Studied plant species are: *Artemisia vulgaris* L., *Ocimum basilicum* L., *Syzygium aromaticum* L., *Thymus vulgaris* L., *Origanum vulgare*, *Rosmarinus officinalis* L ..

Essential oils: Essential oils are obtained by steam distillation which is accomplished using a Clevenger-type device [7] and have been packaged in glass bottles stained and stored away from light in a 4 ° C [8].

***Candida albicans* strain:** Yeast derived from a reference strain of *Candida albicans* ATCC 90029.

Culture medium: Culture medium used is the Sabouraud-chloramphenicol (0.5 g / L) [9].

Antifungal Reference: Reference antifungal studied is amphotericin B [10].

Evaluation of the anti-*Candida albicans* activity: The technique used is macromethod in liquid medium as recommended by the NCCLS (National Committee for Clinical Laboratory Standards) [11].

Determination of Minimum Inhibitory Concentration (MIC): MIC determination was done according to the macrodilution technique in liquid medium, cascading series of dilution in 0.2% agar solution was performed [12].

RESULTS AND DISCUSSION

Essential oils have investigated an inhibiting power higher than 100%, however, amphotericin B has an inhibitory power less than 100% which is of the order of 82.25% (Table 1 and 2).

Table-1 Essential oils inhibition rate on the growth of *Candida albicans*

Essential oil	Growth Inhibition of *Candida albicans* rate (%)
Artemisia vulgaris L.	110.62
Ocimum basilicum L.	112.65
Syzygium aromaticum L.	100.60
Thymus vulgaris L.	112.64
Origanum vulgare	108.32
Rosmarinus officinalis L.	101.25

Table-2 Inhibition rate of amphotericin B on the growth of *Candida albicans*

Antifungal	Growth Inhibition of *Candida albicans* rate (%)
Amphotéricin B	82.25 %

The associations; *Syzygium aromaticum* / *Thymus vulgaris* L. and *Origanum vulgare* / *Rosmarinus officinalis* L. retain inhibitor power over 100% , that of *Artemisia vulgaris L.* / *Ocimum basilicum L.* association decreased while remaining close to 100% (Table 3).

Table-3 Inhibition rate of the combination of essential oils on the growth of *Candida albicans*

Essential oil	Growth Inhibition of *Candida albicans* rate (%)
Artemisia vulgaris L. + Ocimum basilicum L.	98.62
Syzygium aromaticum L. + Thymus vulgaris L.	110.75
Origanum vulgare + Rosmarinus officinalis L.	102.27

Table-4 Inhibition rate of the growth of *Candida albicans* at different concentrations

Essential oil	Inhibition rate of the growth of *Candida albicans* (%) Essential oil concentration % (v/v)			
	0.125	0.25	0.5	1
Artemisia vulgaris L.	12.36	32.45	103.61	109.32
Ocimum basilicum L.	29.38	59.92	89.64	114.27
Syzygium aromaticum L.	5.37	68.87	101.06	116.24
Thymus vulgaris L.	15.07	70.23	82.03	113.23
Origanum vulgare	16.24	71.01	92.03	117.29
Rosmarinus officinalis L.	0	2.35	84.29	101.84

This rate of inhibition is a function of concentration; it is strictly proportional and reached its maximum at concentrations of 0.5 to 1% (v/v) (Table 4 and 5).

Table-5 Inhibition rate of the growth of *Candida albicans* at different concentrations

Antifungal	Inhibition rate of the growth of *Candida albicans (%)*			
	Antifungal concentration % (v/v)			
	0.125	0.25	0.5	1
Amphotéricin B	13.45	52.37	94.07	95.58

Minimum inhibitory concentrations are used to classify these essential oils into three categories (Table 6 and 7):

1- E.O with a strong anti-Candida power: it is the case of *Ocimum basilicum* L., *Syzygium aromaticum*, *Thymus vulgaris* L. and *Origanum vulgare* with a MIC of 0.25%.

2- E.O with a mean anti-Candida power: *Artemisia vulgaris* L. and amphotericin B have a MIC of 0.5%.

3- E.O with a low anti-Candida power: in this category we find *Rosmarinus officinalis* L. with an MIC of 1%.

Table-6 Minimum inhibitory concentrations of different essential oils

Essential oil	MIC of essential oil % (v/v)
Artemisia vulgaris L.	0.5
Ocimum basilicum L.	0.25
Syzygium aromaticum L.	0.25
Thymus vulgaris L.	0.25
Origanum vulgare	0.25
Rosmarinus officinalis L.	1

Table-7 MIC of amphotéricin B

Antifungal	MIC of amphotéricin B % (v/v)
Amphotéricin B	0.5

The combination of essential oils has identified some associations such partial antagonism low level (Table 8), however those between essential oil and amphotericin B showed a potentiating synergy (Table 9).

Table-8 MIC of the combination of essential oils

Essential oil	MIC in essential oil % (v/v)
Artemisia vulgaris L. + Ocimum basilicum L.	0,5
Syzygium aromaticum L. + Thymus vulgaris L.	0,5
Origanum vulgare + Rosmarinus officinalis L.	1

Table-9 MIC of the combination of essential oils with amphotericin B

Combination antifungal and essential oil	MIC in combination % (v/v)
Amphotéricin B +*Artemisia vulgaris* L.	0.5
Amphotéricin B + *Ocimum basilicum* L.	0.25
Amphotéricin B + *Syzygium aromaticum* L.	0.25
Amphotéricin B + *Thymus vulgaris* L.	0.25
Amphotéricin B + *Origanum vulgare*	0.25
Amphotéricin B + *Rosmarinus officinalis* L.	0.5

Anti-candida activity of essential oils has been demonstrated by several authors [13-16], some studies showed synergistic interactions of these products [17] or in combination with some antifungal drugs [18-19].

Essential oils act on different levels [13]:
Alteration of the cell wall;
Alteration of membrane proteins;
Degradation of the cytoplasmic membrane;
Leakage of the cell contents;
Cytoplasmic coagulation;
Exhaustion of the force of movement of protons.

Amphotericin B binds to sterols in the cell membrane of sensitive fungi, making it permeable and causes its lysis. Selectivity is based on the fact that amphotericin B binds to sterol content in the membranes of fungi; ergosterol (then the human cell membranes containing cholesterol) [10].

Synergistic action between essential oils and amphotericin B may be explained by the simultaneous action on the membrane of *Candida albicans* which facilitates the penetration of essential oils, amphotericin B alone sometimes causes partial deformation of the morphology *Candida albicans* and explain the dormancy phenomenon [20].

The partial antagonism between certain essential oils may be due to some physical and chemical incompatibilities.

CONCLUSION

Anti-candida power of essential oils is relevant and suggests to drugs based on these products. Synergy with antifungal drugs can reduce the doses of these and enhance the therapeutic effect which results in a better balanced risk / benefit. Problem with these substances is the poor tolerance and lack of pharmacological and toxicological data, which may be promising more thorough and targeted studies.

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