

Full Length Research Paper

Effect of essential oil of *Cinnamomum zeylanicum* on some pathogenic bacteria

HADRI Zouheyr^{1,2*}, ALLEM Rachida^{1,2} and Marin Gerald Perry³¹University Hassiba Benbouali of Chlef, Hay Salem, route nationale N° 19, 02000 Chlef, Algeria.²Laboratory of Local Natural Bioresources, University of Chlef, Algeria.³AgroParisTech, 1 Rue des Olympiades, 91300 Massy, France.

Received 21 November, 2013; Accepted 5 February, 2014

Several *in vitro* studies have demonstrated antibacterial activity of essential oils extracted from different medicinal plants. In this work, we performed extraction of essential oil from the bark of *Cinnamomum zeylanicum* by steam distillation, the identification of their compounds was carried out by gas chromatography coupled with mass spectrometry. The antibacterial effect was evaluated on seven isolates of pathogenic bacteria. The results of the chromatographic technique showed that the prevailing compound was cinnamaldehyde at a rate of 91.042%. The diameters of inhibition zones ranged from 19.5 to 30.5 mm. The minimum inhibitory concentrations were between 0.05 and 0.2%. The best antibacterial activity was observed against the Gram-positive isolates.

Key words: Essential oil, *Cinnamomum zeylanicum*, pathogenic bacteria, inhibition.

INTRODUCTION

Medicinal plants are an inexhaustible source of substances and bioactive natural compounds. Essential oils make up a significant market share in the world of cosmetics, health and well-being in the culinary field (Rai et al., 2012).

Essential oils have a very broad spectrum of inhibition of bacteria including Gram-positive and Gram-negative bacteria (Remmal et al., 1993). They are considered as a potential source of natural bioactive molecules. They were the subject of several studies for their possible use as an alternative for the treatment of infectious diseases (Bouhdid et al., 2006).

Cinnamomum zeylanicum is native to India and Sri

Lanka, grown in parts of Africa, South-east India, Indonesia, Seychelles, South America and the Caribbean (WHO, 1999). Their essential oil content varies in the bark and leaves (Ehlers et al., 1995). The cinnamon essential oil and its components (cinnamaldehyde and eugenol) have antibacterial activity (Chang et al., 2001) as the mechanism of activity of cinnamaldehyde is involved in the inhibition of cell wall synthesis (Lambert et al., 2001), inhibition of biosynthetic enzymes (Walsh et al., 2003) and rapid inhibition of ATP or depletion (Gill and Holley, 2004).

This work illustrates the growth inhibitory effect of the essential oil of *C. zeylanicum* against some pathogenic bacteria.

*Corresponding author. E-mail: hadlmd@hotmail.com. Tel: +213552274299.

MATERIALS AND METHODS

Pathogenic bacteria

Helicobacter pylori were isolated from gastric biopsy obtained from a patient suffering from an ulcer. The confirmation of the presence of *Helicobacter pylori* was done by rapid urea test (Cassel-Beraud et al., 1996) which showed change of the color of the indole urea from orange to pink or red. Next, *Helicobacter pylori* were isolated from gastric biopsy grinding with 0.5 ml of nutrient broth and plating on petri dishes containing agar medium chocolate. After incubation for 3 days at 37°C in an anaerobic jar (Bourgeois et al., 1996), purification was done on chocolate agar medium. Biochemical identification was made using API 20 E. This work was made in laboratory of microbiology in university of Chlef-Algeria.

Proteus sp and *Bacillus cereus*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Citrobacter freundii* were isolated from the stools of patients with digestive disorders and infectious diarrhea. These bacteria have been identified in a laboratory for microbiological analysis in Algeria.

Extraction of essential oils

The *C. zeylanicum* essential oil was extracted from the bark rolls originating from Indonesia. The extraction of essential oil (EO) of *C. zeylanicum* was performed by steam distillation of Clevenger-type. A mass of 10 g *C. zeylanicum* was mixed with 50 ml of distilled water (Senhaji et al., 2004) and the distillation time was 5 h (Weiss, 2002).

Analysis of the oil *Cinnamomum zeylanicum* by gas chromatography coupled to mass spectrometry

The operating conditions used for GC-MS analysis (Table 1) was: Camera: Perkin Elmer GC/MS, Model: Clarus 500; analyzer: quadrupole and the serial number was: 650N4110408.

Antibacterial assay

Antibacterial spectrum of essential oils

The growth inhibitory effect of essential oils of *C. zeylanicum* was determined by the agar diffusion method (Okeke et al., 2001). Prior to their use in the inhibition tests, pathogenic bacteria (*E. coli*, *S. aureus*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Proteus sp*, and *Bacillus cereus*) were activated by Selenite cysteine broth. *Helicobacter pylori* was seeded in the agar broth buffered (Guiraud, 1998). All tubes were then incubated at 37°C for 24 h.

The Petri dishes containing the Mueller Hinton agar medium were flooded with 1 ml of the culture of pathogenic bacteria (10^9 CFU). After drying for 30 minutes at 37 °C, filter paper discs (6 mm diameter) impregnated with 50 µl of essential oil were deposited on the surface of the agar. Filter paper discs were impregnated with the solvent Hexane (Hexane used for recovery of the essential oil in extraction) as control. The petri dishes were preincubated for 2 to 4 h at 4°C for the dissemination of the inhibitor and are then incubated for 24 h at 37°C (Vaughan et al., 1992).

Antibiogram

Discs of filter paper impregnated with antibiotics [Amoxicillin, Clarithromycin, Cefazolin and Imipenem (5 mg/ml)] were deposited on the surface of an agar medium previously inoculated with pure cultures of strain to be studied. Upon application of the disks,

antibiotics diffused uniformly. After incubation (24 h at 37°C), the discs around circular inhibition zones correspond to no culture (Guerin and FaubléeCarret, 1999).

Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration is defined as the lowest concentration that can inhibit the growth of bacteria (Senhaji et al., 2004). The essential oil was emulsified by a 0.2% agar solution and then diluted to one-tenth in the same solution. Essential oil concentrations were 0.01, 0.004, 0.002, 0.001 and 0.0002% (v / v). Seeding was done by inundation 1 ml of bacterial culture, the incubation was carried out for 24 h at 37°C (Remmalet al., 1993).

Determination of MIQ (Minimum Inhibitory Quantities)

The minimum inhibitory quantities (MIQ) are defined as the smallest amount of the oil for which no visible growth was compared to the control without essential oil (Billerbeck et al., 2002). This method relies on the evaluation of the inhibitory activity of volatile essential oils in a given incubation temperature. Petri dishes were freshly prepared by dispensing 20 ml of Mueller Hintonagar medium containing bacteria. A filter paper (80 mm diameter) was placed at the bottom of the cover of each petri dish. Just before closing the box, the essential oil tested at different concentrations (0, 10, 20, 40, 80, and 160 µl) was placed in the center of the paper. The boxes were then closed immediately. After incubation at 37°C for six days, the growth was compared to the control.

RESULTS

Extraction efficiency and characteristics

The steam distillation provided essential oil yield of about 1.38%. The oil was a yellow liquid with strong, irritating and persistent odor, which is characteristic to cinnamon.

The chromatographic analysis of the essential oil

The chromatogram of *C. zeylanicum* had 23 peaks, of which 6 were very important. These predominant peaks were subjected to mass spectrometric identification. The results showed the presence of cinnamaldehyde with a major peak (91.042%). The second compound was cinnamyl acetate (8.586%), alpha copaene at a rate of 0.221%. Caryophyllene oxide and bornyl cinnamate represented respectively the rate 0.05 and 0.016% (Table 2). Chromatography of the crude oil gave 6 fractions. Fractions 1, 2, 4 and 6 showed the oxygenates. Fractions 3 and 5 represented the compounds of the hydrocarbon oil of *C. zeylanicum*.

Antibacterial effect of the essential oil of *Cinnamomum zeylanicum*

According to the results, the onset of inhibition diameter of 19.5 to 30.5 mm (Table 3) reflects an excellent antibacterial activity of the EO on the bacterial strains

Table 1. The operating conditions used for GC-MS analysis.

Operating conditions for gas chromatography	
Autosampler	automatic
injected volume	1 µL
carrier gas	helium
column used	Elite-5ms (30 m length, diameter 0.25 µm)
Injection Temperature	250°C
Temperature of transfer line	200°C
Temperature programming	initial temperature: 100°C for 1 mn; ramp: 4°C/mn 260°C for 1 mn
Operating conditions of the mass spectrometry	
Ionization Mode	electron impact
Ionization potential	70 ev
Source temperature	200°C
Scan (m/z)	20-500UMA

Table 2. Compounds of the essential oil of *Cinnamomum zeylanicum*.

Compound	Percentage	MM	Empirical formula
Cinnamaldéhyde	91.042	132	C ₉ H ₈ O
Cinnamylacetate	8.586	176	C ₁₁ H ₁₂ O ₂
Alpha-copaene	0.221	204	C ₁₅ H ₂₄
Caryophylleneoxide	0.05	220	C ₁₅ H ₂₄ O ₂
Bornyl cinnamate2	0.016	132	C ₉ H ₈
Di-n-octylPhthalate	0.085	390	C ₂₄ H ₃₈ O ₄

Table 3. Diameter in mm of the inhibition zones of the essential oil of *Cinnamomum zeylanicum* towards pathogenic strains after 24 h of incubation at 37°C.

Test bacteria	Test 1	Test 2	Test 3	Average
<i>H. pylori</i>	25.5	25	24.5	25
<i>E. coli</i>	23.5	20.5	21	21.66
<i>Proteus sp</i>	18.5	22	21	20.5
<i>Bacillus cereus</i>	31	28	29	29.33
<i>K. pneumoniae</i>	21	18	19.5	19.5
<i>C. freundii</i>	22	22.5	21	21.83
<i>S. aureus</i>	29	31	31.5	30.5

*The disc diameter (6 mm) is included in all tests.

studied. The mean diameters of inhibition zones are 30.5 mm (*S. aureus*), 29.33 mm (*Proteus sp*), 25 mm (*H. pylori*), 21.83 mm (*K. pneumoniae*), 21.66 mm (*E. coli*), 20.5 mm (*C. freundii*) and 19.5 mm (*Bacillus cereus*). Hexane does not have an inhibitory effect against pathogenic bacteria.

Antibiogram

According to the results, all bacterial strains studied had sensitivity to the antibiotic tested screws. The diameters of the inhibition zones varied from one bacterium to another, ranging from 15.33 mm to 30.33 mm (Table 3).

Cefazolin showed a good effect on the four bacteria (*E. coli*, *Proteus sp*, *K. pneumoniae* and *S. aureus*). *B. cereus* was also sensitive to Cefazolin (15.33 mm). The sensitivity of *H. pylori* to Clarithromycin and Amoxicillin corresponded to an average diameter of 16.66 mm. Imipenem was another antibiotic that showed its effectiveness with *C. freundii* whose diameter was 26.33 mm.

Minimum inhibitory concentration and minimum inhibitory amount

The essential oil of *C. zeylanicum* had a significant inhibitory activity for the bacteria that cause digestive disorders. It was very effective towards *Proteus sp*, *C. freundii* and *S. aureus* at a concentration of 0.2%. Inhibitions were also observed with *H. pylori* at concentration of 0.1%, *E. coli* and *K. pneumoniae* at 0.05%, *B. cereus* at 0.03%. The minimum inhibitory amount for *Proteus sp*, *B. cereus* and *E. coli* was 40 μ L, however, it was 20 μ L for *H. pylori*, *K. pneumoniae*, *S. aureus* and *C. freundii*.

DISCUSSION

The results show a good rate of *C. zeylanicum* essential oil (1.38%). Krishnamoorthy et al. (1996) and Weiss (2002) found the values that ranged from 0.4 to 2.8%. The essential oil in this analysis shows a predominance of oxygenates (99.763%) relative to the other identified compounds. Hydrocarbon compounds represent a rate of 0.237%. Among the compounds of this oil, hydrocarbon sesquiterpenes (Alpha copaene) and oxygen (Caryophyllene oxide) represent a rate of 0.271%.

Based on the analysis of specific compounds (6 compounds) by GC-MS, aromatic compounds (cinnamaldehyde and cinnamyl acetate) have a very high rate (99.628%). Raina et al. (2001) and Prabuseenivasan et al. (2006) found that cinnamaldehyde is the major component of the EO of *C. zeylanicum*. Raina et al. (2001) and Vangalapati et al. (2012) showed that this essential oil contains alpha copaene, Caryophylleneoxide, Di-n-octylphthalate and Bornyl cinnamate.

Cinnamaldehyde acts on bacteria by inhibiting the biosynthetic enzymes (Walsh et al. 2003). It can also have an effect on a specific enzyme of *H. pylori* (urease). According to Prabuseenivasan et al. (2006), the inhibitory effect of EO of *C. zeylanicum* may be related to the reduction of intracellular pH of this bacterium (Oussalah et al., 2006).

According to the results, the Gram-positive bacteria are more sensitive to the essential oil of *C. zeylanicum* than Gram-negative bacteria (Table 3). These results are consistent with those found by Bishnu et al. (2009). Some molecules of oil (cinnamaldehyde and cinnamyl) bind to membrane proteins and inhibit peptidoglycan synthesis, the essential component of the bacterial cell wall, thereby increasing their antibacterial effect.

In the work of Hoque et al. (2008), a diameter of 12.5 mm was found when studying the effect of the EO of *C. zeylanicum* against *B. cereus*, our results show a diameter of 29.33 mm. According to the same research, the essential oil of clove does not show a significant effect against the same bacteria (14.2 mm).

The presence of sesquiterpenes in the essential oil of *C. zeylanicum* is significant such as Alpha copaene (0.221%) and Caryophyllene oxide (0.05%). Langenhoven (2006) shows a substantial antibacterial activity of sesquiterpenols on *Citrobacter freundii* at relatively low concentrations of the essential oil of *C. zeylanicum*. They act on the enzymes involved in respiration and alter membrane permeability by direct action on membrane phospholipids pathogenic bacteria. At high concentrations, they cause a total loss of homeostasis, leading to the destruction of cell membranes.

C. zeylanicum EO has a very good effect against *E. coli* (21.66 mm). This may be related to the reduction of intracellular pH of this bacterium (Oussalah et al., 2006; Rayour et al., 2003). The comparison between the diameters of the inhibition zones of antibiotics and the diameters of the *C. zeylanicum* EO shows that *S. aureus*, *K. pneumoniae*, *B. cereus* and *E. coli* are more susceptible to antibiotics than the EO. However, *H. pylori*, *Proteus sp* and *C. freundii* are more sensitive to the EO.

The variation in sensitivity to antibiotics (Table 4) and essential oils depends on the effect of the active ingredients of pathogenic strains. The *C. zeylanicum* EO contains several biologically active compounds that inhibit pathogenic bacteria.

The inhibition of bacterial growth depends on the volatile compounds of the essential oil of *C. zeylanicum* (Prabuseenivasan et al., 2006). Phytochemical and pharmacological studies to better understand the antibacterial activity of EO and their mode of expression still need to be carried out. It was also found out that the minimum inhibitory amount for *H. pylori*, *K. pneumoniae*, *S. aureus* and *C. freundii* is lower than that needed to inhibit *Proteus sp*, *B. cereus* and *E. coli*. The optimal activity against *H. pylori* is due to the aldehydes of *C. zeylanicum* EO (cinnamaldehyde). Thus, aldehydes such as eugenol at a concentration ranging from 0.5 to 1% showed an inhibitory effect against Gram-positive and gram-negative bacteria (Walsh et al., 2003). According to Nanasombat and Lohasupthawee (2005), a concentration of 4.17% was sufficient to exert an inhibitory effect on *C. freundii* and *E. coli*, 1.25% for *K. pneumoniae*, and 2.5% for *B. cereus* (Gupta et al., 2008). Results of Prabuseenivasan et al. (2006) show that a concentration of 0.32% has an inhibitory effect on *S. aureus* and *Proteus vulgaris* about 0.16%.

Conclusion

These results suggest that the essential oil of *C. zeylanicum* can be used in the prevention of gastro-intestinal disorders due to its antibacterial activity. This varies from one

Table 4. Diameter in mm of inhibition zones of antibiotic towards pathogenic strains after 24 h of incubation at 37°C.

Test bacteria	Test 1	Test 2	Test 3	Average
<i>H. pylori</i>	18	16	16	16.66
<i>E. coli</i>	30	31	30	30.33
<i>Proteus</i> sp	28	29.5	28	28.5
<i>B. cereus</i>	18.5	12.5	15	15.33
<i>K. pneumoniae</i>	32.5	26.5	28	29
<i>C. freundii</i>	25.5	27	26.5	26.33
<i>S. aureus</i>	27	24.5	24	25.16

*The disc diameter (6 mm) is included.

bacterium to another and depends on the nature of the chemical compounds of this essential oil.

The results of the chemical analysis by gas chromatography coupled to mass spectrometry show that cinnamaldehyde chemotype is the most abundant compound. The mechanism of action of the essential oil may be due to inhibition of biosynthesis enzymes or rapid inhibition of ATP. These results indicate the important role of the essential oil of *C. zeylanicum* in phytotherapy. A thorough molecular study is needed to understand the mechanisms of action of its active ingredients.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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