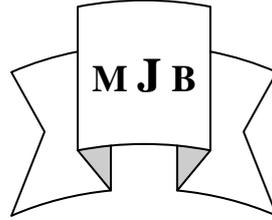


## ***In vitro* Antibacterial Activity of *Eucalyptus microtheca* and *Cinnamomum zeylanicum* Crude Extracts Against *Pseudomonas aeruginosa* Isolates**

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### **Abstract**

In the present research, antibacterial activity of leaves of *Eucalyptus microtheca* and barks of *Cinnamomum zeylanicum* crude extracts were tested against clinically important bacterial isolates besides environmental isolates. Crude extracts of the two plants were analysed for potential antibacterial activity against *Pseudomonas aeruginosa* isolates. The antibacterial activity was determined in alcoholic (ethanolic and methanolic) and aqueous extracts using agar well diffusion method. All crude extracts of both plants found to exhibit an *in vitro* antibacterial activity against all *Pseudomonas aeruginosa* isolates though the pattern of inhibition activity depended mainly upon the plant extract and bacterial isolate. It was found that of the two tested plants, eucalyptus alcoholic extracts were more effective than those of cinnamon, and of all crude extracts the ethanolic extract of eucalyptus was shown to be the most effective. It was also found that the pathogenic *P. aeruginosa* isolates, which involved in burn and wound infections, were more sensitive to all crude extracts than environmental isolates, though all bacterial isolates were inhibited by all crude extracts. Of the two antibiotics tested it was found that pathogenic isolates were the most sensitive bacterial isolates to Ciprofloxacin and far less sensitive to Chloramphenicol. The phytochemical analysis of the crude extracts of eucalyptus and cinnamon revealed the presence of a wide variety of bioactive constituents which may involve in the antibacterial activity.

**Key Words:** Medicinal plants, phytochemical, antibacterial, *Pseudomonas aeruginosa*, isolate, plant extracts, well diffusion method, antibiotics

**الفاعلية التثبيطية لمستخلصات اليوكالبتوس *Eucalyptus microtheca* والدارسين**

***Pseudomonas aeruginosa* ضد عزلات من بكتريا *Cinnamomum zeylanicum***

### **الخلاصة**

في هذا البحث اختبرت الفاعلية التثبيطية لمستخلصات اوراق اليوكالبتوس *Eucalyptus microtheca* وقلف الدارسين *Cinnamomum zeylanicum* ضد عزلات بكتيرية مرضية واخرى بيئية. اختبرت الفاعلية التثبيطية للمستخلصات الخام للنباتين ضد عزلات البكتريا *Pseudomonas aeruginosa*. استعملت مستخلصات كحولية (الميثانول والايثانول) ومستخلصات مائية لتقييم الفاعلية التثبيطية ضد العزلات البكتيرية باستعمال طريقة الحفر. اظهرت جميع المستخلصات لكلا النباتين فاعلية تثبيطية ضد جميع عزلات البكتريا *Pseudomonas aeruginosa* في خارج الجسم الحي بالرغم من ان نمط الفاعلية التثبيطية اعتمد على نوع المستخلص النباتي والعزلة البكتيرية. وجد بان المستخلصات الكحولية لليوكالبتوس كانت اكثر تأثيرا من الدارسين، ومن بين جميع المستخلصات المختبرة اظهر المستخلص الايثانولي لليوكالبتوس الاكثر تثبيطا. وجد كذلك بان عزلات البكتريا المرضية، والتي تسهم في اصابات الحروق الجروح، اظهرت حساسية عالية لجميع المستخلصات من العزلات البيئية، برغم من ان جميع عزلات البكتريا *P.*

*aeruginosa* قد ثبتت بواسطة جميع المستخلصات. اظهرت الاختبارات بان العزلات المرضية كانت اكثر حساسة للمضاد الحيوي Ciprofloxacin من العزلات البيئية واطهرت اقل حساسية للمضاد الحيوي Chloramphenicol. التحليل الكيميائي لمستخلصات اليوكالبتوس والدارسين اظهر وجود مدى واسع من المركبات الحيوية الفعالة والتي قد يكون لها دور في الفاعلية التثبيطة ضد العزلات البكتيرية.

**كلمات مفتاحية:** نباتات طبية، الكيمياء النباتية، مضاد البكتريا، *Pseudomonas aeruginosa*، عزلة، مستخلصات نباتية، طريقة الحفر، مضادات حيوية

## Introduction

Plants have attracted researchers all over the world as a source of medicinal treatment because of the active compounds that present in their parts. Recently these plants of medicinal importance are increasingly being investigated by researchers because of their antimicrobial activity. The antimicrobial activities attributed to compounds synthesized by plants which are known by their active ingredients for instance the phenolic compounds which are part of the essential oils [1]. Several studies have been conducted on antimicrobial activity of plants in different parts of the world [2,3] in an effort to discover new antimicrobial compounds from various plants and their species. These novel compounds may represent an alternative to synthetic chemicals such as drugs and antibiotics, which may exhibit side effects. The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents.

The genus *Eucalyptus* is well known for its bioactive compounds [4] and is a source for several unique secondary metabolites such as essential oils, glycosides and flavenoids which show a variety of biological activities, such as those of antibacterial, antioxidants, HIV inhibitors and others [5,6]. Antimicrobial properties of eucalyptus species are continuously being reported from different parts of the

world. Eucalyptus is an aromatic tree that belongs to the family Myrtaceae. It contains about 600 species and shrubs [7]. Several species of Eucalyptus are used in traditional medicine as an antiseptic against microorganisms and against infections of the upper respiratory tract, such as cold influenza and sinus congestion. [8]. Cinnamon is a spice tree obtained from the inner bark of several trees from the genus *Cinnamomum*. Cinnamon trees are native to South East Asia such as India, Sri Lanka and Bangladesh [9]. A study carried out in 2007 suggested that specific plant terpenoids contained within cinnamon have potent antiviral properties [10]. Another study carried out in 2008 showed that cinnamon barks have an antiviral therapeutic effect [11].

*Pseudomonas aeruginosa* is a gram-negative bacterium and abundant in various types of moist environments such as soil and water and can adapt to numerous other environment. *Pseudomonas aeruginosa* is also an opportunistic pathogen that infects patients in hospitals particularly in burns patients where the skin host defense is destroyed. It can cause serious infections in immunocompromised hosts which include patients with severe burns [12], cystic fibrosis patients and cancer patients [13,14]. The burn is considered as one of the major health problems in the world, the infection of which results in severe complications in patients who have sustained burns [15].

The aim of this research is to evaluate for the activity of *Eucalyptus microtheca* leaves and *Cinnamomum zeylanicum* barks to inhibit the growth of *P. aeruginosa*. In the present research, the selection of plant parts from eucalyptus and cinnamon is based on the fact that these plant parts contain active ingredients such as essential oil, alkaloids, flavonoids and tannin which are active against bacteria and other microorganisms. In this research, the *in vitro* antibacterial activity of the crude extracts of eucalyptus leaves and cinnamon barks were investigated.

## **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 (F.von Muell) [16] and cinnamon as *Cinnomomum zeylanicum* L (Breyn) [17]. 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 (ethanol and methanol) and aqueous crude extracts for leaves of *Eucalyptus microtheca* and barks of *Cinnamomum zeylanicum* were prepared to test against *P. aeruginosa* isolates. The method of Harborne [18] was used to process the ethanolic, methanolic and aqueous extracts.

Stock solutions and various dilutions (concentrations) of alcoholic extracts were prepared in 40% ethanol and 40% methanol. Each stock solution 100mg ml<sup>-1</sup> was prepared by dissolving 1g of dried ethanolic or methanolic extract in 10 ml of ethanol or methanol and three dilutions of ethanolic and methanolic solutions were prepared (0.1, 0.2 and 0.3) 100mg/ml. This was done by adding 1, 2 and 3 ml of stock solution to 9, 8 and 7ml of sterilized distilled water as the final volume for each dilution was 10 ml. Stock solutions of aqueous extract of eucalyptus and cinnamon were prepared by dissolving 1 g of dried aqueous extract in 10 ml of sterilised distilled water (1.0) 100mg ml<sup>-1</sup> and no further dilutions were made. 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 4° C until ready for use.

### **Bacterial Isolates**

The microorganisms used in this research were four Gram-negative of *Pseudomonas aeruginosa* bacterial isolates. Two were pathogenic isolates which were isolated from patients with wounds and burns in local hospital. These bacterial isolates were selected for their potential to cause skin and wound infections. The other two bacterial isolates were isolated from soil sample (garden) and water sample (lake). All test *Pseudomonas aeruginosa* isolates were identified by using AP 20E [19]

### **Antibacterial Test**

The bacterial isolates of *P. aeruginosa* cultures were grown in pseudomonas selective medium (with added Cetromide Fucidin Chloratidine CFC) and tested on Muller-Hinton agar. Cultures were incubated at 37°C for 24 h. The cultures were adjusted to achieve 1.5x10<sup>6</sup> colony forming units (CFU/ml).

Agar well diffusion method [20] was used to test for the inhibition activity of the extracts against the *P. aeruginosa* isolates. The bacteria were cultured on 20 ml Muller-Hinton agar in petri-dishes. An inoculum suspension of 0.1ml ( $1.5 \times 10^6$ ) cell/ml bacterial broth was spread uniformly over this medium by using the spreader and allowed to solidify on the agar medium for 15 minutes. Wells of 5 mm in diameter were made on the surface of cultured medium by using sterilized cork borer. Extract of 50  $\mu$ l from each plant crude extracts 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 37° C for 24 h. Inhibition activities of the extracts were determined by measuring the inhibition zones formed around the wells in millimeter.

Two wide spectrum antibiotic disks of Chloramphenicol (C) 30  $\mu$ g/disk and Ciprofloxacin (CIP) 5  $\mu$ g/disk (MAST DIAGNOSTICS UK) were used to test for their antibacterial activity against bacterial isolates growth. The 6 mm diameter antibiotic disks were placed on the surface of cultured medium. All tests were accomplished in triplicate.

Samples of 50  $\mu$ l of 40% ethanol and 40% methanol 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 mcirotheca* and barks of

*cinnamomum zeylanicum* were screened using the method used by Harborne [18]. The constituents analysed for are: alkaloids, flavonoids, tannins, and terpenes, steroids, saponins and essential oil. Results of phytochemical analysis revealed that the alcoholic extracts of ethanol and methanol of eucalyptus and cinnamon have similar constituents. The author of this research was able to extract essential oil from both leaves of *Eucalyptus mcirotheca* and barks of *cinnamomum zeylanicum* by using Clavenger apparatus.

#### **Statistical Analysis**

The statistical Analysis System - SAS- was used to analyse the results [21]. The results compared statistically to least significant difference (LSD) to level 0.05

#### **Results and Discussion**

##### **Phytochemical analysis**

Phytochemical analyses presented in table-1 showed that various bioactive constituents are present in the leaves of *Eucalyptus mcirotheca* and barks of *Cinnamomum zeylanicum*. The alcoholic extract of eucalyptus found to have flavonoids, tannins and alkaloids, and all the former components as well as saponins found in alcoholic extract of cinnamon. The aqueous extract of eucalyptus found to have flavonoids and tannins and all the former components in addition to saponins, terpenes and steroids were detected in aqueous extract of cinnamon. Alkaloids were absent in aqueous extracts of both plants. Essential oil was observed in both plant extracts.

**Table 1** Phytochemical analysis of alcoholic and aqueous extracts of eucalyptus leaves and cinnamon barks

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

**Note:** + = Present, - = Absent

### Antibacterial Activity of alcoholic and aqueous extracts

Results of this research showed that both alcoholic and aqueous extracts of leaf of *Eucalyptus mcrotheca* and bark of *Cinnomoum zeylanicum* were active in inhibiting the growth of bacterial isolates. Results observed in table-2 and table-3 revealed that the zones of inhibition were increased as the concentrations of ethanolic and methanolic extracts of eucalyptus and cinnamon increased and the final crude extract concentration (1.0) 100mg/ml exerted the highest antibacterial activity.

Results in table -2 revealed that both alcoholic extracts of eucalyptus found to inhibit the growth of all studied bacterial isolates at all concentrations (0.1, 0.2, 0.3 and 1.0) 100mg/ml. Burn and wound isolates were the most affected by both alcoholic extracts with zones of inhibition sizes recorded between (14-29 mm) by ethanolic and (12-25 mm) by methanolic extracts, whereas the least inhibition activity exhibited against water isolate which recorded between (9-20 mm) by ethanolic and (6-17 mm) by methanolic extracts. Burn isolate was significantly inhibited at all concentrations by both extracts.

Result in table-3 showed that there were differences in inhibition activity of cinnamon at different

concentrations in both ethanolic and methanolic extracts against bacterial isolates. Burn isolate showed inhibition activity at all concentrations (0.1, 0.2, 0.3 and 1.0) 100mg/ml, whereas wound, soil and water isolates showed no inhibition activity at concentrations (0.1) 100mg/ml by ethanolic and (0.1, 0.2) 100mg/ml by methanolic extracts. Burn isolate was found significantly inhibited by both ethanolic and methanolic extracts with zones of inhibition sizes recorded between (13-23 mm) by ethanolic extract and (9-21 mm) by methanolic. Soil and water isolates were the least affected which recorded between (0.0-17 mm) by both extracts.

Aqueous extract, as shown in table-4, was found effective against all bacterial isolate with zones of inhibition recorded between (12-17 mm). Cinnamon aqueous extract exhibited slightly higher inhibition activity (14-17 mm) to compare to eucalyptus (12-16 mm).

Burn and wound isolates were found to have the highest inhibition activity with zone size ranging between (16 mm and 17 mm). The least inhibition activity was shown against soil and water isolates (13-15 mm) and (12-14 mm) respectively.

Results in table-5 showed zones of inhibition activity of ethanolic, methanolic and aqueous extracts based

on final extract concentration (1.0) 100mg/ml. Both ethanolic and methanolic extracts of the eucalyptus showed significant inhibitory activities over aqueous extract. Ethanolic and methanolic extracts significantly

inhibited the growth of burn isolate over all other isolates. The diameters of inhibition zone of the alcoholic extracts against the bacterial isolates varied from (17-29 mm) and of aqueous extracts from (12-17 mm).

**Table 2** The average diameter of inhibition zones (mm) of eucalyptus alcoholic extracts against four *Pseudomonas aeruginosa* isolates

Bacteria isolate	Ethanol				LSD	Methanol				LSD
	0.1	0.2	0.3	1.0		0.1	0.2	0.3	1.0	
Burn	14	18	20	29	6.23*	12	15	18	25	5.73*
Wound	13	15	17	24	5.41*	11	13	15	23	5.94*
Soil	12	15	17	22	5.77*	9	11	13	18	5.02*
Water	9	11	14	20	6.24*	6	9	11	17	4.37*
LSD	3.27*	5.0*	4.81*	4.69*	--	4.53*	3.93*	5.61*	5.88*	--

(P<0.05), \*=significant, NS= no significant

**Table 3** The average diameter of inhibition zones (mm) of cinnamon alcoholic extracts against four *Pseudomonas aeruginosa* isolates

Bacteria isolate	Ethanol				LSD	Methanol				LSD
	0.1	0.2	0.3	1.0		0.1	0.2	0.3	1.0	
Burn	13	15	16	23	4.63*	9	11	14	21	4.52*
Wound	0.0	6	9	18	7.02*	0.0	0.0	8	17	5.39*
Soil	0.0	7	9	17	6.78*	0.0	0.0	7	17	5.39*
Water	0.0	7	8	17	6.75*	0.0	0.0	7	17	5.39*
LSD	4.50*	4.20*	5.16*	4.94*	--	3.50*	4.20*	4.83*	3.12*	--

(P<0.05), \*=significant, NS= no significant

**Table 4** The average diameter of inhibition zones (mm) of eucalyptus and cinnamon aqueous extracts against four *Pseudomonas aeruginosa* isolates

Bacterial isolate	Aqueous		LSD
	Eucalyptus	Cinnamon	
Burn	16	17	2.15ns
Wound	16	17	2.15ns
Soil	13	15	2.48ns
Water	12	14	2.44ns
LSD	2.81*	2.50*	--

(P<0.05), \*=significant, NS= no significant

**Table 5** The average diameter of inhibition zones (mm) of alcoholic and aqueous extracts of eucalyptus and cinnamon against four *Pseudomonas aeruginosa* isolates

Bacterial isolate	Eucalyptus			LSD	Cinnamon			LSD
	Ethanol	Methanol	Aqueous		Ethanol	Methanol	Aqueous	
Burn	29	25	16	4.2*	23	21	17	3.4*
Wound	24	23	16	3.8*	18	17	17	2.1ns
Soil	22	18	13	2.9*	17	17	15	2.3ns
Water	20	17	12	4.5*	17	17	14	2.7*
LSD	4.18*	5.31*	3.14*	--	5.49*	2.81*	2.35*	--

(P<0.05), \*=significant, NS= no significant

**Antibiotics Activity Test**

Both Chloramphenicol (C) and Ciprofloxacin (CIP) antibiotics were tested against *P. aeruginosa* isolates. Results in table-6 showed that all *P. aeruginosa* isolates were inhibited by both antibiotics. All bacterial isolates were highly sensitive to Ciprofloxacin (24-37 mm) and far less sensitive to Chloramphenicol (9

mm) each. Burn isolate showed the highest sensitivity to Ciprofloxacin (37 mm) followed by wound isolate (33 mm), then water isolate (25 mm) and soil isolate (24 mm). Both burn and wound isolates showed significant inhibition activity to Ciprofloxacin over water and soil isolates. All bacterial isolates showed some resistance to Chloramphenicol.

**Table 6** The average diameter of inhibition zone (mm) of antibiotics against four *Pseudomonas aeruginosa* isolates

Bacterial isolate	Chloramphenicol	Ciprofloxacin	LSD
Burn	9	37	5.63*
Wound	9	33	5.19*
Soil	9	24	4.64*
Water	9	25	4.59*
LSD	N.S	5.71*	--

(P<0.05), \*=significant, NS= no significant

**Discussion**

Results of phytochemical analysis revealed that the crude extracts of both leaves of *Eucalyptus microtheca* and barks of *Cinnamomum zeylanicum* table-1 exerted potential *in vitro* antibacterial activity against all *Pseudomonas aeruginosa* isolates. The antibacterial activity demonstrated in this research against *P. aeruginosa* isolates may be due to the presence of potent compounds in the extracts. Several investigators attributed the presence of the secondary metabolites in plant parts to antimicrobial activity

[22,23]. Karou et al., for example, in 2006 [24] attributed the observed antimicrobial activities to the presence of some bioactive compounds like alkaloids, tannins, saponins, terpenes and essential oils in plant extracts. It was also shown by a number of investigators that essential oil of eucalyptus and cinnamon and their major components to possess antimicrobial activity against bacteria and other microorganisms [25,26]. Essential oil and its major components possess toxicity against a wide range of microorganisms. Some essential oils

contain active components which influence certain metabolic functions of microbial cells. The presence of bioactive components with essential oil in eucalyptus and cinnamon extracts may act collectively to produce the antibacterial activity shown in this research, and the observed antibacterial activity may be as a result of the several components act synergistically to bring the overall activity effect. Thus the inhibitory effects of eucalyptus and cinnamon extracts against the *P. aeruginosa* isolates may be due to the combination of several components present in the extracts.

Results obtained in this research indicated that organic (alcoholic) extracts exhibited more potent antibacterial activity than aqueous extracts. Both plant alcoholic extracts showed higher antibacterial activity against individual bacterial isolates than their aqueous extracts. It was found by some researchers that alcohol solvent to be a better solvent for extraction of antimicrobially active substances compared to water [27]. Two possibilities that may consider for the higher antibacterial activity of alcoholic extract are the nature of bioactive compounds which may be enhanced in the presence of the extract [28] and the high volatility of ethanol or methanol which tends to extract more active compounds from the sample than water [29]. Other investigators showed that some antimicrobial substances could only be extracted by organic solvents, suggesting that organic solvents are clearly better solvents of antimicrobial agents [30].

The results obtained in this research agreed with several investigators who found that alcoholic extracts of eucalyptus, cinnamon and other medicinal plants to exhibit greater effect against bacteria and other

microorganisms than aqueous extracts [31,32,33].

More observed results showed that Pathogenic isolates were found more sensitive to all crude extracts than the environmental isolates. Results of the present research (tables 2,3,4) showed that both plant crude extracts exhibited higher inhibition activity against pathogenic isolates than environmental isolates. However alcoholic extracts showed stronger antibacterial activity against pathogenic isolates than aqueous extracts [34]. When comparing the inhibition activity of eucalyptus and cinnamon crude extracts with the inhibition activity of antibiotics it showed that there were clear differences especially with Ciprofloxacin which showed stronger antibacterial activity against burn and wound isolates than the two plant extracts, whereas Chloramphenicol showed low activity against all studied bacterial isolates. Since, multidrug resistance of some bacteria is a major medical problems, screening of natural products in a search for new antibacterial agents that would be active against these bacteria is needed.

### Conclusion

Results of the present research demonstrated that leaves of *Eucalyptus microtheca* and barks of *Cinnamomum zeylanicum* extracts possessed antibacterial potentials. Both plant extracts revealed a fine antibacterial activity. Crude extracts of *Eucalyptus microtheca* and *Cinnamomum zeylanicum* were found to be effective against all the tested *P. aeruginosa* isolates and the alcoholic extracts of both plants exerted appreciable antibacterial effect against pathogenic *P. aeruginosa* isolates. The use of natural compounds of plant origin against bacteria may represent an alternative to chemical synthetic

compounds such as drugs and antibiotics. Thus the extracts of higher plant can be important source of antibacterial agent against various bacterial pathogens.

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