J. Agric. Sci. Mansoura Univ., 34 (3): 1745 - 1756, 2009

ANTIFUNGAL PROPERTIES OF SOME MEDICINAL PLANTS AGAINST UNDESIRABLE AND MYCOTOXIN-PRODUCING FUNGI

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ABSTRACT

Antifungal effects of essential oils obtained from five aromatic plants (lemon grass, brasil, sage, clove and rosemary) purchased from a local market in Egypt, were extracted to obtain the oil fraction and screened in vitro against three different pathogenic fungi, *Aspergillus flavus* (*A. flavus*), *Asprgillus parasiticus* (*A.parasiticus*) and *Asprgillus ochraceus* (*A. ochraceus*). The oil fraction from each tested plant, was carried out using water extraction and used to determine its inhibitory effects on both the fungal isolates. Also, the MIC of each oil extract and its content of the phytochemicals of phlenoles, flavonoides and terpenoids were determined. Data exhibited wide range of the inhibitory action occurred against the tested microorganisms where, bioactivity of the five plants reached its maximum using clove and its minimum using sage extracts against the tasted microorganisms. No bioactivity was observed in the treatment(s) of sage extract against the mold *Asp. ochraceus*. The other three extracts of rosemary, brasil and lemon grass showed roughly the same bioactivity. Also, data obtained from MIC and semi-quantitative determinations confirmed the results obtained from the antimicrobial bioactivity study. Except sage extract, the MIC of the other four extracts were ranged between 0.35-1.5 mg/ml, reached its maximum using clove and its minimum using lemon grss extracts. As well, the semi-quantitative determination of the phytochemicals of phenols, flavonides and terpenoids proved that clove extract had the highest concentration of terpenoids, while sage extract showed traces from the three phytochemicals. The other three extracts of basil, rosmary and lemon grass showed roughly the same content of the sum of the three phytochemicals. These effects against the tested microorganisms indicated the possible ability of each essential oil as a food preservative.Therefore, it is suggested that further work be performed on food to test the antifungal and antibacterial properties of these oil fractions.

Keywords: Spices- Molds- Aflatoxins- Ochratoxin-A- Sage- Rosemary- Ginger.

INTRODUCTION

Securing the safety of food and feed is one of the most important factors in the welfare of human and live stock. It is estimated that there are 250-500 thousand species of plants are spreaded on earth (Borris, 1996). A relatively small percentage (1-5%) of these plants are used as foods and feeds (Meorman, 1996).

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Speculations as to how and why a selected number of plant species came into use for either food or drug is of concern and fascinating. Recently, there are an increasing receptive to the use of antimicrobial and other drugs derived from plants instead of the synthesized products (Cowan, 1999). Useful antimicrobial photochemicals can be divided into several categories. Plants have certain limits to synthesize aromatic substances, most of which are phenols, flavonoids and terpenoids. These natural chemical substances serve as plant defense mechanisms against the invasion of microorganisms, insects and herbivores (Schultez, 1978). Others give plants their appropriate odors and others are responsible for plant pigment (Cowan, 1999). Plant volatile oils have been well known since antiquity as possessing biological activities. Chief amongst these are their antibacterial, antifungal and antioxidant properties (Deans and Waterman, 1993). The use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is gaining momentum, both for the growing interest of consumers in ingredients from natural sources and also because of increasing concern about potentially harmfull synthetic additives (Reische, *et al.*, 1998).

The plants of clove, sag, basil, rosemary and lemon grass are commonly used individually or simultaneously as food enhancers or to gain certain tastes. Initial screening of potential antibacterial and antifungal compounds from plants could be performed with pure substances or crude extracts (Silva *et al.*, 1996). The methods used for the two types of bacteria and fungal organisms are similar. The two most commonly used screens to determine antimicrobial susceptibility are the broth dilution assay (Hess *et al.*, 1995) and the disc or agar well diffusion assay (Navarro *et al.*, 1996). Other variations of these recommended methods could be also applied. .Hence compositions and concentrations of compounds within the distinct types of spice extract preparations would differ (Lienert *et al.*,1998; Nass *et al.*,1998; Vilegs *et al.*, 1997), the efficacy of the extracts may in turn be altered depending on the preparation method employed. Given these considerations, The main target of this study aims to study the bioactivity of some edible plants against undesirable and toxin producing fungi of Aspergillus flavus, A. parasiticus and A. Ochraceus. Lemon grass (*Cymbopogon citratus*), Basil (*Ocimum basilicum*), (*Rosmariinus officinalis*), Sage (*Salvia officinalis*), Clove (*Syzygium aromaticum*), were the plants under consideration in this study.

MATERIALS AND METHODS

1. Microorganisms :

Pure cultures of molds used for the testing purpose were provided from Mycotoxin lab and Microbial Dept., National Research Center, Dokki , Cairo, Egypt . The fungal strains of *A. flavus* (NRRL-2999), *A. parasiticus* and *A. ochraceus* which are identified as aflatoxin(s) and ochratoxin-A producing strains were obtained and subcultured in nutrient Yeast Extract Sucrose media (YES), while sensitivity test agar was used in antifungal sensitivity testing. The inoculated YES media were inoculated at 28 ± 1° c for 10 days, before sensitivity testing and evaluating the efficacy of the studied plants.

2. Mycotoxins standard

Aflatoxins (B1 and G1) and Ochratoxin A were obtained from Sigma Chemical Co. St. Louis MO, USA.

3. Preparation of the conidial suspension :

The inoculum was produced by growing the organism on potato dextrose agar (PDA) slants for 7 days at 28°C ± 2°C. Conidia were harvested from 7-day-old cultures by pouring a sterile 0.01% aqueous solution of Tween 80 onto the culture plates and scraping the plate surface with a bent glass rod to facilitate the release of conidia.The conidial suspension thus obtained had conidial concentration of 10^6 conidia/ml.

4. Plant materials and essential oil extract preparations :

Samples of mature fresh green leaves of Lemon grass (*Cymbopogon citratus*), Rosemary (*Rosmariinus officinalis*), Sage (*Salvia officinalis*), Clove (*Syzygium aromaticum*), and a hole plant of Basil (*Ocimum basilicum*), were obtained locally (Medicinal, Aromatic Plant Research Center). Before use, freshly-collected samples were identified at the Plant Protection Department, National Research Center,Dokki, Giza, Egypt. Such samples were air dried, grinded and powdered before extraction. 200 gms, each of the powdered plants were separately extracted for getting the essential oils (EO), by the hydrodistillation method using Clevenger's apparatus (Lamaty *et al.*,1987). The recovered oils were dried over anhydrous sodium sulphate and stored in darkness at 4°C for further use in an air-tight containers. The yield of the essential oils as percent of plant material weight was as follows: 0.57%, 6.2%, 0.42%, 0.36% and 0.50% for the EO from C. citratus, O. basilicum, R. officinalis, S. officinalis and S. aromaticum respectively. The filtrate, for each, was used as the test extract.

5. Study of the antifungal properties of the five essential oils

5-1. Mould growth and mycoyoxin production

Yeast extract sucrose (YES) broth medium which is known to support mold growth and toxin production (Davis *et al.* , 1966.) was used as a basal medium in this study. Fifty millimeter of medium was dispensed into each of a series of 250-ml Erlenmeyer flasks. The medium was autoclaved at 121°C for 15 min., and the cooled, 0.0, 0.1, 0.2, or 0.3% for each plant extract, was added. Then, the medium was inoculated with 1 ml of the appropriate spore suspension and incubated for 10 days at 28°C ± 2°C.

5- 2.Determination of Minimum Inhibitory Concentration (MIC)

The MIC were determined by the microdilution agar plate method (Skandamis *et al.*, 2001). Aliquots of 90 ml tempered Trypton Soy Agar (TSA) were agitated vigorously with essential oil to achieve the following final oil concentrations: 3.0, 2.8, 2.5, 2.2, 2.0, 1.5, 1.0, 0.6, 0.5, 0.25, 0.15, 0.10, 0.05 and 0.025 ml/100ml. Approximately 15 ml of each of these mixtures was transferred with 1ml of each inoculum to agar plates. The plates were incubated at 27°C for 72h and the numbers of colonies were determined. Each assay was performed by duplication in two separate experimental runs.

6. Phytochemical analysis of extract(s)

A test portion of each of the obtained five plant extracts were prepared and applied for Phytochemical screening as described by (Trease *et al.*, 1993) based on the facts that phenols are a wide group of phenylpropane

compounds in the highest oxidation state, while flavonoides are hydroxylated phenolic substances and terpenoids are synthesized from acetate units. Semi quantitative assay of the three phytochemicals were conducted according to (Harbourne 1973).

8. Mycotoxins analysis

Aflatoxin analysis: Mycelia of cultures were carefully overlaid with chloroform (50 ml) and kept 24 hr in dark then several steps were carried out according to the method of (*Bauer et al.,1983*). Quantitative determination of aflatoxins B1 and G1 was performed on silica gel D.G-plates according to (*the AOAC methods,1980*).

Ochratoxin analysis: Dry weight of mycelia mats from liquid media were obtained by decanting of the culture broth and drying the mat at 65 – 70° C for 24 hr. Ochratoxin A was estimated qualitatively by thin layer chromatography (TLC) and confirmed with ammonia foams and determined flourodenstrometry according to the (*AOAC methods*, 1980).

Calculations:

Percent reduction in growth or mycotxin production was calculated by the following equation:

Percent reduction = 100 – {(A1 / A0) *100}.

Were: A1 = the amount obtained by treatment.

A0 = the amount obtained by control.

9. Statistical analysis

Significant differences between treatments and strains sensitivity were analysed using the M-STAT programme at 99% level of confidence. Mean separation test was done using the Least Significance Difference (LSD), (Nissen, O., 1990).

RESULTS AND DISCUSSION

1. Study of the antifungal properties of the five essential oils

1.1 Antifungal activity of the extracted oils against mold growth and aflatoxin production

The antifungal properties of the five selected essential oils against three fungal species were recorded as inhibition of fungal mycelial growth and mycotoxin production, and summarized in Tables from one to six. The results revealed that the selected essential oils showed antifungal activity with varying magnitudes. Out of five essential oils tested, four oils showed antifungal activity against all tested strains. Clove, lemongrass, rosemary and basil oils exhibited significant inhibitory effect, and showed promising inhibitory activity even at low concentration, whereas sage oil was least active against the tested fungi. The inhibitory action by these extracts was found at concentration 0.1 % and increased as the concentration in the medium increased, to reach maximum at concentration 0.3%.The inhibitory action against growth and aflatoxin production decreased in the following order, clove > lemongrass> rosemary >basil > sage (Tables, 1, 2, 3, 4). These results are in line with those obtained by (Baratta *et al.,* 1998), who found that essential oils of rosemary, sage, lourel and oregano showed a

high degree of inhibition against *A. Niger* and the highest and broadest activity was shown by oregano and sage was the least effective . (Bankole And JODA, 2004) found that The essential oil of lemon grass at 0.1 and 0.25% (v/w) and ground leaves at 10% (w/w) significantly reduced deterioration and aflatoxin production in shelled melon seeds inoculated with toxigenic *A. Flavus* and at higher dosages (0.5 and 1.0% v/w), the essential completely prevented aflatoxin production. Significant differences in the antifungal activities levels, as shown in Tables (1, 2, 3, 4), may be due to the differences in the content of known antimicrobial compounds in each EO as earlier determined by (Lamaty *et al.*, 1987, Amvam *et al.* 1998). Also (Farag *et al.*,1989, Tassou *et al.*, 2000) discussed this correlation of the antifungal activity with the content of antimicrobial compounds of the EO. Studying the antifungal effect of five different EOs including thyme oil against A. parasiticus, (Farag *et al.*,1989) concluded that the inhibitory effect of the oils was mainly due to the most abundant components. (Amvam *et al.* 1998, Menut and Valet, 1985) reported a higher content of thymol in the EO from O. gratissimum (46.5%) compared to the EO from T. vulgaris (27.5%). On the other hand, similarity in some of the EO in their inhibitory effect on growth or aflatoxin accumulation by *A. Flavus* and / or *A. Parasiticus* may be mainly attributed that substances responsible for antifungal activity in these oils may be similar in their chemical structure and concentration. Other investigators reported that diterpenes, carnosic acid from rosemary and curcumene for ginger were the major constituents detected in water extract of these plants (Chen *et al.*, 1992, Dhirendra and Mishra, 1990).

Table 1: Effect of the extracted oils on *A. flavus* growth

Extract level, %	Mycelial dry weight, g / 50 ml medium				
	Lemon grass	Basil	Sage	Clove	Rosemary
Control, 0.0 %	1.89^{Aa} ± 0.012	1.89^{Aa} ± 0.012	1.89^{Aa} ± 0.012	1.89^{Aa} ± 0.012	1.89^{Aa} ± 0.012
0.1	0.85^{Ba} ±0.017	1.50^{Bb} ±0.071	1.60^{Bb} ±0.064	0.00^{Bc} ±0.0	1.54^{Bb} ±0.011
0.2	0.31^{Ca} ±0.03	1.53^{Bb} ±0.0.34	1.43^{Bb} ±0.057	0.00^{Bc} ±0.0	0.00^{Cc} ±0.0
0.3	0.00^{Da} ±0.0	0.40^{Cb} ±0.055	1.18^{Cc} ±0.032	0.00^{Ba} ±0.0	0.00^{Ca} ±0.0
$LSD_{5\%}$	0.17				

N.B.1- Different capital litters in column denote significant differences (p ≤ 5%) between means in the same extract and visa versa. 2- The same small litters in rows denote no significant differences (p ≤ 5%) between extracts and visa versa.

Table 2: Effect of the extracted oils on aflatoxin production by *A. flavus*.

Extract level, %	Aflatoxin production, µg/50 ml medium				
	Lemon grass	basil	Sage	Clove	Rosemary
Control, 0.0 %	1265^{Aa} ± 1038	1265^{Aa} ± 1038	1265^{Aa} ± 1038	1265^{Aa} ± 1038	1265^{Aa} ± 1038
0.1	392^{Ba} ±6.15	1012^{Bb} ±10.13	1075^{Bc} ±12.3	0.00^{Bd} ±0.0	1005^{Bb} ±9.5
0.2	208^{Ca} ±6.71	772^{Cb} ±11.1	945^{Cc} ±15.2	0.00^{Bd} ±0.0	0.00^{Cd} ±0.0
0.3	0.00^{Da} ±0.00	315^{Db} ±19.7	640^{Dc} ±23.5	0.00^{Ba} ±0.0	0.00^{Ca} ±0.0
$LSD_{5\%}$	39.6				

N.B.1- Different capital litters in column denote significant differences (p ≤ 5%) between means in the same extract and visa versa. 2- The same small litters in rows denote no significant differences (p ≤ 5%) between extracts and visa versa.

Table 3: Effect of the extracted oils on *A.parasiticus* growth.

Extract level, %	Mycelial dry weight, g / 50 ml medium				
	Lemon grass	basil	Sage	Clove	Rosemary
Control, 0.0 %	$1.54^{Aa} \pm 0.036$	$1.54^{Aa} \pm 0.036$	$1.54^{Aa} \pm 0.036$	$1.54^{Aa} \pm 0.036$	$1.54^{Aa} \pm 0.036$
0.1	$0.4^{Ba} \pm 0.012$	$0.93^{Bb} \pm 0.029$	$1.50^{Bc} \pm 0.014$	$0.0^{Bd} \pm 0.0$	$1.34^{Bc} \pm 0.036$
0.2	$0.2^{Ba} \pm 0.00.018$	$0.86^{Bb} \pm 0.038$	$1.32^{Bc} \pm 0.047$	$0.0^{Bd} \pm 0.0$	$1.06^{Ce} \pm 0.012$
0.3	$0.0^{Ca} \pm 0.0$	$0.59^{Cb} \pm 0.011$	$0.97^{Cc} \pm 0.076$	$0.0^{Bd} \pm 0.0$	$0.33^{De} \pm 0.017$
$LSD_{5\%}$	0.18				

N.B.1- Different capital litters in column denote significant differences ($p \leq 5\%$) between means in the same extract and visa versa. 2- The same small litters in rows denote no significant differences ($p \leq 5\%$) between extracts and visa versa.

Table 4: Effect of the extracted oils on aflatoxin production by *A. parasiticus*.

Extract level, %	Aflatoxin production, µg/50 ml medium				
	Lemon grass	basil	Sage	Clove	Rosemary
Control, 0.0 %	$1305^{Aa} \pm 13.1$	$1305^{Aa} \pm 13.1$	$1305^{Aa} \pm 13.1$	$1305^{Aa} \pm 13.1$	$1305^{Aa} \pm 13.1$
0.1	$325^{Ba} \pm 10.9$	$1260^{Bb} \pm 17.6$	$780^{Bc} \pm 10.4$	$0.0^{Bd} \pm 0.0$	$1160^{Be} \pm 14.1$
0.2	$185^{Ca} \pm 17.5$	$1080^{Cb} \pm 11.3$	$675^{Cc} \pm 9.7$	$0.0^{Bd} \pm 0.0$	$780^{Ce} \pm 8.3$
0.3	$0.0^{Da} \pm 0.0$	$580^{Db} \pm 19.7$	$340^{Dc} \pm 32.4$	$0.0^{Ba} \pm 0.0$	$235^{Dd} \pm 16.8$
$LSD_{5\%}$	43.3				

N.B.1- Different capital litters in column denote significant differences ($p \leq 5\%$) between means in the same extract and visa versa. 2- The same small litters in rows denote no significant differences ($p \leq 5\%$) between extracts and visa versa.

1.2. Antifungal activity of the extracted oils against mold growth and ochratoxin production

Results obtained from Tables 5 and 6 revealed that mycelial growth and ochratoxin A-production were comparatively in significant differences ($p \geq 0.05$), but with avariable degree, by the difference in the oil source in the medium. The inhibitory action increased as the oil concentrations in the medium increased from 0.1 – 0.3%. The amount of dry weight of mycelium and ochratoxin A- accumulation,. Concentrations of basil, sage and rosemary oils were comparatively weaker in their inhibitory effect than that of clove and lemon grass oils. Our results are contrary with those observed on sage oil, by (Basilico and Basilico, 1999, Sokovic *et al.*, 2002., Abdel-Fattah And Abo-Seree, 2004). Sokovic *et al.*, 2002, reported that oils obtained from Greek sage exhibited low antifungal activity against *A. ochraceus*. Also, (Basilico and Basilico, 1999) found that sage oil at concentration 1000 p.p.m in YES broth incubated for up to 21 days at 25°C, showed no important effect on *A. ochraceus* NRRL 3174. Fluctuation in results occurred may be attributed to differences in the extract used depending on the preparation method and/or concentration employed (Lienert *et al.*,1998, Vilegs *et al.*, 1997).

A very little is known about the possible antifungal activity against *A. ochraceus* by the tested oils. As known literatures about antifungal properties of sage, rosemary and basil still little, their inhibitory action observed in this work might be associated with their phenolic constituents, which namely diterpenes. In this respect, (Tang *et al.*, 2000), reported that alcohol extracts of rosemary and sage contain active antioxidative factors such as phenolic diterpenes, flavonoids and phenolic acids. On the other hand, (Campo *et al.*,

2000) found that antimicrobial activity of rosemary extract was linked to the compounds extracted with hexane, which are presumably phenolic diterpenes. (Abdel-Fattah And Abo-Seree, 2004), observed that compounds isolated from sage and rosemary, had antifungal activity against *A.flavus, A.parasiticus and A.ochraceus*, and the major constituents of these compounds was found to be curcumine (diterpene).

Table 5: Effect of the extracted oils on mold growth by *A. ochraceus*.

Extract level, %	Mycelial dry weight, g / 50 ml medium				
	Lemon grass	basil	Sage	Clove	Rosemary
Control, 0.0 %	$1.20^{Aa} \pm 0.018$	$1.20^{Aa} \pm 0.018$	$1.20^{Aa} \pm 0.018$	$1.20^{Aa} \pm 0.018$	$1.20^{Aa} \pm 0.018$
0.1	$0.036^{Ba} \pm 0.008$	$1.24^{Ab} \pm 0.074$	$1.30^{Ab} \pm 0.018$	$0.00^{Ba} \pm 0.00$	$1.20^{Ab} \pm 0.042$
0.2	$0.00^{Ba} \pm 0.00$	$0.34^{Bb} \pm 0.065$	$0.54^{Bc} \pm 0.037$	$0.00^{Ba} \pm 0.00$	$0.35^{Bb} \pm 0.070$
0.3	$0.00^{Ba} \pm 0.00$	$0.15^{Cb} \pm 0.019$	$0.23^{Cb} \pm 0.034$	$0.00^{Ba} \pm 0.00$	$0.17^{Cb} \pm 0.053$
$LSD_{5\%}$	0.14				

N.B.1- Different capital litters in column denote significant differences ($p \leq 5\%$) between means in the same extract and visa versa. 2- The same small litters in rows denote no significant differences ($p \leq 5\%$) between extracts and visa versa

Table 6: Effect of the extracted oils on ochratoxin A- production by *A. ochraceus*.

Extract level, %	Ochratoxin A- production, mg / 50 ml medium				
	Lemon grass	basil	Sage	Clove	Rosemary
Control, 0.0 %	$3985^{Aa} \pm 46.2$	$3985^{Aa} \pm 46.2$	$3985^{Aa} \pm 46.2$	$3985^{Aa} \pm 46.2$	$3985^{Aa} \pm 46.2$
0.1	$1195^{Ba} \pm 24.11$	$4105^{Bb} \pm 18.9$	$3990^{Bb} \pm 21.3$	$0.00^{Bc} \pm 0.00$	$1155^{Ba} \pm 15.2$
0.2	$0.00^{Ca} \pm 0.00$	$1136^{Cb} \pm 11.7$	$3148^{Cc} \pm 27$	$0.00^{Ba} \pm 0.00$	$0.00^{Ca} \pm 0.00$
0.3	$0.00^{Ca} \pm 0.00$	$840^{Db} \pm 31.5$	$3045^{Cc} \pm 23.7$	$0.00^{Ba} \pm 0.00$	$0.00^{Ca} \pm 0.00$
$LSD_{5\%}$	166				

N.B.1- Different capital litters in column denote significant differences ($p \leq 5\%$) between means in the same extract and visa versa. 2- The same small litters in rows denote no significant differences ($p \leq 5\%$) between extracts and visa versa.

2. The MIC of the five plant extracts against the tested organisms

Minimum inhibitory concentration (MIC) for selected five oils against the tested organisms showed a broad spectrum (between 0.2 to 7.5 mg/ml) of bioactivities ranged between the two extremes of clove as a maximum and sage as a minimum (Table 7). Clove oil showed maximum activity with MIC values ranging from 0.35 to 0.50 mg/ml followed by lemongrass oil with MIC values ranging from 0.40 to 0.60 mg/ml against all the tested strains, whereas remaining oils, except sage, showed moderate MIC values. Adegoke and Odesola (1996) reported similar MIC values (0.25 ml/100ml) for lemon grass against *A. flavus*. On the other hand, Sacchetti *et al.* (2005) studied MIC values for lemon grass and found that concentration between 0.2 and 0.3 ml/100ml were microbiocidal. Data exhibited that standard antibiotic gentamycin (the MIC values varying between 0.2 – 0.35 mg/ml) had stronger bioactive effect on the fungal strains compared with all the 5 plant extracts, at least 25 folds more than sage exctract (Table 7). Cowan (1999) reported that the antimicrobial effects of any plant products or resources was mainly due to its contents of phytochemicals and other bioactive metabolites.

Table 7: The MIC of the 5 plant extracts and gentamycin against the studied fungal strains.

Microorganism(s)	MIC (mg/ml)					
	Clove	Sage	Rosmary	Brasil	Lemon grass	Gentamycin
Against fungal strains						
A. flavus	0.35	6.50	0.60	1.50	0.40	0.20
Asp. parasiticus	0.50	5.30	0.60	0.90	0.50	0.20
Asp. ochraceus	0.40	7.30	1.30	1.50	0.60	0.35

3. Phytochemical synergies

Many workers reported that some phytochemical had the ability to inhibit microbialgrowth (Benny and Venitha, 2004) working on seven chineseherbswith toxic effect on some of both fungal and bacterial species; (Tsukiyama et al., 2002) working on licorice and Listeria monocytogenes; (Abdel-Fattah and Abo-Seree, 2004) working on three herbs against Aspergillus sp. and (Sema *et al.*, 2005) working on cardamom extract and the pathogens of E. coli, S. aureus and M. leutus. The bioactivity of clove extract showed almostly the same effects towads both fungal bacterial species, while the antibiotic standard showed more powerful effect towards bacterial studied strains. Similarly, the other four plant extracts showed almostly the same bioactivity towards both fungal and bacterial species. (Lin *et al.*, 2004) demonstrated that the usage of plant extracts as antimicrobials showed efficient bioactivity which mainly due to phytochemical synergies.

Data obtained from the semi-quantitative assay showed that clove extract contained the highest level of terpenoids, while sage extract contains only traces amounts of each of the three determined phytochemicals (Table 8). The other three plant extracts revealed different concentrations of phytochemicals, but the total amount seems to be roughly the same (1-1.5 µg/ml), as well the three extracts contained detectable amounts of terpenoids. (Aureli *et al.*, 1992) found that terpenoids present in essential oils of plants are useful in the control of listeria monocytogenes containing food. The mechanism of action of such phytochemical is not fully understood, but is speculated to involve membrane disruption (Mendoza *et al.*, 1997). However, many authors agreed the obtained results which refered to the bioactivity of terpenoids containing plants against bacteria (Tassou *et al.*, 2000 and Taylor *et al.*, 1996) and against fungi (Rao *et al.*, 1993 and Rana *et al.*, 1997).

Table 8: Semi-quantitative assay of the 5 plant extracts (µg/ml)

Plant extracts	phytochemicals		
	Phenols	Flavonoids	Terpenoids
Clove	Traces	Traces	1.5 – 2.0
Sage	Traces	Traces	Traces
Rosmary	> 0.5	Traces	0.5 – 1.0
Basil	Traces	Traces	1.0 – 1.5
Lemon grass	Traces	0.5 – 1.0	> 0.5

It is worthy to mention that the recent work focused on three phytochemicals only, while such bioactive substances include other groups and families as alkaloids, lectins, polypeptides, tannins, quinones and some

secondary metabolites (Cowan,1999). The most bioactive plant extract (clove) showed the highest content of terpenoids, while sage extract revealed traces of each of terpenoids, phenols and falvonoids. The rosmary, basil and lemon grass extracts showed approximately the same quantities of the three phytochemicals which reflecting almostly the same bioactivity on both fungal and bacterial species.

The collicted data from Tables 1 to 8 clearly indicate that olthough mold growth and mycotoxin production were significantly reduced by the presence of basil and sage oils in the media; there were no considerable antifungal properties of these oils. And this is because the appearent reduction over control, by these oils was less than 50% (Tables 1 - 6). It is of interest to note that compound is considered as a positive inhibitor if it altered growth and / or mycotoxin biosynthises by 50% or more (Masimango *et al.*, 1978). Subsequet to these results, the inhibitory activity of clove oil was found to be the most positive inhibitor, especially against *A. ochraceus*. These results suggested that herbs and spices could be used as a guide in the continuing search for new natural products with potential antifungal properties.

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التأثير المعاكس لبعض النباتية الطبية ضد بعض الفطريات غير المرغوبة والقادرة علي إفراز سموم فطرية

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هذه الدراسة استهدفت اختبار التأثير المعاكس لخمسة مستخلصات نباتية ضد ثلاثة أنواع من الفطريات هى اسبرجلس فلافس، اسبرجلس باراسيتكس، اسبرجلس اوكراشيس، وجميع السلالات المختبرة معرفه بمقدرتها علي إنتاج سموم ميكروبية وبأنها كائنات غير مرغوبة. والنباتات الخمس موضوع الدراسة هى القرنفل، المريمية، الريحان، حصالبان، حشيشة الليمون تم جمعها من الأسواق المحلية واستخلاص محتواها الزيتي. استخدمت المستخلصات الزيتية بصورتها الخام للتعرف علي التأثير التثبيطي وأيضا "أقل تركيز تثبيطى MIC" للمستخلصات الخمسة علي السلالات الفطرية المختبرة، بالإضافة إلى أجراء فحوص وصفيه وشبه كميه للتعرف علي محتوي المستخلصات الخمسة من الفينولات والفلافونات والتربينات. ودلت النتائج علي تفاوت درجات التأثير المعاكس للمستخلصات المستخدمه لوحظ أعلاها عند المعاملة بمستخلصات القرنفل تجاه السلالات الميكروبية جميعا وهو ما انعكس علي مساحات التثبيط للنموات الفطرية. في المقابل لوحظ أقل نشاط حيوى لمستخلصات المريميه التي أظهرت نشاطا محدودا للغاية علي جميع السلالات بصفه عامه وقد أنخفض هذا النشاط إلي قيم صفرية عند المعاملة به تجاه فطر الاسبرجلس أوكراشيس. المستخلصات الثلاثة ريحان، حصالبان، الريحان أظهرت نشاطا حيويا متقاربا الي حد كبير وجميعها كانت أقل من نشاط القرنفل. نتائج تحديد "أقل تركيز تثبيطى MIC" وكذلك نتائج الفحص الوصفي وشبه الكمى للمكونات الفعالة أكدت فاعلية المستخلصات المستخدمة ونشاطها الحيوى، وفيما عدا مستخلصات المريميه فأن الفعل التثبيطى لوحظ عند تركيزات تتراوح بين ٠,٢ – ١,٥ ملليجرام لكل ملليلتر من مستنبتات الأختبار، كان حدها الأدنى عند المعاملة بالقرنفل وحدها الأعلى عند المعاملة بحشيشة الليمون. دلت أيضا نتائج الفحص للمواد الفعالة علي ارتفاع محتوي مستخلصات القرنفل من التربينات ووجود أثار من الفلافونات والفينولات بينما لم تحتوي مستخلصات المريميه علي نسب يمكن تقديرها من المواد الثلاثة الفعالة، أما المستخلصات الثلاثه للريحان وحصالبان وحشيشة الليمون فقد تقاربت نسب المواد الفعالة بها وهو ما يعكس تقارب التأثير التثبيطى لكل منها. وهذه التأثيرات ضد السلالات المختبرة تؤكد إمكانية إستخدام هذه الزيوت كمواد حافظة ، ولكن هذا يتطلب مزيداً من البحث بالتجربة على بعض الأغذية.