Antifungal Activity of *Cinnamomum zeylanicum* and *Eucalyptus microtheca* Crude Extracts Against Food Spoilage Fungi

Sameer N. Mahmoud
Biotechnology Research Center, Al Nahrain University

ABSTRACT:

The antifungal activity of barks of *Cinnamomum zeylanicum* and leaves of *Eucalyptus microtheca* crude extracts were tested *in vitro* by agar well diffusion method against *Penicillium digitatum* and *Aspergillus niger* fungi. Alcoholic (methanolic and ethanolic) and aqueous crude extracts were tested to evaluate for their antifungal activities. Two broad spectrum antibiotics, Chloramphenicol and Ciprofloxacillin, were used for comparison. Both plant crude extracts demonstrated antifungal activity. When compare extracts of the two plants, *C. zeylanicum* extracts showed higher inhibition activity than *E. microtheca* extracts. Alcoholic extracts of both plants significantly inhibited the mycelial growth of *P. digitatum* and *A. niger* fungi more than their aqueous extracts. Methanolic extracts of both plants showed higher inhibition activity than ethanolic extracts. Based on final concentration, the alcoholic extracts of both plants showed moderate to strong (15-29) mm antifungal activity against both fungi. Methanolic extracts exhibited the highest antifungal activity (19-29) mm, followed by ethanolic extracts (15-27) mm, while the least activity was observed by the aqueous extract (6-15) mm. *P. digitatum* was the most affected by all crude extracts and antibiotics. The Phytochemical analysis revealed the presence of wide range of bioactive constituents like flavonoids, tannins, alkaloids, saponins, terpenes, steroids and essential oil. The ability of the extracts to inhibit the growth of the two fungi is an indication of the antifungal potential of cinnamon and eucalyptus parts, which makes them candidate for production of antifungal agents.

Key words: Antifungal, well diffusion method, extracts, antibiotics, inhibition zone, phytochemical

الفاعلية التثبيطية لمستخلصات الدارسين واليوكالبتوس ضد الفطريات المسببة لتلف الاغذية

سمير ناجي محمود
مركز بحوث التقنيات الاحيائية، جامعة النهرين

الخلاصة :

اختبرت الفاعلية التثبيطية لمستخلصات قلف الدارسين (*Cinnamomum zeylanicum*) واوراق اليوكالبتوس (*Eucalyptus microtheca*) خارج الجسم الحي بواسطة طريقة الحفر ضد الفطرين *Penicillium digitatum* و *Aspergillus niger* . اختبرت مستخلصات كحولية (الميثانول والايثانول) ومائية لتقييم فاعليتها التثبيطية ضد الفطريات. استعمل المضادين الحيويين ، Chloramphenicol و Ciprofloxacillin للمقارنة. اظهرت

ISSN 2072-3875

مستخلصات الدارسين واليوكالبتوس فاعلية تثبيطية ضد الفطريات. بمقارنة مستخلصات كلا النباتين، اظهرت مستخلصات الدارسين فاعلية تثبيطية اعلى من مستخلصات اليوكالبتوس. المستخلصات الكحولية لكلا النباتين ثبطت معنويا نمو غزل الفطرين *P. digitatum* و *A. niger* اكثر من المستخلصات المائية للنباتين. مستخلصات الميثانول لكلا النباتين اظهرت فاعلية تثبيطية اعلى من مستخلصات الايثانول. اظهرت المستخلصات الكحولية ، استنادا الى التركيز الاعلى، فاعلية تثبيطية متوسطة الى قوية (15-29) مليمتر ضد كلا الفطرين. كما اظهرت مستخلصات الميثانول اعلى فاعلية تثبيطية (19-29) مليمتر، يليها مستخلصات الايثانول (15-27) مليمتر، بينما اقل فاعلية تثبيطية لوحظت مع المستخلصات المائية (6-15) مليمتر. اظهر الفطر *P. digitatum* تثبيطا واضحا مع مستخلصات النباتين وكذلك مع المضادين الحيويين. التحليل الكيميائي للنباتين اظهر وجود مدى واسع من المركبات الحيوية الفعالة : الفلافونيدات والتانين والقلويدات والتربين والصابونين والستيرويدات والزيت الطيار. ان قدرة المستخلصات على تثبيط نموات الفطرين هو دليل على القوة التثبيطية لاجزاء النباتين الدراسين واليوكالبتوس اذ تجعلهما مرشحين لانتاج مواد ضد الفطريات.

كلمات مفتاحية: ضد الفطريات ، طريقة الحفر ، مستخلصات ، مضادات حيوية ، منطقة التثبيط ، الكيمياء النباتية

Introduction

There is evidence that Neanderthals living 60,000 years ago in present-day Iraq used plants such as hollyhock (Stockwell, 1988),(Thomson, 1978); these plants are still widely used in ethnomedicine around the world. Recently many efforts have been made to discover new antimicrobial agents from various species of medicinal plants. Screening of such plants may result in the discovery of valuable compounds that can be used against pathogenic microorganisms. Plants produce a variety of chemical compounds for defense and communication, and can trigger their own chemical offensive against the attacking pathogens. These chemicals may have general or specific activity against key target sites in bacteria, fungi and viruses. Thus, plant based secondary metabolites, which have defensive role may be exploited for disease control or the management of storage problems associated with food spoilage microorganisms.

Cinnamon is a spice tree contains several bioactive compounds that can be used against a wide range of microorganisms. Cinnamon bark crude extract has constantly been reported to have antifungal activity. This activity was attributed mainly to the presence of cinnamaldehyde and eugenol comoupounds (He et al., 2005). Eucalyptus as well contains several chemical compounds that play several roles in the plant such as defense against insect, vertebrate herbivores and protection against UV radiation and against cold stress. Both cinnamon barks and eucalyptus leaves represent important source of compounds like flavonoids, tannins, glycosides, saponines, alkaloids and essential oils with biological activities such as bacteriostatic, fungistatic and anti-inflammatory. Terpenoids, which form most of the essential oil giving eucalyptus foliage its characteristic smell. Cinnamon and Eucalyptus species possess a strong antimicrobial potential and their volatile oils are used as antibacterial and antifungal agents in creams, soaps and toothpastes (Lis-Balchin et al., 2000).

ISSN 2072-3875

Penicillium digitatum and *Aspergillus niger* are two fungal pathogens causing food spoilage. The green mold of citrus, caused by the fungus *Penicillium digitatum* is considered one of the most economically important postharvest agent of citrus in citrus growing regions of the world. Citrus is grown in over 100 countries on six continents (Saunt, 2000). Infections may lead to the spoilage of almost all kinds of mature citrus fruits (Plaza *et al*., 2004). Wounds on the fruit, inflicted during harvest and subsequent handling, are infected by spores of this pathogen. *Aspergillus niger* can contaminate agricultural products at different stages including pre-harvest, harvest, processing and handling, thus causing considerable economic losses due to spoilage with the consequence of possible accumulation of mycotoxins. For example, black rot of onions associated with *A. niger* is responsible for serious losses of onion bulbs in the field and in storage. *Aspergillus niger* is usually found in common mesophilic environments such as soil, plants, and enclosed air environments. Ochratoxin A is food-contaminating mycotoxin produced by *Aspergillus* spp (Varga *et al.* 2004). Human contact with this mycotoxin usually occurs through consumption of food which has not been stored and taken care of appropriately ((Schuster et al., 2002),(May and Adam, 1997). Studies have shown that less than 10% of the *A. niger* strains were tested positive for ochratoxin A under conditions that were favorable (Schuster et al., 2002).

The heavy usage of pesticides in agriculture to overcome the pre-harvest and post-harvest problems was resulted in many toxic epidemics and resistance to fungicide among fungal pathogens, therefore alternative control methods are needed (Pramila and Dubey, 2004). The uses of plant-derived products as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance (Lee *et al*., 2007)**.** The result of different studies provided evidence that some medicinal plants might be potential source of new antifungal agents**.** Plant extracts and their essential oils are one of several non- synthetic chemical control options that have recently received attention for controlling plant diseases (Soylu *et al.* 2005), (Abad *et al.* 2007).

This research was aimed at evaluating the antifungal activity of alcoholic and aqueous extracts of barks of *Cinnomomum zeylanicum* and leaves of *Eucalyptus microtheca* against *Pepnicillium digitatum* and *Aspergilus niger in vitro* in order to determine their antifungal inhibition activity. Only *in vitro* methods were conducted in assessing the antifungal potential of the crude extracts of cinnamon and eucalyptus.

Materials and Methods

Plant Parts

Fresh leaves of eucalyptus were collected from the campus of the University of Baghdad and cinnamon barks were bought from local market in Baghdad. Both plants were authenticated by Dr Ali H. E. Al Musawi at the Department of Biology- College of Science -University of Baghdad. Eucalyptus was identified as *Eucalyptus microtheca* L

ISSN 2072-3875

(F.von Muell) and cinnamon as *Cinnamomum zeyl*anicum L (Breyn). The leaves of eucalyptus were washed and air-dried in room temperature for two weeks and then ground into small pieces by using electric grinding machine. Cinnamon barks were ground into powder. Both plant parts were kept in plastic bags until used.

Extraction Method

Alcoholic (methanolic and ethanolic) and aqueous crude extracts of barks of cinnamon and leaves of eucalyptus were prepared to test against *Penicillium digitatum* and *Aspergillus niger*. The method of (Harborne, 1984) was used to process the methanolic, ethanolic and aqueous extracts.

Stock solutions and various concentrations (dilutions) of alcoholic extracts were prepared in 40% methanol and 40% ethanol. Each stock solution (100 mg/ml which is equal to (1.0)% mg/ml as the final concentration) was prepared by dissolving 1g of dried methanolic or ethanolic extract in 10 ml of methanol or ethanol, and three concentrations of methanolic and ethanolic solutions were prepared (0.1, 0.2 and 0.3)% mg/ml. This was done by adding 1, 2 and 3 ml of stock solution to 9, 8 and 7 ml of sterilized distilled water respectively as the final volume for each concentration was 10 ml. Stock solutions of aqueous extract of both cinnamon and eucalyptus were prepared by dissolving 1 g of dried aqueous extract in 10 ml of sterilised distilled water and no further concentrations were made. All stock solutions were sterilised through 0.20 µm Millipore filter. The crude extracts were then transferred into clean sterilised glass vessels and stored in refrigerator at 4º C until ready for use.

Tested Fungi

The fungi used in this research were *Penicillium digitatum* and *Aspergillus niger*. *P. digitatum* was isolated from spoiled orange *(Citrus sinensis* L.) and *A. niger* isolated from soil sample. The spoiled oranges were collected from local market in Baghdad and soil samples were collected from vegetable field in Baghdad. The fungal cultures were studied using morphological and staining techniques to identify the two fungi (Mahdi, 1991),(Pitt, and Hocking. 1997).

Antifungal Tests

Fungal cultures of *P. digitatum* and *A. niger* were cultured on Potato Dextrose Agar medium (PDA) (Himedia Labs Pvt.Ltd. India). Cultures were incubated at 28°±2º C for (3-5) days. Spore suspension was used to inoculate the PDA medium. The method used to prepare spore suspension was that used by (Faraj, 1990).

Agar well diffusion method (Bauer et al, 1966) was used to test for the inhibition activity of the extracts against *P. digitatum* and *A. niger*. The fungi were cultured on 20 ml PDA in petri-dishes. An inoculum of 0.1ml fungal suspension was spread uniformly over this medium by using the spreader and allowed to solidify on the agar medium for

ISSN 2072-3875

15 min. Wells of 5 mm in diameter were made on the surface of cultured medium by using sterilised cork borer and each well was filled with certain concentration (0.1, 0.2, 0.3 and 1.0)% mg/ml of each tested crude extract. Wells were distributed evenly on the medium in the Petri-dish of 9 cm in diameter. Extract of (50) µl from each plant crude extract was added into each hole on the medium and allowed to stand on the bench for one hour for proper diffusion. Cultures were incubated at 28º ±2 C for (3-5) days. Inhibition activities of the extracts were determined by measuring the inhibition zones formed around the wells in millimeter. Two wide spectrum antibiotic disks of Chloromphenicol (C) (30) µg/disk concentration and Ciprofloxacin (CIP) (5) µg/disk concentration (Mast Diagnostics UK) were used to test for their antifungal activity against the two fungal growths. The (6) mm diameter antibiotic disks were placed on the surface of cultured medium. The plates were observed for presence of zones of inhibition around the discs from day 3 to 5. All tests were accomplished in triplicates.

Samples of (50) µl of 40% methanol and 40% ethanol were used in the same manner as negative control. The controls were the solvents used for preparation of plant stock alcoholic extracts and they showed no inhibitions in preliminary studies.

Phytochemical Analysis

The phytochemical of alcoholic and aqueous extracts of leaves of eucalyptus and barks of cinnamon were screened using the method used by Harborne (Harborne, 1984). The constituents analysed for are: flavonoids, tannins, alkaloids, saponins, terpens and steroids. Results of phytochemical analysis revealed that the alcoholic extracts of ethanol and methanol of both eucalyptus and cinnamon had similar constituents. The author of this research was able to extract essential oil from both plants using Clavenger apparatus.

Statistical Analysis

The Statistical Analysis System - SAS- was used to analyse the results (Cary, 2004). The results compared statistically to least significant difference (LSD) to level (0.05).

Results

Phytochemical analysis

Phytochemical analysis of *Cinnamomum zeylanicum* and *Eucalyptus microtheca* crude extracts (Table1) confirmed the presence of several bioactive compounds. Favonoids, tannins and alkaloids were detected in alcoholic extract of eucalyptus, and all the former constituents as well as saponins were detected in alcoholic extract of cinnamon. Flavonoids, tannins, saponins, terpens and steroids were detected in aqueous extract of cinnamon and flavonoids and tannins in aqueous extract of eucalyptus. Alkaloids were only detected in alcoholic extracts of both plants. Essential oil was detected in both plant crude extracts.

ISSN 2072-3875

Table (1): Phytochemical analysis of alcoholic and aqueous extracts of cinnamon barks and eucalyptus leaves

Constituents	Cinnamon		Eucalyptus	
	Alcoholic	Aqueous	Alcoholic	Aqueous
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Alkaloids	+	-	+	-
Saponins	+	+	-	-
Terpens	-	+	-	-
Steroids	-	+	-	-
Essentoal oil	+	+	+	+

Note: + = Present, - = Absent

The effect of the crude alcoholic and aqueous extracts of cinnamon barks and eucalyptus leaves against mycelial growth of *Penicillium digitatum* and *Aspergillus niger* are presented in Tables (2, 3, 4). The antifungal activity was determined by measuring the diameters of inhibition zone produced by the crude extracts against the two fungi. The method used by Monks et al (2002) was used to classify the antifungal activity as no activity, weak, moderate and strong. Less than 7 mm no activity, more than 7-10 mm weak activity, more than 10-16 mm moderate activity, more than 16 mm high or strong activity.

Antifungal activity of cinnamon alcoholic extracts

The results in table (2) showed that both cinnamon methanolic and ethanolic extracts exhibited strong antifungal activity (21-29) mm against both *P. digitatum* and *A. niger* based on the final concentration (1.0)% mg/ml. The mycelial growths of both fungi were inhibited at all concentrations (0.1, 0.2, 0.3, 1.0)% mg/ml and the zones of inhibition were increased in diameter as the concentrations of both extracts increased. Methanolic extract exhibited higher inhibition activity than ethanolic extract at all concentrations. The final concentration exhibited the highest antifungal activity against both fungi. *P. digitatum* was the most affected by both plant alcoholic extracts. The range of diameters of inhibition zone for mthanolic extract against *P. digitatum* recorded between (12-29) mm and against *A. niger* recorded (9-25) mm, whereas the range of diameters of inhibition zone for ethanolic extract against *P. digitatum* recorded between (10-27) mm and against *A. niger* recorded (8-21) mm. Based on the final concentration both methanolic and ethanolic extracts exhibited significant reduction in mycelial growth of *P. digitatum* compared with that against *A. niger*.

ISSN 2072-3875

Antifungal activity of eucalyptus alcoholic extracts

The results in table (3) showed that both methanolic and ethanolic extracts exhibited moderate to strong antifungal activity (15-21) mm against both fungi based on the final concentration and, as with cinnamon, the inhibition increased as the concentrations of both extracts increased. Methanolic extract showed higher inhibition activity than ethanolic extract against both fungi. Methanolic and ethanolic extracts exhibited higher inhibition activity against *P. digitatum* than *A. niger* at all concentrations (0.1, 0.2, 0.3, 1.0)% mg/ml. The range of diameters of inhibition zone for mthanolic extract against *P. digitatum* recorded between (8-21) mm and against *A. niger* recorded (0.0-19) mm, whereas the range of diameters of inhibition zone for ethanolic extract against *P. digitatum* recorded between (8-19) mm and against *A. niger* recorded (0.0-15) mm. Based on the final concentration, ethanolic extract showed significant reduction in mycelial growth of *P. digitatum* compared with that against *A. niger*. Results showed that there were no inhibition activity at concentrations (0.1,0.2)% mg/l in both extracts.

Table (2): The average diameter of inhibition zone (mm) of cinnamon bark alcoholic extracts against *P digitatum* and *A niger*

Fungus	%Methanol				%Ethanol			
	0.1	0.2	0.3	1.0	0.1	0.2	0.3	1.0
P. digitatum	12	15	17	29	10	13	16	27
A. niger	9	11	13	25	8	8	11	21
LSD	3.41 ns	3.26*	3.18*	3.16*	2.75 ns	3.81*	3.55*	4.19*

(P<0.05), *=significant, ns= no significant

Table (3): The average diameter of inhibition zone (mm) of eucalyptus leaves alcoholic extracts against *P digitatum* and *A niger*

fungus	% Methanol				%Ethanol			
	0.1	0.2	0.3	1.0	0.1	0.2	0.3	1.0
P. digitatum	8	11	13	21	8	10	12	19
A. niger	0.0	6	10	19	0.0	6	8	15
LSD	2.50*	2.71*	3.55 ns	4.01 ns	2.05*	2.65*	3.60*	4.33*

(P<0.05), *=significant, ns= no significant

Antifungal activity of aqueous extracts

The results in table (4) represent zones of inhibition activity of aqueous extracts of cinnamon and eucalyptus based on the final concentration (1.0)% mg/ml. Results in table (4) showed that cinnamon aqueous extract exerted higher inhibition activity than that of eucalyptus. Aqueous extracts of cinnamon and eucalyptus exhibited no activity to moderate inhibition activity (6-15) mm. Aqueous extract showed higher inhibition

ISSN 2072-3875

activity against *P. digitatum* (9-15) mm than *A. niger* (6-9) mm. Cinnamon aqueous extract significantly reduced the mycelial growth of *P. digitatum* compared with that against *A. niger*.

Table (4): The average diameter of inhibition zone (mm) of cinnamon and eucalyptus aqueous extract against *P. digitatum* and *A. niger*

Fungus	%Aqueous (1.0)% mg/ml		*LSD*
	Cinnamon	Eucalyptus	
P. digitatum	**15**	**9**	**3.49***
A. niger	**9**	**6**	**3.25 ns**
LSD	**3.49***	**3.25 ns**	**----**

(P<0.05), *=significant, ns= no significant

Antifungal activity of alcoholic and aqueous extracts

Results in (table 5) represent zones of inhibition activity for both alcoholic and aqueous extracts of both plants based on final concentration (1.0)% mg/ml. All crude extracts of both plants showed broad antifungal activity against the tested fungi (table 5). Based on the final concentration (table 5), the diameters of inhibition zone of the alcoholic extracts against the tested fungi varied from **(**15) mm to (29) mm and of aqueous extracts from (6) mm to (15) mm. alcoholic extracts exhibited higher and significant antifungal activity against both fungi than their aqueous extracts. Results in (table 5) revealed that alcoholic extracts of both plant showed significant reduction in mycelial growth of *P. digitatum* and *A. niger* when compared with their aqueous extracts. All cinnamon crude extracts (alcoholic and aqueous) showed significant inhibition activity against *P. digitatum* compared with that against *A. niger*, whereas only the ethanolic extract of eucalyptus exerted significant inhibition activity against *P. digitatum.*

Antibiotics Activity Test

P. digitatum and *A. niger* were tested against two broad spectrum antibiotics; Chloramphenicol (C) and Ciprofloxacillin (CIP). The results in table (5) revealed clear sensitivity of *P. digitatum* to both antibiotics with inhibition zone recorded between (22-36) mm, whereas *A. niger* showed some resistance with inhibition zone recorded between (6-7) mm. Both antibiotics significantly inhibited the growth of *P. digitatum.*

Discussion

Results obtained in this research showed that the crude extracts of both barks of *Cinnamomum zeylanicum* and leaves of *Eucalyptus microtheca* possessed potential *in vitro* antifungal activity against *Penicillium digitatum* and *Aspergillus niger*. The antifungal activity of cinnamon and eucalyptus crude extracts can be recognized owing to

ISSN 2072-3875

their content of phytochemical constituents. Several researchers linked the presence of the bioactive compounds in plant parts to the antimicrobial properties of plant extracts (Owolabi et al., 2007),(Alam, 2009).The inhibitory effects of cinnamon and eucalyptus crude extracts against the two studied fungi may, for that reason, be due to the presence of these bioactive constituents.

Phytochemical analysis of the crude extracts of cinnamon and eucalyptus (table 1) indicated the presence of a wide variety of secondary metabolites. The presence of flavonoids, alkaloids, tannins, saponins, terpens, steroids and essential oil in cinnamon or eucalyptus crude extracts may be collectively or individually responsible for the observed antifungal activity. Nevertheless, the various constituents of plant extracts with the essential oils may act synergistically to bring the overall antimicrobial activity. It was reported that the presence of cinnamaldehyde, eugenol, cinnamic acid, which are compounds of the essential oil, in the bark of cinnamon to show antifungal activities (Gill and Holly, 2004). These bioactive constituents posses both antifungal and antibacterial properties. Several investigators showed eugenol to exert antifungal activity against *Aspergillus* spp. and *Penicillium* spp. in various foods (Vazquez *et al*., 2001). Cinnamaldehyde is a major bioactive constituent in the bark of cinnamon and some reports indicated that cinnamaldehyde killed 80% of fungi and bacteria (McCann, 2003). Cinnamaldehyde, eugenol and cinamic acid in addition to flavonoids, alkaloiks, tannins and saponins recognized by some investigators as antifungal agents (Rojas et al.,1992). Among the various constituents of eucalyptus essential oil is 1,8-cineole which is a characteristic compound of the genus *Eucalyptus* and found by some researchers that this compound was largely responsible for a variety of its antimicrobial and pestcidal effect (Santos and Rao, 2000), (Nafiseh et al., 2011).

Both cinnamon and eucalyptus are rich in essential oils. Several researchers attributed the presence of essential oil and its various constituents in cinnamon and eucalyptus extracts for the inhibition of a wide range of microorganisms including fungi and bacteria (Bachir and Mohamed, 2008),(Suree and Lohasupthawee, 2005**).** Essential oils are made up of many different volatile compounds and the make up of the oil quite often varies between plant species. It seems that the antimicrobial effects are the result of many constituents acting synergistically. This means that the individual compounds by themselves are not as effective.

Thus the antifungal activity of the cinnamon and eucalyptus extracts observed in this study against the *P. digitatum* and *A. niger* may be due to the presence of different phytochemical constituents in the extracts. In this research cinnamon extracts exerted higher antifungal activity than eucalyptus extracts and this may be related to the content of bioactive compounds in cinnamon which are more potent against fungi than those in eucalyptus.

The results obtained in the present research (tables 2,3,4) showed that alcoholic extracts of barks of cinnamon and leaves of eucalyptus were more effective than their

ISSN 2072-3875

aqueous extracts in inhibiting the mycelial growth of *P. digitatum* and *A. niger*. This finding is in agreement with previous investigation which stated that the extracts of garlic can inhibit mould growth, and the effectiveness of this inhibition is related to the solvent used in the extraction (Irkin and Korukluoglu, 2007). The higher antifungal activity of alcoholic extracts explains the nature of the bioactive compounds which may be enhanced in the presence of the alcoholic extract (Ghosh et al, 2008). Furthermore, the high volatility of alcoholic, methanolic or ethanolic, extract tends to extract a wider range of antimicrobial compounds from the sample than the aqueous solvent (Ibekwe et al, 2001),(Dutta,1993,).

The large sizes of zone of inhibition produced by alcoholic extracts as was shown by the results of present research is an indication of the effectiveness of the bioactive constituents of the plant extracts against the tested fungi. Alcoholic extracts of cinnamon and eucalyptus showed greater antifungal activity against *P. digitatum* and *A. niger* than their aqueous extracts and both plant methanolic extracts exhibited higher inhibition activity than ethanolic extracts. The results obtained in this research agreed with several investigators who found that alcoholic extracts of cinnamon, eucalyptus and other medicinal plants to exhibit greater effect against fungi and other microorganisms than aqueous extracts (Dash and Murthy, 2011). Results obtained by (Ghassan and Al-Najar,2008) revealed that the methanolic fraction of cinnamon bark showed the highest antifungal activity against four *P. digitatum* isolates as compared with the same fraction from other plants, followed by hexane and aqueous fractions respectively. In another study carried out against *A. alternata* fungus, it was found out that methanolic extract of eucalyptus and other medicinal plants to exhibited impressive antifungal effects in inhibiting the mycelia growth, whereas aqueous extracts had either less or no effects (Zaker and Mosallanejad, 2010). In another study carried out to investigate the antifungal activity of 13 herbs and spices *in vitro* against *Aspergillus niger*, *A. oryzae* and *penicillium* sp., it was found that the crude ethanolic extracts of the three plants *Piper betel*, *Boesenbergia pandurata* and *Andrographis paniculata* exhibited antifungal activity against all tested fungi (Penkhae et al., 2005)

The aim of using the wide spectrum antibiotics was to compare the inhibition activity of plant extracts with those antibiotics against the two fungi. When comparing the inhibition activity of cinnamon and eucalyptus crude extracts with the inhibition activity of antibiotics it showed that there were differences especially with Ciprofloxacillin which exhibited stronger inhibition activity against *P. digitatum* (36) mm than all plant crude extracts, whereas *A. niger* showed high resistance to both antibiotics (6-7) mm, as well as to aqueous extracts (6-9) mm.

Conclusion,

The results of the present research showed the potential antifungal activity of the barks of *Cinnamomum zeylanicum* and leaves of *Eucalyptus microtheca* against the *Penicillium digitatum* and *Aspergilus niger*, which is an indication of the pesticidal value of the plant extracts. This research suggests that the two plant extracts may possess some compounds with antifungal properties against fungi.

ISSN 2072-3875

Table (5): The average diameter of inhibition zone (mm) of final extract concentrations (1.0)% of cinnamon and eucalyptus alcoholic and aqueous extracts and antibiotics against *P. digitatum and A. niger*

Fungus	Cinnamon			LSD	Eucalyptus			LSD	Antibiotics		
	%Methanol	%Ethanol	%Aqueous		%Methanol	%Ethanol	%Aqueous		Chloramphenicol	Ciprofloxacillin	LSD
P. digitatum	29	27	15	4.9 *	21	19	9	5.3 *	22	36	4.79 *
A.. niger	25	21	9	6.2 *	19	15	6	4.8 *	6	7	1.88 ns
LSD	3.16*	4.61*	3.49*	--	4.01 ns	3.49*	3.25 ns	--	5.17*	6.09*	---

(P<0.05), *=significant, ns= no significant

ISSN 2072-3875

References :

Abad, M.J., Ansuategui M., Bermejo P. 2007. Active antifungal substances from natural sources. Arkivoc. (VII): 116–145.

Alam Sher. 2009. Antimicrobial activity of natural products from medicinal plants. *Gomal* . Journal of Medical Sciences. Vol.7, No.1: 72-78.

Bachir Raho Ghalem, Benali Mohamed. 2008. Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis*. African Journal of Pharmacy and Pharmacology. Vol. 2(10): 211-215.

Bauer, RW, Kirby MDK, Sherris J. C., Turck M. 1966. Antibiotic susceptibility testing by standard single disc diffusion method. Am. J. Clin. Pathol., 45: 493-496.

Cary, N.C. 2004. SAS. Statistical Analysis System. User's Guide. Statistical. Version 7th ed. SAS. Inst. Inc. USA.

Dash, G. K. and Murthy P.N. 2011. Antimicrobial Activity Of Few Selected Medicinal Plants. International Research Journal Of Pharmacy (IRJP) 2 (1): 146-152.

Dutta, A.C. 1993. Botany for Degree Students. 5th edition. Oxford University Press, Oxford. Pp. 810-844.

Faraj, M. K. 1990. Regulation of mycotoxin formation in *Zea mays*. PhD thesis, Department of Bioscience and Biotechnology. University of Strathclyde, Glascow, UK.

Ghassan, J. Kanan, Rasha A. Al-Najar . 2008. *In vitro* Antifungal Activities of Various Plant Crude Extracts and Fractions Against Citrus Post-harvest Disease Agent *Penicillium digitatum*. Jordan Journal of Biological Sciences. Volume 1, Number 3, September. Pages 89 – 99.

Gill, AO and Holly RA. 2004. Mechanisms of bactericidal action of Cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. Appl. Environ. Microbiol. 70: 5750-5755.

Ghosh, A, Das B.K., Roy A., Mandal B., Chanda G. 2008. Antibacterial activity of some medicinal plants extracts. Journal of Natural Medicine., 62: 259-263.

Harborne, J. B. 1984. Phytochemical method. A guide to modern techniques of plnats analysis. 2nd Ed. Chapman and Hall. Prss. London.

He, ZD., Qiao CF., Han QB., Cheng CL., Xu HX., Jiang RW., Pui-Hay BP., and Shaw PC. 2005. Authentication and quantitative analysis on the chemical profile of Cassia bark (*cortex cinnamomi*) by high pressure liquid chromatography. J. Agri. Food Chem. 53: 2424-2428.

Ibekwe, V.I., Nnanyere N.F. and Akujobi C.O. 2001. Studies on Antibacterial activity and phytochemical qualities of extracts of orange peels. Int. J. Environ., Health and Human Development. 2(1):41-46.

Irkin, R. and Korukluoglu M. 2007. Control of *Aspergillus niger* with garlic, onion and leek extract. Afri. J. Biotech. 6(4): 384-387.

ISSN 2072-3875

Lee, SO., Choi GJ., Jang KS. and Kim JC. (2007). Antifungal Activity of Five Plant Essential Oils as Fumigant Against Postharvest and Soilborne Plant Pathogenic Fungi. Plant Pathol. J. 23(2): 97-102.

Lis-Balchin, M., Hart SL., Deans SG. 2000. Pharmacological and antimicrobial studies on different tea-tree oils (Melaleuca alternifolia, Leptospermum scoparium or Manuka and Kunzea ericoides or Kanuka), originating in Australia and New Zealand. Phytother Res. 14: 623–62

Mahdi Majid Al-Shukri.1991. Principles of Fungi and Their Plant Diseases.1st edition. The Ministry of Higher Education and Scientific Research, University of Baghdad. Published in Arabic Language. 431p.

May, G. and Adams T. 1997. "The Importance of Fungi to Man". Genome Research. Volume 7. p. 1041-1044.

McCann, J. 2003. Herbal Medicine Handbook, 2nd Edition. Philadelphia: Lippincott.

Monks, N.R., C. Lerner, A.T. Henriques, F.M. Farias, E.S.E. Schwartmannand B. Mothes. 2002. Anticancer, anticemotactic and antimicrobial activities of marine sponges collected off the Coast of Santa Cstarina, Southern Brazil. J. Exp. Mar. Biol. Ecol., 281: 1-12.

Nafiseh Katooli, Raheleh Maghsodlo and Seyed Esmaeil Razavi. 2011. Evaluation of eucalyptus essential oil against some plant pathogenic fungi. Journal of Plant Breeding and Crop Science. Vol. 3(2), pp. 41-43, February.

Owolabi, OJ., Omogbai EKI., Obasuyi O. 2007. Antifungal and antibacterial activities of the ethanolic and aqueous extracts of *Kigelia Africana* (Bignoniaceae) stem bark. Afr. J. Biotechnol., 6(14): 1677-1680.

Penkhae Wanchaitanawong, Piyamat Chaungwanit, Ngamtip Poovarodom and Sunee Nitisinprasert. 2005. *In vitro* Antifungal Activity of Thai Herb and Spice Extracts against Food Spoilage Fungi. Kasetsart J. (Nat. Sci.) 39: 400 – 405.

Pitt, J. I. and Hocking A. D. 1997. Fungi and food spoilage. Blackie Academic and Professional, London.

Plaza, P., Sanbruno A., Usall J., Lamarca N., Torres R., Pons J. and Vinas I. 2004. Integration of curing treatments with degreening to control the main postharvest diseases of Clementine mandarins. Post. Biol. Technol. 34(1): 29-37.

Pramila, T. and Dubey NK. 2004. Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Post. Biol. Technol. 32: 235-245.

Rojas, A., Hernandez L., Pereda-Miranda R., Mata R. 1992. Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. J. Ethnopharmacol. 35: 275–283.

Santos, FA., Rao VS. 2000. Anti-inflammatory and antinociceptive effects of 1,8-cineole, a terpenoid oxide present in many plant essential oils. Phytother Res. (4): 240-244.

ISSN 2072-3875

Saunt, J., 2000. Citrus Varieties of the World. Sinclair International Ltd., Norwich, England.

Schuster, E., Dunn-Coleman N., Frisvad J. and van Dijck P. 2002. "On the safety of *Aspergillus niger* – a review". Applied Microbiology and Biotechnology. Volume 59. pp. 426-435.

Soylu, E.M., Tok F.M., Soylu S., Kaya A.D., Evrendilek G.A. 2005. Antifungal activities of essential oils on post harvest disease agent *Penicillium digitatum*. Pakistan. J. Biol. Sci. 8 (1): 25–29.

Stockwell, C. (1988). Nature's pharmacy. London, United Kingdom: Century Hutchinson Ltd.

Suree Nanasombat and Pana Lohasupthawee. 2005. Antibacterial activity of crude ethanolic extracts and essential oils of spices against Salmonellae and other Enterobacteia. Kmitl Sci. Tech. J. Vol. 5 No. 3: 527-538 Jul-Dec.

Thomson, W. A. R. (1978). Medicines from the Earth. Maidenhead, United Kingdom: McGraw-Hill Book Co. editor.

Varga, J., Juhász A., Kevei F., Kozakiewicz Z. (2004). Molecular diversity of agriculturally important *Aspergillus* species. European Journal of Plant Pathology. 110: 627–640.

Vazquez, I.B., C. Fente, C.M. Franco, M.J. Vazquez and A. Cepeda. 2001. Inhibition effects of eugenol and thymol on *Penicillium citrinum* strains in culture media and cheese. Int. J. Food Microbiol. 67: 157-163.

Wayne, Pa .2000. National Committee for Clinical Laboratory Standards (NCCLS). Performance standard for antimicrobial disk susceptibility test. Approved standard NCCLS document M2-A7.

Zaker, M. , H. Mosallanejad. 2010. Antifungal Activity of Some Plant Extracts on *Alternaria alternata*, the Causal Agent of Alternaria Leaf Spot of Potato. Pakistan Journal of Biological Sciences. 13: 1023-1029.

ISSN 2072-3875