

Antimycotic efficiency of essential oils and ethanol extracts of some medicinal plants in Egypt

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Abstract

The antimycotic activity of essential oils and ethanol extracts of seven species of medicinal plants namely; garlic, galangal, cinnamon, eucalyptus, elecampane, basil and clove were assayed against the growth of five pathogenic fungi; *Aspergillus flavus*, *A. niger*, *A. ostianus*, *Alternaria alternata* and *Fusarium solani* in addition to *Candida albicans* (pathogenic yeast) by disc diffusion method. The results revealed that garlic essential oil had broad-spectrum activity against all tested fungi followed by clove, cinnamon, elecampane, basil, galangal and eucalyptus. Clove ethanol extract had broad-spectrum activity against all tested fungi followed by elecampane and cinnamon. Minimum inhibitory concentration (MIC) and qualitative phytochemical screening were carried out for the wide spectrum highly active antimycotic extracts. Garlic, cinnamon, elecampane and clove essential oils and cinnamon, elecampane and clove ethanol extracts were chosen to undergo the minimum inhibitory concentration (MIC) determination and qualitative phytochemical screening. Both clove essential oil and ethanol extract, compared to the other extracts, exhibited the best antimycotic activity and lowest MIC in concentration.

Key words: Medicinal plants, antimycotic, essential oils, ethanol extracts, MIC, phytochemical screening.

Introduction

The World Health Organization defined a medicinal plant as any herbal preparation produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological process which may be produced for immediate consumption or as a basis for herbal products (WHO, 2001). Phytochemicals often referred to as "secondary metabolites" chemical compounds formed during the plant normal metabolic processes, they were first described at the beginning of the 19th century (Cordell, 1995). The most important of these bioactive compounds of plants are alkaloids, flavanoids, quinones, phenolic compounds, saponins, tannins, coumarins, glycosides, gums, polysaccharides, terpenes and other chemical compounds (Leon *et al.*, 2001; Okwu, 2004; Edeoga *et al.*, 2005; Al-Zubaydi *et al.*, 2009).

In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms have been developed due to indiscriminate use of commercial antimicrobial drugs/chemical commonly used in the treatment of infectious diseases (Anwar *et al.*, 2009). In addition, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immunosuppression and allergic reactions (Ahmad *et al.*, 1998; Ababutain, 2011). This situation has forced scientists to search new antimicrobial substances in various sources like medicinal plants (Kumar *et al.*, 2006; Bolivara *et al.*, 2011). Higher plants have been shown to be a potential source for new antimicrobial agents (Kuete *et al.*, 2009; Renisheya *et al.*, 2011).

Medicinal plants have been a source of wide variety of biologically active compounds for many centuries and used extensively as crude

material or as pure compounds for treating various disease conditions (Borris, 1996). The presence of antifungal compounds in higher plants has long been recognized as an important factor in disease resistance. Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases. In addition, plant extracts might have inhibitors to enzymes from the invading pathogens, and the effects of different phenolic compounds on the germination and growth of many fungal pathogens have been reported (Siva *et al.*, 2008).

Mould growth is commonly controlled using synthetic antimicrobials; however, natural antimicrobials had also demonstrated important antifungal properties (Lopez-Malo *et al.*, 2000). Superficial fungal infections, dermatomycoses, are probably the most common communicable fungal disease affecting humans. They have become a serious problem in immunocompromised patients (Pujol *et al.*, 2002). *Candida albicans* is the most common causative agent of oral candidiasis (Barchiesi *et al.*, 1993). Oral candidiasis is the earliest and most frequent fungal infection in the HIV-infected patients (Reichart, 2003).

Plants that are traditionally used in the treatment of fungal infections or related ailments could be a good source for new, safe, biodegradable and renewable antifungal drugs (Hamza *et al.*, 2006). As aromatic plants, herbs and spices have been used for ages both as flavouring agents and as preservatives of food, they may be effective sources of biodegradable fungitoxicants without harmful

side effects (Sokovic *et al.*, 2009). Reports by several authors (Zohri *et al.*, 1995; Youssef, 1995; Youssef and El-Maghraby, 2000; Pradeep *et al.*, 2003; Reddy *et al.*, 2009) support the fact that extracts of certain spices and herbs of medicinal importance exhibit antifungal and antidermatophytic property. These natural antifungal agents can be potentially exploited in controlling the growth of fungi and consequently, inhibiting aflatoxin formation (Yin and Cheng, 1998).

The search for natural sources of antifungal additives that are safe and efficient to be used in food has shown that extracts and essential oils from spices, herbs, and other plants carry antifungal activity (Fan and Chen, 1999; Juglal *et al.*, 2002; Sokovic *et al.*, 2009). Some compounds have been cited as being capable of inhibiting toxigenic species growth, as well as mycotoxin production (Gowda *et al.*, 2004; Marin *et al.*, 2004; Sanchez *et al.*, 2004).

The current study deals with the inhibitory effects of each of essential oils and ethanol extracts of seven different medicinal plant kinds tested on growth of five pathogenic fungi in addition to *Candida albicans* as pathogenic yeast. Minimum inhibitory concentration (MIC) and qualitative phytochemical screening were carried out for the wide spectrum highly active antimycotic extracts.

Materials and Methods:

Collection of Medicinal Plant Samples:

Seven species of medicinal plants belonging to six taxonomic botanical families were collected from local retail markets of drugs, spices and herbs "Attarin" in Egypt (table, 1).

No.	Arabic name	Latin name	English name	Plant part used	Family name
1	الثوم	<i>Allium sativum</i> L.	Garlic	Bulblets	Liliaceae
2	خلنجان	<i>Alpinia galanga</i> Will.	Galangal	Rhizomes	Zingiberaceae
3	القرقة	<i>Cinnamomum zeylanicum</i> L.	Cinnamon	Bark	Lauraceae
4	الكافور	<i>Eucalyptus globulus</i> Labill.	Eucalyptus	Leaves	Myrtaceae
5	عرق جناح	<i>Inula helenium</i> L.	Elecampane	Rhizomes	Asteraceae
6	الريحان	<i>Ocimum basilicum</i> L.	Basil	Seeds	Lamiaceae
7	القرنفل	<i>Syzygium aromaticum</i> (Linn.) Merr. & Perry.	Clove	Floral buds	Myrtaceae

Table (1): Different investigated species of medicinal plants collected during this study, Arabic name, Latin name, English name, Plant part used and Family name of each kind used.

Preparation of Plant Extracts:

Sequential extraction method was employed to extract the plant powders using n-hexane and ethanol 95% (Pandey, 2007).

Antimycotic Activities of Essential Oils and Ethanol Extracts of Different Kinds of Medicinal Plants:

a) Agar disc diffusion method:

The antimicrobial activities of different plant extracts were carried out by agar disc diffusion method (NCCLS, 1993). Disc diameter was 5 mm. Nystatine was used as positive control, while n-hexane, Dimethylsulfoxide (DMSO) and ethanol were used as negative control.

Sabouraud's dextrose and 1% glucose-Czapek's plates previously inoculated with a spore suspension of yeasts and moulds, respectively were used for antimycotic activity.

b) Organisms:

Five isolates of fungi were used as test organisms. These isolates were *Aspergillus flavus* Link, *A. niger* Van Tieghem, *A. ostianus* Wehmer, *Alternaria alternata* (Fries) Keissler and *Fusarium solani* (Mart.) Saccardo. In addition to the previous fungi, one isolate of *Candida albicans* (Robin) Berkhout was used as the test organism.

Determination of Minimum Inhibitory Concentration (MIC):

The diameter of the inhibition zone around the disc, measured in millimeter, is used as positive bioactivity. MIC was determined according to (Lamikanra, 1999) and employed by Ayoola *et al.* (2008a,b).

Qualitative Phytochemical Analysis:

Phytochemical screening for the presence of glycosides, alkaloids, tannins, flavonoids, saponins, terpenoids and coumarins was undertaken using standard qualitative methods as described by Trease and Evans (1989), Fadeyi *et al.* (1989), Harbone (1991), Sofowora (1993), Finar (2003) and Parekh *et al.* (2006).

Results:

Essential oils and ethanol extracts were tested against the growth of pathogenic fungi

Aspergillus flavus, *A. niger*, *A. ostianus*, *Alternaria alternata* and *Fusarium solani* in addition to *Candida albicans* (pathogenic yeast) by disc diffusion method. The data recorded in tables (2 & 3) revealed that essential oils were more active on the mycotic growth than ethanol extracts.

Garlic essential oil had wide spectrum highly antimycotic activity against all tested fungi followed by clove, cinnamon, elecampane, basil, galangal and eucalyptus. Ethanol extracts occupied the second order in antimycotic activity. *Aspergillus flavus* was sensitive to cinnamon and clove, with different degrees, while *Aspergillus niger* and *Aspergillus ostianus* were sensitive to clove, cinnamon, elecampane, basil and galangal ethanol extracts (table, 2).

Alternaria alternata was the most sensitive fungus to the ethanol extracts. Its growth was affected by clove, elecampane, basil, galangal, cinnamon and eucalyptus. Clove, basil, elecampane and cinnamon had antimycotic activity with different degrees upon the growth of *Fusarium solani*.

Garlic ethanol extract was not effective against the growth of all tested fungal species. Clove essential oil was the highly active against *Candida albicans*, followed by cinnamon, garlic, basil essential oils. Elecampane, eucalyptus and galangal essential oils had weak activity on *Candida albicans*. The ethanol extract of clove was the highly active against the growth of *Candida albicans*, while cinnamon came second. Elecampane, basil and eucalyptus were also effective against *Candida albicans* (table, 3).

Based on the results in table (4), the MICs of garlic essential oil on the growth of *Aspergillus flavus*, *A. niger*, *A. ostianus*, *Alternaria alternata*, *Fusarium solani* and *Candida albicans* were 94.19, 109.64, >500, 58.1, 66.7 and 500 mg/ml. For cinnamon essential oil, the MICs were >500, 500, 82.22, 81.28, 208.93 and 148.93 mg/ml. Moreover, the data showed that the MICs of elecampane essential oil were >500, 500, >500, 94.18, 75.85 and 62.51 mg/ml, and for clove were

85.86, 37.41, 46.77, 111.43, 33.88 and 31.11 mg/ml.

On the other hand, the results in table (4) presented the MICs of the ethanol extracts of cinnamon, clove and elecampane against the preceding tested fungi and showed that the MICs of cinnamon were 500, 500, 112.2, 100, 151.35 and 63.1 mg/ml, elecampane 250, 500, 500, 63.1, 23.44 and 123.02 mg/ml and clove 6.22, 158.49, 500, 11.48, 114.81 and 98.62 mg/ml.

From the results outlined in table (5), we can conclude that the phytochemical analysis of garlic essential oil showed the presence of flavonoids, terpenoids and glycosides. As well as, clove essential oil contained the same

constituents in addition to coumarins. All tested phytochemicals were found in the essential oil of cinnamon except tannins and saponins, while elecampane essential oil contained flavonoids and alkaloids in addition to terpenoids.

On the other hand, terpenoids were detected in all tested ethanol extracts, while flavonoids and coumarins were absent in cinnamon and clove, respectively. Both glycosides and coumarins were present in cinnamon, while flavonoids, alkaloids, coumarins and terpenoids were present in elecampane ethanol extract. Clove ethanol extract also had flavonoids, glycosides and tannins.

Medicinal plant	Aspergillus flavus	Aspergillus niger	Aspergillus ostianus	Alternaria alternata	Fusarium solani	Candida albicans 48 h
Garlic	40	27	30	44	42	20
Galangal	8	8	8	17	15	9
Cinnamon	25	16	20	17	30	21
Eucalyptus	8	9	9	12	15	11
Elecampane	14	16	13	15	20	11
Basil	14	10	11	14	12	14
Clove	28	35	40	40	28	26
Nystatine	10	7	NI	12	10	8
n-hexane or DMSO	NI	NI	NI	NI	NI	NI

Table (2): Antimycotic activities of the essential oils of the investigated plants against fungal strains [After 72 h]. Inhibition zone diameter in millimeter (mm).

NI = No Inhibition

Medicinal plant	Aspergillus flavus	Aspergillus niger	Aspergillus ostianus	Alternaria alternata	Fusarium solani	Candida albicans 48 h
Garlic	NI	NI	NI	NI	NI	NI
Galangal	NI	8	7	12	NI	NI
Cinnamon	7	9	20	11	10	24
Eucalyptus	NI	NI	NI	10	NI	7
Elecampane	8	9	8	40	12	9
Basil	NI	7	7	13	14	8
Clove	20	25	25	40	28	25
Nystatine	10	7	NI	12	10	8
Ethanol or DMSO	NI	NI	NI	NI	NI	NI

Table (3): Antimycotic activities of the ethanol crude extracts of the investigated plants against fungal strains [After 72 h]. Inhibition zone diameter in millimeter (mm).

NI = No Inhibition

Type of extract	Medicinal plant	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Aspergillus ostianus</i>	<i>Alternaria alternata</i>	<i>Fusarium solani</i>	<i>Candida albicans</i>
n-hexane (essential oils)	Garlic	94.19	109.64	>500	58.1	66.7	500
	Cinnamon	>500	500	82.22	81.28	208.93	148.93
	Elecampane	>500	500	500	94.18	75.85	62.51
	Clove	85.86	37.41	46.77	111.43	33.88	31.11
Ethanol 95%	Cinnamon	500	500	112.2	100	151.35	63.1
	Elecampane	250	500	500	63.1	23.44	123.02
	Clove	6.22	158.49	500	11.48	114.81	98.62

Table (4): MICs (mg/ml) of essential oils and ethanol extracts of investigated medicinal plant species with wide spectrum highly antimycotic activities against tested fungi.

Type of extract	Medicinal plant	Flavonoids	Alkaloids	Terpenoids	Glycosides	Coumarins	Tannins	Saponins
n-hexane (essential oils)	Garlic	+	-	+	+	-	-	-
	Cinnamon	+	+	+	+	+	-	-
	Elecampane	+	+	+	-	+	-	-
	Clove	+	-	+	+	+	-	-
Ethanol 95%	Cinnamon	-	-	+	+	+	-	-
	Elecampane	+	+	+	-	+	-	-
	Clove	+	-	+	+	-	+	-

Table (5): Phytochemical screening of the bioactive compounds in wide spectrum highly active antimycotic extracts.

+ = Present - = Absent

Discussion:

The object of the present experiment was to examine the antimycotic activity of each of essential oils and ethanol extracts of seven different medicinal plant species assayed on the growth of six pathogenic moulds representing fungi (five isolates; *Aspergillus flavus*, *A. niger*, *A. ostianus*, *Alternaria alternata* and *Fusarium solani*) in addition to pathogenic yeast species (*Candida albicans*). Minimum inhibitory concentration (MIC) and qualitative phytochemical screening were carried out for the wide spectrum highly active antimycotic extracts. In the testing procedures used in this investigation, the moulds were grown under near optimum conditions with controlled temperature and adequate nutrients.

The results obtained during this investigation elucidated clearly that generally, pathogenic fungi were more resistant to treatment with different extracts tested. Essential oils were more effective antimycotic agents than ethanol extracts. This conclusion is in full agreement to that previously recorded by several researchers (Youssef, 1995; Thanaboripat *et al.*, 2004, 2005).

The data recorded in current investigation revealed that all essential oils had highly broader antimycotic activity against all tested fungi and these were; basil, cinnamon, clove, elecampane, eucalyptus, galangal and garlic. On the other hand, three ethanol extracts showed highly broader antimycotic activity against all tested fungi (cinnamon, clove and elecampane), whereas ethanol extract of basil

had moderate antimycotic activity against five fungal species out of six tested. Basil essential oil (*Ocimum basilicum*) exhibited inhibition activity against *Fusarium graminearum*, *F. culmorum*, *Aspergillus flavus*, *A. oryzae*, *A. brasiliensis* (Dobre *et al.*, 2011) and *A. niger* (Hussain *et al.*, 2008).

Data in current study showed that cinnamon essential oil and ethanol extract had highly antifungal activity against all tested fungi. Cinnamon is rich in essential oils and tannins, which inhibit microbial growth (Chang 1995). The major constituent possessing antifungal activity in *C. zeylanicum* bark and leaf oils were found to be cinnamaldehyde and eugenol, respectively (Mishra *et al.*, 2009). In addition other compounds having fungicidal property have also been reported to be present in bark and leaves (Ranasinghe *et al.*, 2002).

Clove buds oil proved to develop the best antifungal activity against *Fusarium graminearum*, *F. culmorum*, *Aspergillus flavus*, *A. oryzae* and *A. brasiliensis* (Dobre *et al.*, 2011). The effects of clove essential oil and its principal component, eugenol, on growth and mycotoxin production by some toxigenic fungal genera such as *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. had been reported by Velluti *et al.* (2003, 2004), Lopez-Malo *et al.* (2005a,b) and Nesci *et al.* (2011). This component was able to inhibit both growth and/or mycotoxin production (Passone *et al.*, 2012).

Ayoola *et al.* (2008b) and Ali *et al.* (2009) reported that clove essential oil possessed antifungal properties against *Candida albicans* and showed a broad spectrum of activity. Phenols are known to have antiseptic properties (Pelczar *et al.*, 1998), which is consistent with the antimicrobial data obtained for these compounds. Caryophyllene had also been shown to possess antimicrobial properties, though not as potent as eugenol (Dorman *et al.*, 2000).

Chloroform and essential oil of elecampane exhibited good antidermatophyte activity while aqueous extract had no effect on tested dermatophytes (Youssef, 1995). The essential oil of eucalyptus leaves has been the object of

several studies about antibacterial, antioxidant, antihyperglycemic and antifungal activity (Derwich *et al.*, 2009).

Janssen and Scheffer (1985) reported that fresh and dried rhizomes of galangal (*Alpinia galanga* Will.) showed antimycotic activity against yeast, fungi and some dermatophytes. *Fusarium graminearum*, *F. culmorum*, *Aspergillus flavus*, *A. oryzae* and *A. brasiliensis* were tested for the antifungal activity of garlic oil. Only *F. culmorum* was inhibited by garlic oil (Dobre *et al.*, 2011). *Allium sativum* and its components (allicin and its derivatives) were well known to possess antimicrobial activity (Harris *et al.*, 2001).

Antifungal activity of clove and cinnamon oils against *A. niger*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Phomopsis viticola* and *Rhizopus stolonifer* showed minimal inhibitory concentration (MIC) for clove: 200, 200, 400, 800, 200 and 200 mg/ml, respectively, whereas the MIC obtained from cinnamon oil were 50, 100, 200, 200, 100 and 800 mg/ml, respectively (Sukatta *et al.*, 2008). Ayoola *et al.* (2008b) and Ali *et al.* (2009) reported that clove essential oil possessed anticandidal property against *Candida albicans* and showed a broad spectrum of activity with minimum inhibitory concentrations (MICs) 0.067 mg/ml and (24µg/ml), respectively. The efficacy of cinnamon and clove oils as antifungal agents, were reported by many researchers (Soliman and Badeaa, 2002; Velluti *et al.*, 2003; Lopez-Malo *et al.*, 2007). Anticandidal activity of crude ethanol extract from clove was evaluated against *Candida albicans* over a wide range of concentrations (50-5000 ppm), and it was proved most active at MIC 800 ppm (DIZ = 25 mm) (Dababneh, 2008).

Qualitative analysis was carried out for screening the presence of major phytochemical constituents such as glycosides, alkaloids, tannins, flavonoids, saponins, terpenoids and coumarins in the highly active antimycotic extracts.

Our results are in full agreement with the previous data of several researchers (Mishra *et al.*, 2009; Pathmanathan *et al.*, 2010; Nan *et*

al., 2012). Phytochemical constituents like alkaloids, phenolics, flavonoids, tannins and saponins are usually responsible for medicinal and antimicrobial importance of herbal plants (Krishnaiah *et al.*, 2009). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties (Cowan, 1999). The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds and flavonoids (Aboaba and Efuwape, 2001).

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الملخص العربي

الفاعلية ضد فطرية لكل من الزيوت الطيارة والمستخلصات الكحولية لبعض النباتات الطبية في

مصر

استهدف البحث تقييم التأثيرات الضد فطرية للزيوت الطيارة ومستخلصات الكحول لسبعة أنواع من النباتات الطبية في مصر على نمو خمسة أنواع من الفطريات الممرضة (أسبرجلس فلافس، أسبرجلس نيجر، أسبرجلس أوستيانس، ألترناريا ألترناتا وفيوزاريم سولاني) ونوعاً واحداً من الخمائر الممرضة (كانديدا ألبيكانس). أظهرت الزيوت الطيارة النشاط الأعلى ضد الفطريات المختبرة، يليها مستخلصات الإيثانول. أظهرت الزيوت الطيارة للثوم، القرفة، عرق جناح والقرنفل بالإضافة إلى مستخلصات الإيثانول للقرفة، عرق جناح والقرنفل أعلى نشاطاً تثبيطياً ضد الفطريات المختبرة. لذلك تم اختيارهم لتحديد أقل تركيز مثبط لنمو الفطريات المختبرة بالإضافة إلى التحليل الوصفي للكشف عن تواجد بعض المنتجات الثانوية الفعالة في تلك المستخلصات النباتية.