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Antimicrobial Activity of Extracts of Bark of *Cinnamomum cassia* and *Cinnamomum zeylanicum*

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ABSTRACT

Objective: To investigate the antimicrobial activities of various solvent extracts of the bark of *Cinnamomum cassia* and *Cinnamomum zeylanicum*. **Methods:** The powdered barks of *C. cassia* and *C. zeylanicum* were extracted using methanol, ethanol and acetone. The antimicrobial activity of methanolic, ethanolic and acetone extracts of barks of *C. cassia* and *C. zeylanicum* were studied using agar well diffusion method against seven ATCC bacterial species (*Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis, Streptococcus pyogenes, Escherichia coli, Salmonella bongori* and *Pseudomonas aeruginosa*). The antimicrobial activity of extracts of *C. cassia* and *C. zeylanicum* were compared with gentamycin. **Results:** The ethanolic and acetone extract of *C. cassia* and *C. zeylanicum* showed greater zones of inhibition than methanolic extract. *C. zeylanicum* inhibited the growth of all seven ATCC strains used in this study whereas, *C. cassia* inhibited the growth of *S. pyogenes, S. aureus, B. cereus, E. faecalis, P. aeruginosa* and *S. bongori*. Gentamycin inhibited the pathogenic group of all ATCC strains used in this study. **Conclusion:** The ethanolic and acetone extracts of bark of *C. Cassia* and *C. zeylanicum* exhibited a broad spectrum of exhibited a broad-spectrum antimicrobial activity.

Key words: Agar well-diffusion method, Cinnamomum, Gentamycin, Minimum Inhibitory Concentration (MIC**).**

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INTRODUCTION

Antimicrobials are "*any substance of natural, semisynthetic or synthetic origin that kills or inhibits the growth of microorganisms but causes little or no damage to the host*".[1] Antimicrobials are linked to adverse effects on consumption by the host which includes allergic reaction and hypersensitivity. In addition to this problem, commercialized antimicrobial drugs have caused multiple drug resistance in hosts and this is a major concern. Due to the evolution of bacteria being resistant towards modern medicine, this has forced to search for a new therapeutic agent that could be effective towards these bacteria. Therefore, it was a necessity to search an alternative drug derived from plants that could treat these diseases without triggering other side effects.[2] The benefits obtained from these medicinal plants are due to their secondary metabolites. These secondary metabolites are steroids, tannins, flavonoids, phenols, alkaloids and these compounds are present in different quantities depending on the part of the plant.[3,4]

Cinnamomum cassia and *Cinnamomum zeylanicum* (family: Lauraceae) are the well-known plant species for its medicinal properties. *C. cassia* also called Chinese cassia is widely cultivated in China. The dried stem bark of *C. cassia* is one of the important food spices and also has considered having medicinal properties, such as antimicrobial, anti-tumorigenic, anti-inflammatory and antidiabetic characteristics.[5] *C. cassia* is commonly used as traditional Chinese medicine for treating dyspepsia, gastritis, blood circulation disturbances and inflammatory diseases.[6] *C. zeylanicum* also called Ceylon cinnamon is widely cultivated in Sri Lanka and has antidiabetic, anti-inflammatory, antibacterial, antiviral and antifungal activities.[7,8] Cinnamon Oil and cinnamaldehyde from the *C. cassia* is showed antimicrobial activity against both gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria including *Escherichia coli*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Vibrio parahaemolyticus and Samonella typhymurium*.[9] The essential oil from the bark of *C. zeylanicum* has antimicrobial activity and highly active against both gram-positive (*Staphylococcus, Streptococcus, Enterococus*) and gram-negative bacteria including *Pseudomonas aeruginosa*.[10] The antimicrobial activity of essential oil from *C. cassia* and *C. zeylanicum* is well documented. The antimicrobial activity of extracts of bark of *C. cassia* and *C. zeylanicum* is not clear. Hence the present study is planned to investigate the antimicrobial activities of various solvent extracts of bark of *C. cassia* and *C. zeylanicum*.

MATERIALS AND METHODS

Collection and identification of barks of *Cinnamomum*

The cinnamon barks were obtained from Penang Spice Garden, Pulau Pinang and the authenticity of the *C. cassia* and *C. zeylanicum* species was confirmed by Dr. Deivanai Subramanian. The bark was cleaned thoroughly and placed in an oven to be dried for 24 h at 40°C.The dried bark was then powdered using a blender and the power was stored in an airtight container.

Extraction of Cinnamomum bark

The powdered barks of *C. cassia* and *C. zeylanicum* were packed in a thimble and placed in a Soxhlet apparatus and extracted using methanol, ethanol and acetone. The extraction was carried out for 24 h at about 55°C–80°C; the extract was filtered through a muslin cloth. The filtrate was concentrated to a dry mass by evaporation under reduced pressure. The extracts of *C. cassia* and *C. zeylanicum* were stored in a desiccator at room temperature until further analysis. The yield of methanolic,

ethanolic and acetone extract of barks of *C. cassia* was found to be ≈22, ≈17 and ≈15% w/v, respectively. The percentage yield of methanolic, ethanolic and acetone extract of *C. zeylanicum*was found to be≈21, ≈19, and ≈12% respectively.

Test organisms

Total of seven pure ATCC bacterial strains were selected and obtained from Bio-focus Saintifik Sdn Bhd with 4 strains being gram-positive (*Staphylococcus aureus*, *Bacillus cereus, Enterococcus faecalis*, and *Streptococcus pyogenes*) and the remaining 3 strains being gram-negative (*Escherichia coli, Salmonella bongori* and *Pseudomonas aeruginosa*).

Antimicrobial Activity of extracts of *C. cassia* and *C. zeylanicum*

Agar well-diffusion method used to evaluate the antimicrobial activity of an extract and this method is almost similar to the disk-diffusion method.[11] The surface of the Petri-plate containing agar is inoculated by streaking the specific bacterial strain over it with even distribution. Sterile cork-borer of the desired diameter is used to create wells on the agar in order for the extracts to be filled into these wells.

Bacterial strains were prepared using nutrient broth and kept in an incubator at 37°C for 24 hrs. Bacterial growth is observed in the nutrient broth which was then diluted to contain 10^5 viable cells in accordance with the Mcfarland standards. A 25 g of Mueller-Hinton Agar (MHA) was dissolved into 200 ml of distilled water and was autoclaved at 121°C for 15 min at 15 Psi. The MHA was then poured into Petri-plates with a volume of 15 ml and left to cool. 150 µl of the bacterial culture were spread equally on the plate and left to dry. 8 mm sterile cork-borer was used to create wells on the agar in order for the extracts ranging from 20–100 µl to be filled into these wells. Four wells were created to fill 3 concentrations (0.5 g, 1 g and 1.5 g) as well as a well for the standard antibiotic gentamycin (1 mg). This process was repeated for the remaining 6 ATCC bacterial strains and the Petri-plates were properly labeled and stored in an incubator at 37°C for 24 hrs.[12] All tests were carried out in triplicates and the mean zones of inhibition were determined. The Minimum Inhibitory Concentration (MIC) was further determined.

Determination of Minimum Inhibitory Concentration

MIC of the extracts was carried out using the broth dilution method. MIC is said to be the lowest concentration of the sample, which inhibits the visible growth of microbes.[13] Serial dilution was carried out accordingly with concentrations of 1000 mg/ml, 500 mg/ml, 250mg/ml, 125 mg/ml and 62.5 mg/ml. Mueller- Hinton (MH) broth was prepared and 1 ml was added into test tubes alongside 100 µl of selected ATCC strains. Then, 1 ml of extracts with the concentrations mentioned above were introduced into the test tubes contained the broth and ATCC strain and mixed for even distribution. These test tubes were then incubated for 36 hrs at 37°C. As a positive control, 1 ml of selected antibiotics was added to the test tube while the negative control only contained selected ATCC strains. The results obtained after incubation were compared to both the positive and negative control. The concentration of the extract that gave clear results (absence of turbidity) was considered as the MIC of the extract.[14]

RESULTS

Preliminary antimicrobial activity of extracts of *C. cassia* and *C. zeylanicum*

Preliminary antimicrobial screening of extract of *C. cassia* (150 mg/ml) and *C. zeylanicum* (150 mg/ml) tested against *E. coli, P. aeruginosa, S. bongori, S. pyogenes, S. aureus, B. cereus and E. faecalis.* The results of preliminary antimicrobial activity of extract of *C. cassia* and *C. zeylanicum* were summarized in Table 1.

Table 1: Results of a preliminary study of the extracts against ATCC cultures.

	ATCC Strains	*C. cassia*			*C. zeylanicum*		
		Methanol	Ethanol	Acetone	Methanol	Ethanol	Acetone
Gram positive bacteria	*Streptococcus pyogenes*	/	/	/	/	/	/
	Staphylococcus aureus	/	/	/	X	/	/
	Bacillus cereus	/	/	/	/	X	/
	Enterococcus faecalis	/	/	/	X	/	/
Gram negative bacteria	*Escherichia coli*	X	X	X	X	/	/
	Pseudomonas aeruginosa	/	/	/	/	/	/
	Salmonella bongori	/	/	/	/	X	/

(/) indicates inhibitory action; (X) indicates absence of inhibitory action.

Antimicrobial activity of extracts of *C. cassia* and *C. zeylanicum*

Following the preliminary screening, all the 7 ATCC strains were tested (in triplicates) using the agar-well method to determine its activity against different concentrations of the extract of *C. cassia* and *C. zeylanicum*. The antimicrobial activity of extracts of *C. cassia* and *C. zeylanicum* on gram-positive and gram-negative organism were presented in Table 2 and 3 respectively. The antimicrobial activity of extract of *C. cassia* and *C. zeylanicum* were compared with gentamycin. Overall, gentamycin inhibited the pathogenic group of all ATCC strains used in this study. The ethanolic and acetone extract of *C. cassia* and *C. zeylanicum* showed greater zones of inhibition than methanolic extract. *C. zeylanicum* inhibited the growth of all seven ATCC strains used in this study whereas, *C. cassia* inhibited the growth of *S. pyogenes*, *S. aureus*, *B. cereus*, *E. faecalis*, *P. aeruginosa* and *S. bongori*.

Minimum Inhibitory Concentration

MIC values (µg/ml) of the different solvent extracts of *C. cassia* and *C. zeylanicum* against the ATCC bacterial strains were summarized in Table 4. The methanolic and ethanolic extracts of *C. cassia* showed turbidity in the broth dilution method whereas, acetone extract of *C. cassia* inhibited the bacterial growth (absence of turbidity) of few ATCC strains. The methanolic, ethanolic and acetone extracts of *C. zeylanicum* inhibited bacterial growth of few ATCC strains.

DISCUSSION

The bacterial strains used were of different taxonomy. In this study, both *C. cassia* and *C. zeylanicum* bark extracts showed antimicrobial activity against gram-positive and gram-negative bacteria. The antibacterial activity has been associated with the presence of active phytoconstituents such as phenols and flavonoids in the bark extract.[15] Cinnamon (*C. zeylanicum* and *C. cassia*) primarily contains vital oils and other derivatives, such as cinnamaldehyde, cinnamic acid and cinnamate. Cinnamaldehyde and trans-cinnamaldehyde are the most important constituents of cinnamon.[16] The antibacterial activity of cinnamon is said to be associated with its major component, cinnamaldehyde. This compound inhibits bacterial acetyl-CoA carboxylase.[17] Acetyl-CoA carboxylase (ACC) functions by catalyzing the first important step in

Table 2: Antimicrobial activity of extracts of *C. cassia* and *C. zeylanicum* against gram-positive bacteria.

Test compound	concentration	Zone of Inhibition (mm)			
		S. pyogenes	*S. aureus*	*B. cereus*	*E. faecalis*
Gentamycin	1mg/ml	26.75 ± 0.57	16.94 ± 0.39	21.72 ± 0.58	22.16 ± 0.35
Methanolic extract of *C. cassia*	50 mg/ml	-	-	-	-
	100 mg/ml	21.5 ± 1.32	-	13.8 ± 1.26	16.0 ± 0.87
	150 mg/ml	21.7 ± 2.08	18.5 ± 1.32	17.7 ± 1.26	18.3 ± 1.53
Ethanolic extract of *C. cassia*	50 mg/ml	-	-	-	-
	100 mg/ml	17.8 ± 1.04	11.7 ± 1.26	16.7 ± 1.26	16.5 ± 1.50
	150 mg/ml	21.8 ± 1.61	16.5 ± 1.32	18.5 ± 0.50	18.2 ± 0.29
Acetone extract of *C. cassia*	50 mg/ml	-	9.8 ± 1.04	-	-
	100 mg/ml	24.0 ± 1.0	22.0 ± 1.00	22.0 ± 2.00	16.2 ± 1.04
	150 mg/ml	28.0 ± 0.87	23.7 ± 1.26	26.2 ± 1.04	24.7 ± 0.29
Methanolic extract of *C. zeylanicum*	50 mg/ml	-	-	-	-
	100 mg/ml	13.16 ± 0.46	-	13.26 ± 0.59	-
	150 mg/ml	16.32 ± 0.71	-	14.21 ± 0.73	-
Ethanolic extract of *C. zeylanicum*	50 mg/ml	-	18.53 ± 0.96	-	-
	100 mg/ml	15.15 ± 1.00	19.54 ± 0.58	-	-
	150 mg/ml	16.34 ± 0.80	20.48 ± 0.53	-	13.51 ± 0.56
Acetone extract of *C. zeylanicum*	50 mg/ml	-	-	15.65 ± 0.34	-
	100 mg/ml	17.41 ± 0.94	-	15.70 ± 0.57	10.10 ± 0.46
	150 mg/ml	20.48 ± 0.51	17.31 ± 0.33	16.31 ± 0.70	14.34 ± 0.65

All the values are Mean ± SD (n = 3).

Table 3: Antimicrobial activity of extracts of *C. cassia* and *C. zeylanicum* against gram-negative bacteria.

Test compound	concentration	Zone of Inhibition (mm)		
		E. coli	*P. aeruginosa*	*S. bongori*
Gentamycin	1mg/ml	16.64 ± 0.54	29.27 ± 0.67	20.72 ± 1.51
Methanolic extract of *C. cassia*	50 mg/ml	-	-	-
	100 mg/ml	-	22.7 ± 0.76	16.1 ± 0.76
	150 mg/ml	-	23.5 ± 1.32	20.5 ± 1.80
Ethanolic extract of *C. cassia*	50 mg/ml	-	-	-
	100 mg/ml	-	28.2 ± 1.04	16.7 ± 1.26
	150 mg/ml	-	28.7 ± 1.53	19.7 ± 2.08
Acetone extract of *C. cassia*	50 mg/ml	-	16.0 ± 1.73	21.8 ± 1.04
	100 mg/ml	-	27.8 ± 1.61	24.8 ± 1.04
	150 mg/ml	-	32.3 ± 2.08	26.0 ± 1.00
Methanolic extract of *C. zeylanicum*	50 mg/ml	-	-	11.22 ± 0.85
	100 mg/ml	-	-	14.23 ± 0.43
	150 mg/ml	-	14.27 ± 1.05	16.14 ± 0.80
Ethanolic extract of *C. zeylanicum*	50 mg/ml	17.31 ± 1.13	-	-
	100 mg/ml	21.15 ± 1.03	14.40 ± 0.67	-
	150 mg/ml	22.56 ± 0.96	17.14 ± 1.01	-
Acetone extract of *C. zeylanicum*	50 mg/ml	-	-	11.12 ± 0.87
	100 mg/ml	10.91 ± 0.74	-	11.33 ± 1.28
	150 mg/ml	12.74 ± 0.58	16.20 ± 1.05	17.77 ± 0.75

All the values are Mean ± SD (n = 3).

Table 4: Minimum Inhibitory Concentration results of the plant extract (µg/ml).

ATCC bacterial strains	*C. cassia*			*C. zeylanicum*		
	Methanol	Ethanol	Acetone	Methanol	Ethanol	Acetone
E. coli	-	-	-	-	250	125
S. bongori	-	-	500	125	-	125
S. pyogenes	-	-	-	125	125	250
S. aureus	-	-	500	-	250	-
P. aeruginosa	-	-	500	-	125	-
B. cereus	-	-	-	125	-	250
E. faecalis	-	-	-	-	-	125

fatty acid biosynthesis which is a metabolic pathway required for several important biological processes which include the synthesis and maintenance of cellular membranes.[18]

The antimicrobial activity of these extracts is said to be to arise principally from the potential hydrophobicity of the extracts in obstructing the proper formation of the bacterial cell membrane and its other important structures which in return leads to ion leakage. It has been suggested that eugenol and cinnamaldehyde inhibit the production of an important and essential enzyme produced by the bacteria which in return causes damage to the cell wall of the bacteria.[19] This could be explained by the principle of their hydrophobicity which may allow the separation of the lipids of the bacterial cell membrane which in return would make them more permeable hence leading to leakage of ions and other cell constituents.[20,21] The bark of cinnamon contains 65 to 80% of cinnamaldehyde and 5 to 10% of eugenol.[16]

The different parts of cinnamon plants studied for their antimicrobial activity. Matan *et al.* reported the effects of cinnamon oils on different bacterial (*Pediococcus halophilus* and *Staphylococcus aureus),* fungal *(Aspergillus flavus, Mucor plumbeus, Penicillium roqueforti* and *Eurotium* sp.*) and* yeast species (*Candida lipolytica, Pichia membranaefaciens, Debaryomyces hansenii* and *Zygosaccharomyces rouxii*).[22] Varalakshmi *et al.* reported the effects of bark extract of *C. zeylanicum* on different bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*. The findings of this study showed that the bark extracts of *C. zeylanicum* inhibiting both gram-positive bacteria and gram-negative bacteria.[23] These study result indicating that cinnamon is a natural antimicrobial agent with a broad spectrum of activity.

The major limitation of the study is that the method of antimicrobial assay. The method of antimicrobial study may affect the result.[24] Also, factors such as choice of bacterial strains selected for the study, their sensitivity, incubation time, as well as temperature which affect the result.[18,24]

CONCLUSION

In conclusion, the results of this investigation revealed the ethanolic and acetone extract of *C. cassia* and *C. zeylanicum* showed greater zones of inhibition than methanol extract. *C. zeylanicum* inhibited the growth of all seven ATCC strains used in this study whereas, *C. cassia* inhibited the growth of *S. pyogenes*, *S. aureus*, *B. cereus*, *E. faecalis*, *P. aeruginosa* and *S. bongori*. The bark of *C. Cassia* and *C. zeylanicum* exhibited a broad-spectrum antimicrobial activity.

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Nil.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ABBREVIATIONS

ACC: Acetyl-CoA carboxylase; **MH:** Mueller- Hinton; **MHA:** Mueller-Hinton Agar; **MIC:** Minimum Inhibitory Concentration.

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