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Cinnamaldehyde Constituent and Screening of Antibacterial Potential in Local *Cinnamomum Zeylanicum* Bark

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Abstract

Cinnamomum belongs to the Lauraceae family which is an imperative traditional herbal medicine that is widely distributed all over the world. Our study aimed to identify the cinnamaldehyde constituent and investigate the antibacterial potential in local Cinnamomum zeylanicum bark. The bark was extracted in Petroleum ether using soxhlet apparatus and separatory evaporator until essential oil of C. zeylanicum bark was produced. Essential oil was injected into the Gas Chromatography and Mass Spectroscopy (GC-MS). Two gram positive culture Staphylococcus aureus, Bacillus subtilis and three gram negative culture which are Pseudomonas fluorescen, Salmonella typhimurium, and Serratia marcescens were used to test antibacterial activity using the agar disc diffusion method. Petroleum ether serves as negative control and tetracyline as positive control. From the results, S. typhimurium (44 mm) was found to be highly sensitive to it is action followed by P. fluorescen (35 mm), S. marcescen (34 mm), B. subtilis (33 mm), and S. aureus (29 mm) when the essential oil of C. zeylanicum had been applied on that bacteria cultures. These showed the antibacterial activity of the essential oil might be effect from cinnamaldehyde which found as major constituent in C. zeylanicum bark assist by other constituents could be potential as natural antibacterial agent in pharmaceutical industries.

Keyword: Antibacterial, Cinnamaldehyde, *Cinnamomum zeylanicum* bark

Introduction

Essential oils and other plants extract have evoked interest as sources of natural products due to their potential uses as alternative remedies for the treatment of many infectious diseases (Tepe *et al,* 2004). Ravikumar, 2010 stated that remedies might be of effective benefit and free from side effects. They are broadly used in pharmaceutical, cosmetic and flavor agent in cooking process. *Cinnamomum zeylanicum* bark is one of various spices used in cooking that serves as natural preservatives. Natural preservatives are the chemical agents derived from plants that prevent the decomposition of products by any means (Singh *et al.*, 2010).

Generally, natural preservatives have higher ability to inhibit microbial growth, oxidation processes and certain enzymatic reactions occurring in the food stuffs (Arora and Kaur, 2007). The effect of this inhibition is come from the phenolic compounds present in spices and herbs might also play a major role in their antibacterial effects (Hara-Kudo *et al*., 2004). Stated by Masih *et al*.(2012), the in vitro antibacterial activities of cinnamon bark (*Cinnamomum zeylanicum*) extracted with ethanol showed the positive result against two gram negative food spoilage bacteria *Pseudomonas sp., Escherichia* coli and two gram positive bacteria *Bacillus subtilis and Staphylococcus aureus.* Cinnamon mainly contains essential oils and important compounds like Cinnamaldehyde, eugenol, cinnamic acid and cinnamate(Hanafy and Hatem, 1991).

The aim of this research work is to identify the Cinnamaldehyde constituent and investigate the antibacterial potential of local *Cinnamonum zeylanicum* bark essential oil against positive and negative bacteria as well as consumed as a spice in Malaysia.

Material and Method

Raw Materials

Samples of *C. zeylanicum bark* was purchased from the local market of Arau, Perlis, Malaysia.

Extraction of Essential Oil of Cinnamon Bark

The fresh *C. zeylanicum bark* was weighed and extracted by the application of Soxhlet apparatus. Petroleum ether is used as solvent. The sample was extracted at temperature below 100ºC for 20-30 extraction cycles, depending on the nature of the sample and the solvent employed. The solvent and oil were mixed together and separated using separatory evaporator. Then, the essential oil was collected and stored in a sealed glass bottle with screw lid cover under refrigeration at 4ºC for further analysis.

Gas Chromatography and Mass Spectroscopy (GC-MS)

The extracted material was taken for Gas Chromatography and Mass Spectroscopy (GC-MS) analysis. The Gas Chromatography and Mass spectroscopy (Agilant 6890/Hewlettpackard 5975) was fitted with electron impact (EI) mode. The Helium was used as the carrier gas at a flow rate of 1mL/min. The temperature was programmed at 80ºC for 5 minutes then increased to 300ºC at the rate of 15ºC/min. The temperature of injector and EI detector (70eV) were 280ºC and 300ºC, respectively. Each plant extract of 29 µL was injected with a Hamilton syringe to the Gas Chromatography and Mass Spectroscopy (GC-MS) manually.

Tested Bacteria

Bacterial stocks were obtained from Microbiology Lab UiTM Perlis. Two gram positive culture *Staphylococcus aureus, Bacillus subtilis* and three gram negative culture which are *Pseudomonas fluorescen, Salmonella typhimurium,* and *Serratia marcescens* have been used.

Medium Preparation

Nutrient Broth (NB): A 1.3 g of Nutrient Broth (NB) was mixed with 1000 mL distilled water using beaker before transferred into Schott Duran bottle. The solution was heated until the solution is clear. Nutrient broth was autoclaved at 121ºC for 15 minutes to kill indigenous microbes and then poured into sterile petri dish and allowed to solidify.

Nutrient Agar (NA): A 28 g of Nutrient Agar was mixed with 1000 mL distilled water using beaker before transferred into Schott Duran bottle. The solution was heated until the solution is clear. Nutrient agar (NA) was autoclaved at 121ºC for 15 minutes to kill indigenous microbes and then poured into sterile petri dish and allowed to solidify.

Assay for Antibacterial Activity

The antibacterial assay was carried out using the disc diffusion method. The test bacteria was first inoculated into tubes of Nutrient Broth separately and incubated at 37ºC for 18 hours. Each of the cultures was then adjusted to 0.5 McFarland turbidity standards and inoculated (100µl) onto Nutrient Agar (NA) plates. The discs diffusion (6 mm diameter) with 100% concentrations of the essential oil was putting on each of the plates containing cultures of the different test bacteria. Petroleum ether only was serve as negative control and tetracyline as positive control. The culture plates are incubated at 37°C for 24 hours. After 24-48 hours, antibacterial activity is determined by measurement of diameter zones of inhibition (mm) around each of the extracts and the antibiotics.

Result and discussion

Identification of volatile components in essential oil

The essential oil of *C. zeylanicum* was the most effective as an antibacterial agent which attributed by the presence of some active constituents in the oils. Analysis of essential oil of *C. zeylanicum* obtained after Soxhlet extraction was done using Gas Chromatography and Mass Spectroscopy (GC-MS) method. There were 14 constituents detected in the essential oil of *C. zeylanicum* shown in the table below (Table 1). From the result, there was *cinnamaldehyde* or also known as 3-phenyl-*2-propenal* which is the major component (68.47%) presence in the essential oil of *C. zeylanicum* bark. From the chromatogram as seen in Figure 1, the cinnamaldehyde component was shown on 18.203 retention time (RT).

Rana *et al*., 2011 revealed that the major constituents in the oil were cinnamaldehyde (68.95%), benzaldehyde (9.94%) and (E)-cinnamyl acetate (7.44%) was also identified by GC–MS techniques. This *cinnamaldehyde* was the predominant active component found in essential oil of *C. zeylanicum* (Simic *et al*., 2004). Earlier studies suggested that the antibacterial activity from essential oil of *C. zeylanicum* was probably due to their major constituent, *cinnamaldehyde* was completely inhibiting the bacteria including gram positive and gram negative bacteria (Mukhtar and Ghori, 2012).

Table 1: The chemical components of essential oil of *C. zeylanicum*

Peak	Retention time, t_R	Component	Percent abundance (%)
1	3.438	4-Undecene, 3-methyl-, (E)-	1.13
2	4.249	Propionic acid, thio-, S-isopentyl ester	0.26
3	4.339	Toluene	15.11
4	6.917	Ethylbenzene	0.29
5	7.246	o-Xylene	0.21
6	7.901	Benzenepropanoyl bromide	0.23
7	16.823	2-Cyclopentene-1-butanal	0.19
8	17.482	p-menth-1-en-8-ol	0.15
9	18.203	Cinnamaldehyde, (E)	2.31
10	**19.810**	**Cinnamaldehyde (3-phenyl-2-propenal)**	**68.47**
11	23.634	Alpha, -Cubebene	0.31
12	25.298	Trans-alpha-Bergamotene	0.15
13	26.530	2H-1-Benzopyran-2-one	10.59
14	30.36	Naphthalene	0.24

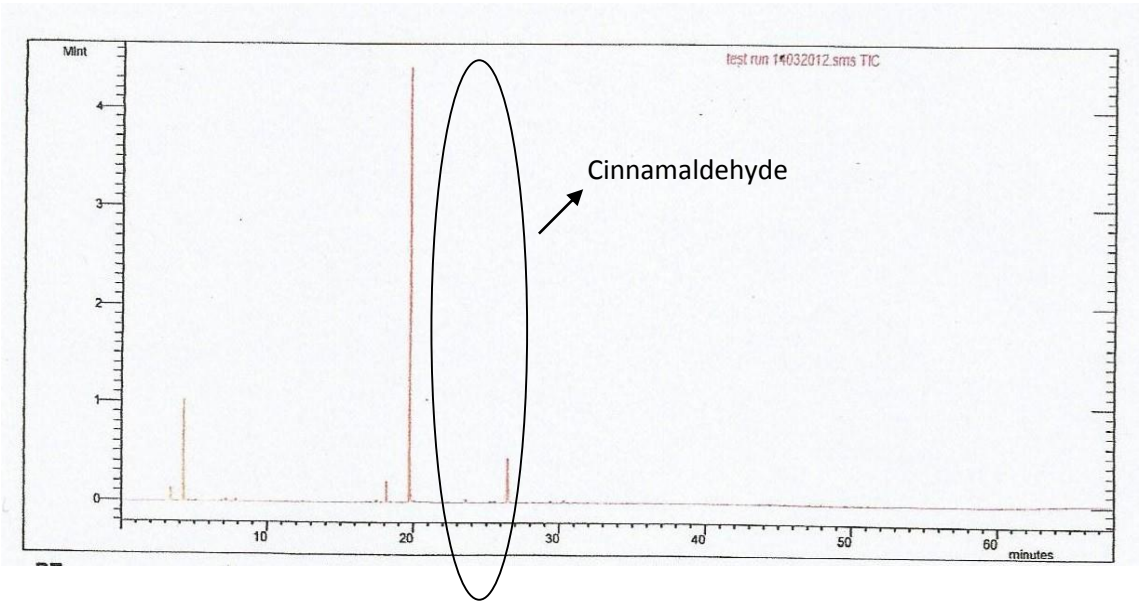


Figure 1: Chromatogram of *C. zeylanicum* essential oil

Antibacterial Activity

The antibacterial activity of essential oil of *C. zeylanicum* against five bacterial species which were *S. aureus*, *P. fluorescen*, *S. marcescen*, *S. typhimurium* and *B. subtilis* are summarized in Table 2.

Table 2: Result of the zone of inhibition of *C. zeylanicum* (mm)

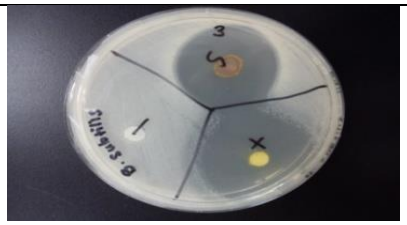
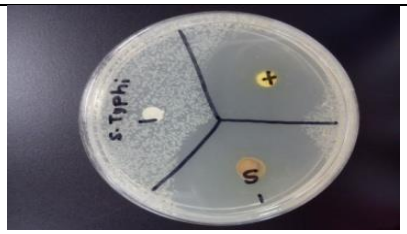
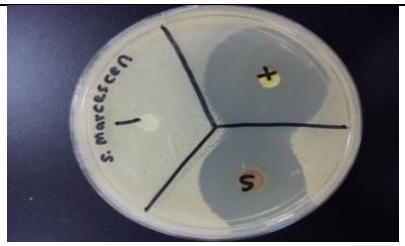
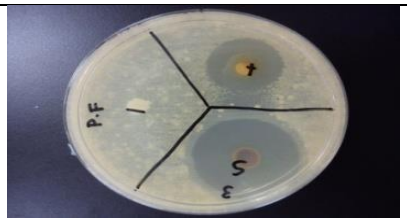
Type of Bacteria	**Zone of inhibition of essential oil of cinnamon (mm)**			
	Plant extraction	Tetracyline	Petroleum ether	
S. aureus	29	34	0.0	
B. subtilis	33	38	0.0	
S. typhimurium	44	40	0.0	
S. marcescen	34	39	0.0	
fluorescen	35	25	0.0	

Table 3: Types of bacteria response range (BRR), in mm

Type of bacteria response range (BRR)	Range, mm
Resistant, R	Less than 10
Intermediate, I	11-14
Susceptible, S	Same or more than 15

From Table 2 above, the bacterial species tested against the essential oil of *C. zeylanicum*, *S. typhimurium* (44 mm) was found to be highly sensitive to it is action followed by *P. fluorescen* (35 mm), *S. marcescen* (34 mm), *B. subtilis* (33 mm), and *S. aureus* (29 mm). These results showed that all bacterial tested are susceptible towards essential oil of *C. zeylanicum* because the diameter inhibition zone range is more than 15. Based on Mukhtar and Ghori (2012), the cinnamon ethanolic extracts are equally effective against both Gram negative and Gram positive bacteria.

The sensitivity toward *C. zeylanicum* extract is reflected to the structure of cell wall and outer membrane of the bacteria (Sandigawad and Patil, 2010). The antibacterial activity has been attributed to the presence of some active constituents in the essential oils and was probably due to major component, cinnamaldehyde and their constituents (Hassan *et al*., 2014). Cinnamon inhibits bacterial acetyl-CoA carboxylase and responsible for major antibacterial activity (Meades *et al.*, 2011). Hassan *et al*., 2014 declared, the antibacterial action is also considered to arise mainly from the potential of hydrophobic essential oils to obstruct the bacterial cell membrane and its structures which leads to ion leakage. Burt, 2004 stated possess of cinnamaldehyde inhibit production of an essential enzyme by the bacteria and/or cause damage to the cell wall of bacteria.

Conclusion

Essential oil of *C. zeylanicum* showed the presence of cinnamaldehyde which is the major component found by Gas Chromatography and Mass Spectroscopy (GC-MS). Consequently, essential oil of *C. zeylanicum* bark gave an excellent result against gram negative bacteria and gram positive bacteria. This indicator, clearly demonstrated that essential oil of *C. zeylanicum* bark with cinnamaldehyde assist by others constituents has a great potential as natural antibacterial agent and being one of plant antibacterial in order to inhibit the growth of bacteria. There is increasing acquaintance acceptability of the use of plant contituents in daily practice.

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