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Antibiosis by Cinnamon Extracts Against Antibio-Resistant Strains

SENHAJI O., FAID M.†[1] AND ELYACHIOUI M.‡
Laboratoire de Recherche et d'Analyses Médicale de la Gendarmerie Royale, Av. Ibn Sina Agdal- BP n° 8087 Rabat,Maroc
†Hassan II Institute of Agronomy and Veterinary Medicine Department of Food Engineering and Technology, P.O. Box 6202, Rabat-Institute, Morocco
‡College of Science, Ibn Toufail University, P.O. Box 133, Kénitra, Morocco
[1]Corresponding author's e-mail: faidmohamed@yahoo.fr

ABSTRACT

Extracts from cinnamon with water, hexan, methanol and ethanol were tested on antibio-resistant Gram positive and negative bacteria, on yeasts and *Leishmania infantum* by the microtitration assay method. Results showed that the hexanic and ethanolic extracts were the most active against both Gram + and – bacteria, and yeasts. The hexanic extract and methanolic extracts showed the highest inhibition. Hexanic extract was also highly inhibitory to parasites. The MIC was not influenced by the extraction for all the extracts so the 5th extracts were almost as inhibitory as the first ones and the second extraction by dilution of 1/50. The hexanic extracts to 1/50 were sufficient to inhibit almost all the species and the ethanolic extract was inhibitory to all the strains by the dilution ranging from 1/10 to 1/80.

Key Words: Cinnamon; Antimicrobial; Microbiology; Antibio-resistant bacteria; Parasites; *Leishmania infantum*

INTRODUCTION

Extracts from many plants used as flavoring and seasoning agents in foods and beverages have been used therapeutically for centuries (Ayres *et al.,* 1980; Charalambous, 1994). However, there is little data on the antimicrobial activities of most plant extracts (Conner & Beuchat, 1984; Davidson & Brancen, 1993). The antimicrobial activity of garlic, onion, cinnamon (*Cinnamon zeylanoides*) and cloves has been studied since the end of the last century and the active components in these herbs were determined (Saxena & Vyas, 1986).

Aromatic and medicinal plants have acquired particular attention in the field of intensive research on the natural antimicrobial compounds. Several plants are known for their antibacterial (Alzoreky & Nakahara, 2003) and/or antifungal (Renée & Harborne, 1994) properties. The use of these plants or their extracts is preferred to chemicals in the field of food preservation. In a previous study, Faid *et al.* (1995) showed that when the whole plant was used, the activity was higher than its oil or extracts with water or other organic solvents. In some cases, the entire plant is not easy to handle and not practical for the user so, the oil is often used as the most active principle of the plant. However, the use of the oil does not represent the whole plant and some other secondary principles are usually lost, which some times may be more active than other more abundant compounds.

In the present study, the antimicrobial activity of the cinnamon extracts with water, methanol, ethanol and hexan has been investigated.

MATERIAL AND METHODS

Extraction with water. Extracts were prepared by mixing 10 g of ground cinnamon with 50 mL of distilled water and were (i) heated until boiling and allowed to cool to 40°C (for warm extract) and (ii) placed in shaker IKA at ambient temperature for 12 h (for cold extract). Both the mixtures were filtered using Whatman No.4 paper.

Extraction with methanol, ethanol and hexan. 10 g of ground cinnamon was mixed with 50 mL of the solvent (methanol, ethanol & hexan) in separate erlenmeyer flasks, which were placed on a shaker (IKA, Germany) for 2 h. The mixtures were then filtered using Whatman No.4 paper and the solid cake was extracted 4 times with the same volume of the solvent as already indicated and the filtrates or extracts for each solvent were kept separated to be used in the inhibitory assays.

Antibiotic sensivity of bacteria. The effect of cinnamon was studied on pure cultures of Gram + and – bacteria, yeasts and parasites. All the strains used were anti-bioresistants. All the strains were tested for their resistance to the antibiotics by the ATB streeps (Biomerieux SA, France) (Table Ia).

Strains used. *Escherichia coli* (5 isolates), *Klebsiella pneumonae* (5 isolates), *Staphylococci aureus* (4 isolates), *Streptococcus agalactiae* (5 isolates), *Pseudomonas aerugenosa* (5 isolates), *Bacillus sp* (3 isolates) *Candida albicans* (3 isolates), *Candida glabrata* (1 isolates), *Leishmania infantum* (1 isolates). The isolates were spot inoculated and incubated at 37°C for 24 h for bacteria, 48 h for yeasts and 5 days for parasites. A control plate of the medium was inoculated in the same conditions as the assays

Table Ia. Antibiotic resistance of the strains used in the antimicrobial activity of cinnamon

Antibiotics Strains	TIC	CIP	TSU	GEN	PEN	TET
E. coli 1	R	R	S	S	R	S
E. coli 2	R	R	R	R	R	S
E. coli 3	R	R	R	R	R	S
E. coli 4	R	R	R	S	R	R
E. coli 5	S	R	R	R	R	R
Klebsiella pneumonae 1	R	R	S	R	R	R
Klebsiella pneumonae 2	S	R	R	R	R	R
Klebsiella pneumonae 3	R	R	R	R	R	S
Klebsiella pneumonae 4	R	S	R	R	R	R
P. aeruginosa 1	R	R	R	R	R	R
P. aeruginosa 2	R	S	R	R	R	R
P. aeruginosa 3	R	R	R	S	R	S
P. aeruginosa 4	R	R	R	R	R	R
P. aeruginosa 5	S	R	R	S	R	R
S. aureus 2	S	R	R	R	R	R
S. aureus 3	R	S	S	R	R	R
S. aureus 4	S	S	R	R	R	S
S. aureus 5	R	R	S	R	R	S
S. aureus 6	R	R	S	R	R	R
St. agalactiae 1	R	R	S	R	R	R
St. agalactiae 2	S	R	R	R	R	R
St. agalactiae 3	R	R	R	R	R	R
St. agalactiae 4	R	R	S	R	R	R
St. agalactiae 5	R	R	S	R	R	R
Bacillus sp 1	S	I	R	R	R	R
Bacillus sp 2	R	R	R	S	R	R
Bacillus sp 3	S	R	S	S	R	S

TIC: Ticarcillin 16-64 mg/L; CIP: Ciporfloxacin 1-2 mg/L; TSU: Cotrimoxazole 2/38 mg/L; GEN: Gentamicin 4 mg/L; PEN: Penicillin 0.25 mg/L; TET: Tetracyclin 4 mg/L

Table Ib. Antifungal resistance of the strains used in the antifungal activity of cinnamon

Antifungal Strains	5FC	AB	MCZ	KET	ITR	FLU
Candida albicans 1	R	R	S	S	R	S
Candida albicans 2	R	R	S	R	R	S
Candida albicans 3	R	R	S	R	R	S
Candida glabrata 1	S	R	R	S	S	R

5FC: 5-Fluorocytosine (2-32 µg/mL); AB: Amphotéricine B (2-8 µg/mL); MCZ: Miconazole (0.5-8 µg/mL); KET: Kétoconazole (0.5-4 µg/mL); ITR: Itraconazole (0.5-4 µg/mL); FLU: Fluconazole (8-64 µg/mL)

Table Ic. Antiparasitic resistance of the strains used in the antiparasitic activity of cinnamon

Antiparasitic Strains	G	F
Leishmania infantum I	R	R

G: Glucantine *30 mg/mL); F: Fongisone (3.125 µg/mL)

but no extract was added.

Determination of minimal inhibitory concentration (MIC). Minimal inhibitory concentration is defined as the lowest concentration that may show an inhibitory zone around the culture. MICs of cinnamon extracts were determined using a broth microdilution test as recommended by NCCLS M27-A (Anonymous, 2000). The medium used was BHI (Brain & Heart Infusion: Sanofi Diagnostic Pasteur). Wells were inoculated with 10 µL of the microbial suspension in saline water. The covered microplates were incubated overnight at 35°C. 10 µL of 2-3-5 Triphenyltetrazolium chloride [TTC] (Sigma) dissolved in sterile water was added aseptically to the microplate wells and incubated at 37°C for 10-30 min. TTC was prepared at a final concentration of 0.4 mg/mL.

RESULTS AND DISCUSSION

Most of the strains used in this study were resistant to antibiotics (Table Ia, Ib, Ic). These were isolated from human samples and might have been involved in some diseases that would imply the use of antibiotics. As it could be pointed out, all the strains showed a normal growth on the medium. There were only few strains, which were sensitive to some antibiotics in every case. All the strains of bacteria were not resistant to penicillin.

Antimicrobial activities in cinnamon extracts by infusion are reported in Table II which shows the inhibition of both Gram + and – bacteria, yeasts and parasites. It could also be pointed out that all the extracts showed the same inhibition on the different strains. In general, most of the strains of *Staphylococcus aureus* and *Klebsiella pneumonia* were sensitive to the extracts. The fifth extract was not inhibitory to most of the strains of *E. coli*, *Pseudomonas aeruginosa* and *Streptococcus agalactiae* and *Bacillus sp.* This is probably due to the low concentration of the

Table II. Inhibitory activity of the infusion extracts of cinnamon

	Strains		E 1	E2	E3	E4	E5
		1	-	-	-	-	-
		2	-	-	-	-	-
	Escherichia coli	3	1/2	1/2	1/2	-	-
		4	1/4	1/2	1/4	-	-
		5	-	-	-	-	-
		1	-	-	-		-
Gram - bacteria		2	1/8	1/4	1/2	1/2	-
	Pseudomonas	3	-	-	-	-	-
	aerugenosa	4	1/8	1/2	1/2	1/2	-
		5	1/8	1/8	1/8	1/4	1/2
		1	-	-	-	-	-
	Klebsiella pneumoniae	2	1/2	1/2	1/2	1/2	1/2
		3	1/8	1/4	1/4	1/2	1/2
		4	1/8	1/4	1/8	1/2	1/2
		1	1/8	1/2	1/2	1/2	1/2
	Staphylococcus	2	1/2	1/2	1/2	1/2	1/2
	aureus	3	1/8	1/4	1/8	1/2	1/2
		4	1/2	1/2	1/2	1/2	-
		1	1/8	1/8	1/8	1/4	-
Gram + bacteria	*Streptococcus*	2	1/4	1/4	1/4	1/2	-
	agalactiae	3	1/2	1/2	1/2	-	-
		4	1/8	1/4	1/8	1/2	-
		5	1/4	1/2	1/2	1/2	1/2
		1	1/4	1/2	1/2	1/2	-
	Bacillus sp	2	1/2	1/2	1/2	-	-
		3	1/2	1/4	1/4	-	-
	Candida albicans	1	1/16	1/8	1/8	-	1/16
	Candida albicans	2	1/16	1/8	1/8	-	-
yeasts	*Candida albicans*	3	1/16	1/8	1/8	-	1/16
	Candida glabrata	1	1/40	1/20	1/20	-	1/16
Parasite	*Leishmania infantum*	1	1/32	1/32	-	-	-

Table III. Inhibitory activity of water extracts of cinnamon

	Strains		E 1	E2	E3	E4	E5
		1	-	-	-	-	1/4
		2	-	-	-	1/4	1/4
	Escherichia coli	3	1/2	1/2	1/2	1/2	1/8
		4	1/2	-	-	-	1/2
		5	-	-	-	-	-
		1	1/4	1/4	1/2	1/2	1/8
Gram - bacteria	*Pseudomonas*	2	-	-	-	-	-
	aeruginosa	3	-	-	-	-	-
		4	-	-	-	-	-
		5	1/4	1/2	1/2	1/2	1/4
		1	-	-	-	-	1/4
		2	-	-	-	-	1/2
	Klebsiella pneumoniae	3	-	-	-	-	1/4
		4	-	-	-	-	1/4
		1	-	-	-	1/4	1/4
	Staphylococcus	2	-	-	-	-	1/2
	aureus	3	-	-	-	1/2	1/2
		4	-	1/2	-	1/4	1/2
		1	-	-	-	-	1/4
		2	-	-	-	-	-
	Streptococcus	3	-	-	-	-	1/8
Gram + bacteria	*agalactiae*	4	1/4	-	-	-	1/8
		5	-	-	-	-	1/4
		1	1/4	-	-	1/4	1/8
	Bacillus sp	2	1/8	-	-	1/4	1/4
		3	1/2	1/4	-	1/4	-
	Candida albicans	1	1/8	1/8	1/16	-	-
	Candida albicans	2	1/8	1/8	1/16	-	-
yeasts	*Candida albicans*	3	1/8	1/8	1/16	-	-
	candida glabrata	1	1/16	1/16	1/16	-	-
Parasite	*Leishmania infantum*	1	1/8	1/8	-	-	-

Table IV. Inhibitory activity of methanolic extracts of cinnamon

	Strains		E 1	E2	E3	E4	E5
		1	1/40	1/20	1/20	-	1/10
		2	1/40	1/20	1/10	-	1/10
	Escherichia coli	3	1/80	1/20	1/20	-	1/10
		4	1/80	1/20	1/20	-	1/10
		5	1/20	1/20	1/20	-	1/10
		1	1/80	1/20	1/20	1/10	1/160
Gram - bacteria		2	1/80	1/20	1/40	-	1/40
	Pseudomonas	3	1/40	-	1/20	1/10	1/20
	aeruginosa	4	1/10	-	1/20	1/10	1/10
		5	1/20	-	1/20	1/10	1/40
		1	1/80	1/10	1/40	-	1/10
	Klebsiella	2	1/40	1/10	1/40	-	1/10
	pneumoniae	3	1/10	1/20	1/20	-	1/20
		4	1/20	1/20	1/160	-	1/160
		1	1/10	1/20	1/20	-	1/10
	Staphylococcus	2	1/10	1/20	1/40	-	1/10
	aureus	3	1/10	1/80	1/80	-	1/10
		4	1/10	1/20	1/20	-	1/10
		1	1/160	1/80	1/160	-	1/80
Gram + bacteria		2	1/80	1/80	1/40	-	1/10
	Streptococcus	3	1/40	1/20	1/40	-	1/20
	agalactiae	4	1/10	1/10	1/20	-	1/10
		5	1/40	1/20	1/40	-	1/20
		1	1/80	1/80	1/40	1/80	1/80
	Bacillus sp	2	1/80	1/80	1/80	1/80	1/40
		3	1/80	1/80	1/80	1/80	1/80
	Candida albicans	1	1/10	1/8	-	-	1/10
	Candida albicans	2	1/10	1/8	-	-	1/10
Yeast	*Candida albicans*	3	1/10	1/8	-	-	1/10
	Candida glabrata	1	1/20	1/16	-	-	1/16
Yeasts	*Leishmania infantum*						
	1		1/8	1/8	-	-	-

remaining active principles. It is rarely observed that both Gram + and – bacteria are sensitive to the same antimicrobial principles and it is known that these bacteria are not sensitive to the same compounds. In our case, all the strains of both bacteria were inhibited by the same concentration. Yeasts were completely inhibited by cinnamon. The extracts by infusion were strongly inhibitory to all strains of yeasts. The infusion showed the highest antileishmanial activity.

The extraction by infusion may indicate a non-convenient method for extracting the active principles from cinnamon. This phenomenon is now being observed and all the procedures used for investigating active principles in plants against microorganisms should take in account this factor.

Results relative to the water extracts without heating (Table III) were quite different from those obtained from infusion. It could be pointed out that the cold water extracts 1, 2, 3 and 4 showed no inhibition in all the strains of bacteria used except for two strains of *Pseudomonas*, one strains of *E. coli,* which was inhibited by all the extracts. The extract 5 was the most active except for most strains of *Pseudomonas*. This could be due to the solubilization of the active compounds, which might require more time in cold water, than by boiling. Temperature may accelerate the extraction but some compounds may be inactivated. The cold water extract 1, 2 and 3 were completely inhibited growth of yeast. The CMIs were ranged between 1/8 and 1/16. The extract 4 and 5 showed no inhibition for all the strains of yeast. Against promastigotes of *Leishmania infantum* all the extracts (1to5) showed also no inhibition.

Extracts of cinnamon by ethanol showed better results than water extracts (Table IV). All the strains were inhibited by even low concentrations compared to water extracts. Antibacterial activity showed that the *Bacillus* strains were the most sensitive and concentration of 1/80 was inhibitory to these strains. It should also be emphasized that all the extracts were active in the inhibition of most strains except for extract 4 which was active only against some strains of *Pseudomonas* and *Bacillus*, which were inhibited by all the extracts at the same concentration. The extract by ethanol was inhibitory for all the strains of yeast the CMIs were ranged between 1/2 to 1/10 against the promastigotes of *Leishmania infantum* only the extracts 1 and 2 have inhibitory effect. But the extracts 3, 4 and 5 were not inhibitory to this parasites.

This can be explained by the solubilization of the active compounds by utilizing the same amount of time in the solvent and also the time required for its extraction. These could not be extracted in the same time during the first extraction, which might be due to the time these compounds to migrate into the solution. The concentration that showed an inhibitory activity was lower than that observed for the water extract. This can be very interesting for the application of these extracts in some microbial

delaying or stopping rather in the medical field or in the food preservation.

Results regarding the effect of methanol extract are reported in Table V. *Escherichia coli* and *Staphylococcus aureus* were the most sensitive to the methanolic extract. This is might be due to the nature of the active principles, which were more soluble in ethanol and other solvent than methanol. Different results were obtained by the methanol extract indicating a net activity on *E. coli, S. aureus* and *S. agalactiae* (Table V). *S. aureus* was more sensitive than other bacteria. The extract 4 showed no activity against all the strains. This would not be easy to explain but some arguments would imply the nature of the chemical compounds that would have antimicrobial properties. Strains of *Klebsiella, Pseudomonas* and *Bacillus* were not inhibited by the methanolic extract. The extraction by methanol was completely inhibited growth of yeasts the CMIs ranged between 1/8 to 1/32 methanol extract was also strongly inhibitory to promastigotes of *Leishmania infantum.* This should be more complicated to explain if the nature of the compounds and their nature could be used to make tentatives about the mechanisms by which the extracts may act in the inhibition of microorganisms.

Extraction by hexan also showed an effect on some strains (Table VI). All the extracts (1 to 5) showed the same pattern on most bacteria except for *Pseudomonas* and Staphylococcus. The extract No.1 and 2 were the most active on *E. coli* and *Klebsiella* with low MICs compared to other extracts (No. 3, 4 & 5). The hexan extract was strongly inhibitory against yeasts and parasites; the CMIs ranged between 1/16 to 1/40 and 1/128, respectively.

Most of the research carried out on cinnamon and other herbs and species has been concerned with the essential oils. Cinnamon is characterized by the most representative compound called o-methoxycinnamaldehyde (Morozumi, 1978), which act with other compounds to achieve the inhibition of microorganisms. This compound is more soluble in ethanol than other solvents such as hexan and methanol. The activity of the ethanolic extracts is mainly due to the o-methoxycinnamaldehyde. Friedman *et al.* (2002) demonstrated that compounds from cinnamon (oil) were among the most active on *E. coli* and *S. enterica,* which was also confirmed by Kalemba and Kunicka (2003), who reported that cinnamon oil was among the strongest antimicrobials from plants and species. Valero and Salmeron (2003) also reported a very strong effect of cinnamon oil against *Bacillus* at 16°C. This may show the interesting combination with refrigeration for psychrotrophic microorganisms in foodstuffs.

In conclusion, this study demonstrated that extracts from cinnamon with water and different solvent have excellent antibacterial, antifungal and antiparasitic activities. This is the first scientific study to report antiparasitic activity of cinnamon.

Table V. Inhibitory activity of the methanolic extracts of cinnamon

	Strains		E 1	E2	E3	E4	E5
		1	1/50	1/50	1/25	-	1/100
		2	1/100	1/50	1/25	-	1/25
	Escherichia coli	3	1/100	1/50	1/10	-	1/25
		4	1/50	1/50	1/25	-	1/25
		5	1/50	1/50	1/25	-	1/25
		1	-	-	-	-	-
Gram - bacteria		2	-	-	-	-	-
	Pseudomonas	3	-	-	-	-	-
	aerugienosa	4	-	-	-	-	-
		5	-	-	-	-	-
		1	-	-	-	-	-
	Klebsiella	2	-	-	-	-	-
	pneumoniae	3	-	-	-	-	-
		4	-	-	-	-	-
		1	1/100	1/50	1/5	-	1/80
	Staphylococcus	2	1/100	1/50	1/25	-	1/10
	aureus	3	1/100	1/50	1/50	-	1/50
		4	1/100	1/50	1/25	-	1/10
		1	1/100	1/80	-	-	-
		2	1/50	1/80	-	-	-
Gram + bacteria	*Streptococcus*	3	1/100	1/25	-	-	-
	agalactiae	4	1/100	1/25	-	-	-
		5	1/100	1/25	-	-	-
		1	-	-	-	-	-
	Bacillus sp	2	-	-	-	-	-
		3	-	-	-	-	-
	C. albicans	1	1/32	1/8	1/8	-	1/16
	C. albicans	2	1/32	1/8	1/8	-	1/16
Yeasts	*C. albicans*	3	1/32	1/8	1/32	-	1/16
	C. glabrata	1	1/40	1/40	1/16	-	1/8
Parasite	*Leishmania infantum*	1	1/8	1/8	-	-	-

Table VI. Inhibitory activity of the hexanic extracts of cinnamon

	Strains		E 1	E2	E3	E4	E5
		1	-	-	-	-	-
		2	1/10	1/80	1/50	1/10	1/50
	Escherichia coli	3	1/80	1/50	1/40	-	1/50
		4	1/80	1/50	1/50	-	-
		5	1/50	1/50	1/25	1/10	1/50
		1	1/50	-	-	-	-
Gram - bacteria		2	-	1/20	-	-	-
	Pseudomonas	3	1/50	-	-	-	-
	aeruginosa	4	-	-	-	-	-
		5	-	-	-	-	-
		1	1/50	1/20	1/10	-	1/50
	Klebsiella	2	1/50	1/20	1/80	1/50	1/10
	pneumoniae	3	1/100	1/80	1/50	1/50	1/50
		4	1/80	1/80	1/40	1/40	1/50
		1	1/25	-	1/10	-	-
	Staplylococcus	2	1/25	-	-	-	-
	aureus	3	-	-	-	-	-
		4	-	1/10	1/50	-	-
		1	1/50	1/10	1/10	1/10	1/10
Gram + bacteria		2	1/80	1/80	1/80	1/80	1/80
	Streptococcus	3	1/100	1/50	1/80	1/50	1/50
	agalactiae	4	-	-	-	-	-
		5	-	-	-	-	-
		1	-	-	-	1/80	1/50
	Bacillus sp	2	1/10	-	1/10	1/80	1/50
		3	1/50	1/50	1/10	1/10	1/10
Yeasts	*Candida ablcans*	1	1/40	1/32	1/16	-	1/16
	Candida ablcans	2	1/40	1/32	1/16	-	1/16
	Candida ablcans	3	1/40	1/32	1/16	-	1/16
	Candida glabrata	1	1/50	1/40	1/30	-	1/8
Parasite	*Leishmania infantum*	1	1/128	1/128	-	-	-

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