Inhibitory effect of the essential oil from Cinnamonleaves against microbial pathogens

[1]Zuraida Ab Rahman, [1]Ayu Nazreena Othman, [2]Norma Hussin, [3]Fatin KamiliaJohari and [4]Amirah Balqis Amir Amran

[1]Biotechnology &NanotechnologyResearchCentre, Malaysian Agricultural Research and Development Institute, MARDI HQ, Persiaran MARDI-UPM, 43400 Serdang Selangor, Malaysia.
[2] Industry Crop Research Centre, Malaysian Agricultural Research and Development Institute, MARDI HQ, Persiaran MARDI-UPM, 43400 Serdang Selangor, Malaysia.
[3]Faculty Of Industrial Science And Technology (Fist), Universiti Malaysia Pahang, Lebuhraya Tun Razak26300 Gambang, KuantanPahang, Malaysia
[4]School of Agriculture Science & Biotechnology, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200, Besut, Terengganu, Malaysia

IJASR 2021
VOLUME 4
ISSUE 4 JULY – AUGUST **ISSN: 2581-7876**

Abstract: *Cinnamomum zeylanicum* is a plant that is used in traditional medicine for the treatment of gastritis, blood circulation disturbance, and inflammatory diseases. As an evaluation of the scientific basis of the use of the plant, research regarding the antimicrobial activities of essential oil of cinnamonwere conducted against some common gram-negative, gram-positive bacteria, and also fungi. The antimicrobial activity of the concentrated extracts was evaluated by the determination of the diameter of the zone of inhibition against both gram-negative and gram-positive bacteria and fungi using the agar well diffusion method. The results showed that all of the bacterial and also fungal pathogens were inhibited by a variety of concentrations of cinnamon leaves essential oil (30 %, 50 %, 70 %, and 100 %). Studies on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts on the test organisms showed that the lowest MIC was demonstrated against *Streptococcus.*pyogenes and the highest MBC was exhibited against *Escherichia coli.*

Keywords: Cinnamomum zeylanicum, microbial pathogens, essential oil, antimicrobial agent

Introduction

The most common antimicrobial agents are plant-based and the usage of plants or plant extracts as a traditional health remedy is favored among 80 % of the world population stretching from Asia to Latin America and even Africa. In recent years, pharmaceutical companies focused on developing natural products extracted from plants with the aim to produce more cost-effective remedies that are affordable to the population. However, the rising incidence of multidrug resistance amongst pathogenic microbes has further necessitated the need to search for the latest antibiotic sources.

Cinnamomum zeylanicum belongs to the family of*Lauraceae*, most noted for its bark that provides the world with the commonly known culinary spice, cinnamon (Hend, 2015). In Malaysia, it is commonly known as “kayu manis” and it thrives in a condition containing almost pure sand. Not only that, but cinnamon also grows well in a sheltered place equipped with heavy, warm moisture with unvarying temperature. The tree usually grows up to 30 feet high and physically has thick outer bark and strong branches. The top side of the leaves is shiny and is usually emerald green (Tomar et al., 2015). Subsequent bruising, cinnamon leaves have a slightly spicy smell with a hot taste when riped and upon boiling it gives off an oily mater which cools and solidified as cinnamon suet (Tomar et al, 2015).

Traditionally in the ancient age, cinnamon's have been used to treat dyspepsia, gastritis, and also disturbance in blood circulation (Yu, Lee, & Jang, 2007). As for essential oils sourced from a variety of herbs, the diversity of the usage is endless ranging from bactericidal, virucidal, and also fungicidal properties (Bakkali et al., 2008; Dubey, Shukla, Kumar, Singh, & Prakash, 2010). Therefore, this study combined both of the benefits of essential oils and cinnamon by conducting this research to study the antimicrobial activity of cinnamon leaves' essential oil towards pathogenic bacteria which can benefit future researches regarding cinnamon and also broaden the spectrum for essential oil studies.

Materials and Methods

Extraction of Essential Oil

In this experiment, cinnamon leaves essential oil sample was supplied by Industry Crop Research Centre, Malaysian Agricultural Research and Development Institute, HQ MARDI Serdang, Malaysia (Figure 1a). The essential oil from *C. zeylanicum* leaves was obtained from Ferquima Ind. e Com. Ltda. (Vargem Grande Paulista – SP, Brazil), and its quality parameters (appearance, color, purity, odor, density –20 °C, and refraction index –20 °C) were described in the accompanying technical report. The extracts of essential oils are extracted by the supplier on an industrial scale using the hydrodistillation procedure. The essential oil was assayed at concentrations ranging from 160 to 0.62 $\mu L mL^{-1}$, and the solutions were prepared according to Souza et al. (2007).

Detection of Antimicrobial Activity via Agar Well Diffusion Method

Detection of antimicrobial activity by agar well diffusion was carried out based on the method proposed by Ahmad & Beg (2001) and Srinivasan *et al.* (2001). Each overnight culture was adjusted to 0.5 McFarland standards before spread onto Mueller Hinton Agar (MHA) plates as shown in Figure 2.2. The sterile cotton swab was dipped into the culture and evenly streak at the plate surface. The swab was rotated several times and pressed firmly on the inside wall of the bottle above the fluid level to remove excess inoculum from the swab. The streaking of the bacteria by the swab was done by rotating 60^{o} each time to ensure the even distribution of the inoculum. Then, the wells were made on the agar spread and loaded with 10 µL of cinnamon leaves essential oil and solvent blanks. The antibiotic used is vancomycin (100mg/mL) as the positive control. The agar plates were further incubated at 37^{o}C overnight and the diameter of clear inhibition zones produced was measured. The zone of inhibition was measured to evaluate the antimicrobial activity and tabulated in Table 2.1 and 2.2.

Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration that prevents the visible growth of microorganisms after overnight incubation (Andrews, 2001). The MICs were determined by a microdilution assay. 50µL of an overnight bacterial inoculum was added into 96-well sterilized microtitre plate (NEST, China) in which contained 50 µL of diluted sample. The microtitre plate was further incubated for 24 hours at 37^{o}C. After overnight incubation, 0.02 mg/mL MTT solution was added to each well. The viability of the bacteria was determined by observing the color changes. If the color turns purple, there is bacterial growth. The antibiotic solution, Chloramphenicol (Duchefa Biochemie, Netherlands) at a concentration of 128mg/L to 0.06mg/L was used as the positive control. In this experiment, the negative control used is MHB only.

Determination of Bactericidal Activity by Microdilution Technique

An overnight bacterial inoculum was added into a 96-well microtitre plate containing 50 µL of diluted sample. After incubation for 24 hours at 37^{o}C, the sample from the test wells was streaked onto the nutrient agar plates (Figure 2.5) and will be further incubated for 24 hours at 37^{o}C to determine the bacterial regrowth. If there is no bacterial regrowth, it showed that the extract samples have bactericidal effects and vice versa for bacteriostatic effect if there is bacterial regrowth (Cosentino et al., 1999; Innsan et al., 2011).

Results and Discussion

The demonstration of antibacterial activity against both gram-positive and gram-negative bacteria may indicate the presence of broad-spectrum antibiotic compounds present in the cinnamon leaves essential oil. In Table 1, it can be seen that as the concentration of cinnamon leaves essential oil increases, the inhibition zone also subsequently increases which translates to the fact that, cinnamon leaves essential oil contained anti-microbial properties. As for bacterial pathogen, *S. typhimurium* seems to have been inhibited even at the lowest concentration of essential oil which is at 30 %, and not only that, at 70 %, the inhibition zone (20mm) is equal to the inhibition zone showed in the plate containing vancomycin 100 mg/mL (Figure 1b). However, as for *V. cholerae,* there was no inhibition zone present for any concentration of cinnamon leaves essential oil but the presence of inhibition zone can be seen in the positive control (Vancomycin 100 mg/mL) measuring at 14 mm. Conversely, alternate findings can be seen in the *V. fluvialis*where 30 % cinnamon leaves essential oil able to inhibit *V. fluvialis*with the readings of inhibition zone at

13 mm and 100 % of cinnamon leaves essential oil showed 19 mm inhibition zone while positive control was incapable of inhibiting *V. fluvialis*. The lowest inhibition zone in 30 % of cinnamon leaves essential oil was recorded for *MRSA* which is at 11 mm and positive control showed a much higher inhibition zone which is 33 mm. Vancomycin 100 mg/mL is seen best in inhibiting *MRSA, S.aureus,* and*S.epidermidis*, however from the results obtained in Table 1 showed that cinnamon leaves essential oil capable to inhibit a wider range of bacteria except for *V. cholerae*and the possiblereason for the anti-microbial properties in cinnamon are due to the presence of cinnamaldehyde and eugenol which are responsible for anti-microbial properties (Schmidt et al. 2006).

Furthermore, according to research conducted by Han et al., (2018), antimicrobial properties increases as the concentration of cinnamon essential oil increases similar to the findings in this study and not only that Han et al., (2018) also stated that cinnamon essential oil can inhibit both *S.aureus*and also *E.coli*. However, the research by Han et al., (2018) highlighted that cinnamon essential oil was more effective in inhibiting *S.aureus*compared to *E.coli*and this study also concluded similar findings where 100 % of cinnamon leaves essential oil is needed to produce a 13 mm inhibition zone compared to *S.aureus*which needed only 50 % of cinnamon leaves essential oil with 12 mm inhibition zone. The reason for this is because *E. coli*is a gram-negative bacteria while *S.aureus*is a gram-positive bacteria. Gram-negative bacteria have an outer lipid membrane while gram-positive bacteria have none and the function of an outer lipid membrane in gram-negative bacteria is to prevent antibiotics from entering which further explains the results obtained. On the other hand, Atki et al., (2019) stated that the combination of cinnamon essential oil with antibiotics such as streptomycin produces a synergistic effect. Synergistic effect contributes to the increase or enhanced activity of cinnamon essential oil in providing anti-microbial effects.

As for fungal pathogen, cinnamon leaves essential oil generally able to inhibit all of the fungal pathogen tested while the positive control was unable to inhibit any fungal pathogens.*A. Fumigatesshowed* the highest reading at 50 %, 70 %, and also 100 % of cinnamon leaves essential oil. On another note, *C.parapsilosis*showed the lowest reading in the inhibition zone among all fungal pathogens regardless of the percentage of cinnamon leaves essential oil. However, cinnamon leaves essential oil still manage to inhibit *C.parapsilosis*when the concentration reaches 70 % and also 100 %. Perdones et al., (2014) reported that cinnamon essential oil contained a high value of antioxidant contents and also have the capability to inhibit several fungal pathogens such as *A. niger, B. cinerea, and R. stolonifera* with the combination of chitosan. Cinnamon oil was also reported to have a total effective ratio of anti-fungal properties for the fungal strains of Candida strains(Wang et al., 2012) which supports the results obtained in Table 1. Furthermore, a report by Gucwa et al., (2018) stated that cinnamon essential oil can be used alongside amphotericin B which are conventional anti-fungal medications to prevent fungal infections of *C. albicans* and *C. glabrata*.

Table 1: Inhibition zone (mm) for the antimicrobial test of cinnamon leaves essential oil

Test Bacteria	Cinnamon leaves essential oil				Negative control (100% pure coconut oil)	Positive control Vancomycin (100mg/mL)
Bacterial pathogen						
	30%	50%	70%	100%		
B. subtilis	-	11	11	12	-	-
MRSA	11	14	16	14	-	33
S. aureus	-	12	13	14	-	37
S. epidermidis	13	14	16	18	-	26
S. pyogenes	-	-	10	11	-	-
E. aerogenes	-	-	10	11	-	-
E. coli	-	-	-	13	-	-
P. aeruginosa	-	-	-	11	-	-
P. mirabilis	-	11	-	12	-	-
S. marcescens	-	11	13	13	-	-
S. sonnei	-	11	11	12	-	-
S. typhimurium	16	18	20	20	-	20
V. cholerae	-	-	-	-	-	14
V. fluvialis	13	16	16	19	-	-

Fungal pathogen						
A. fumigates	14	22	26	26	-	-
A. niger	13	14	18	19	-	-
C. albicans	16	14	32	18	-	-
C.parapsilosis	-	-	16	16	-	-

Generally, significant results are shown in Table 2 where a general pattern of 25 % of MIC was recorded for *MRSA, S.epidermism E.aerogenes, E.coli, P.aeruginosa, P.mirabilis, S.sonnei, and V.fluialis*. However, the lowest MIC recorded were for *S.aureus*which indicates that only a small amount of essential oil (6.25 %) is needed to inhibit *S.aureus*. However, *S.aureus*illustrated a general percentage in terms of MBC and the general percentage would be 50 % which are also recorded for other bacterial pathogens excluding *MRSA* (25 %), *S. pyogenes* (25 %),*S.sonnei*(100 %), and *E.coli*(100 %). Similar results are seen in both Table 1 and Table 2 for *V. cholerae,* where the results showed no significant result. Both MIC and MBC are needed to further determine the antibiotic properties where in this case, is the cinnamon leaves essential oil. A lower MIC indicates that the cinnamon leaves essential oil is capable of inhibiting the pathogens which can be seen from Table 2 where all of the pathogens are susceptible to the cinnamon leaves essential oil excepts for *V. cholerae*. Other than that, the highest MBC (100 %) were recorded for *S.sonnei*and*E.coli*but the MIC was both recorded at 25 %. Although, a huge gap is seen in the percentage of MIC and MBC of *S.sonnei*and*E.coli*cinnamon leaves essential oil are still considered bactericidal for the bacteria because the readings of MBC are not more than four times of the MIC. For the fungal pathogens, both *C.parapsilosis*and *C.albicans*have complementary MIC and MBC which are good indicators that cinnamon leaves essential oil to have the ability to suppress the growth of the fungal pathogens.

Zhang et al., (2016) stated that after adding essential oil according to the MIC, distinct changes were observed in the bacterial cells due to cell damage, and not only that, when cinnamon essential oil was added according to the MBC level, the bacteria cells were destroyed. This strengthens the fact that cinnamon essential oil when added according to the MIC and also MBC can inhibit bacteria. Moreover, a report mentioned that there was a significant relationship between cinnamon essential oil with MIC and MBC, and not only that the report also highlighted the stability of and effectiveness of cinnamon oil compared to another essential oil which *Origanum compactum* or also known as oregano (Chahbi et al., 2020). Other than that, according to Ács et al., (2018), the cinnamon essential oil was reported to have the highest effectiveness against Haemophilus spp. with MIC recorded at 0.06 mg/ml and this strengthen the fact that cinnamon essential oil can help to hinder microorganisms.

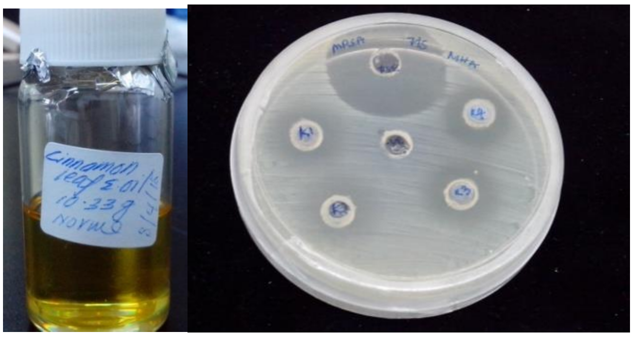

Figure 1.a: Cinnamon leaves essential oil
Figure 1.b: The inhibition zone produced by the agar well diffusion test

Table 2: MIC (%) and MBC (%) values of cinnamon leaves essential oil against test microorganisms

Microorganisms	MIC (%)	MBC (%)
Bacterialpathogen		
B. subtilis	12.5	50
MRSA	25	25
S. aureus	6.25	50
S. epidermidis	25	50
S. pyogenes	50	25
E. aerogenes	25	50
E. coli	25	100
P. aeruginosa	25	50
P. mirabilis	25	50
S. marcescens	12.5	50
S. sonnei	25	100
S. typhimurium	12.5	50
V. cholerae	-	-
V. fluvialis	25	50
Fungal pathogen		
A. fumigates	Not tested	Not tested
A. niger	Not tested	Not tested
C. albicans	3.125	3.125
C.parapsilosis	6.25	6.25

Conclusion

In conclusion, cinnamon leaves essential oil exhibited significant antimicrobial properties against all of the bacteria tested except *V. cholera*. The high antimicrobial activity and inhibitory concentration of the essential oil towards thepathogenic bacteria indicate that many potential products can be produced and derived from cinnamon leaves essential oil. The demonstration of a broad spectrum of antimicrobial activity by *Cinnamomum zeylanicum*may helps future researches not only towards cinnamon's antimicrobial properties but also anti-fungalproperties.

References

1. Ács, K., Balázs, V. L., Kocsis, B., Bencsik, T., Böszörményi, A., & Horváth, G. (2018). Antibacterial activity evaluation of selected essential oils in liquid and vapor phase on respiratory tract pathogens. *BMC Complementary and Alternative Medicine*, *18*(1), 1–9. https://doi.org/10.1186/s12906-018-2291-9
2. Ahmad, I; Beg, A. Z. 2000. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology. 74,* 113-123
3. Bakkali, F; Averbeck, S; Averbeck, D. & Idaomar, M. 2008. Biological effects of essential oils-a review. *Food and Chemical Toxicology, 46,* 446-475.
4. Bhanu, P; Akash, K; Prashant, K. M. & Dubey, N. K. 2015. Plant essential oils as food preservatives to control moulds, mycotoxin and contamination and oxidative deterioration of agri-food commodities – potentials and challenges. *Food Control, 47,* 381-391.
5. Chahbi, A., Nassik, S., El Amri, H., Douaik, A., El Maadoudi, E. H., Boukharta, M., & El Hadrami, E. M. (2020). Chemical Composition and Antimicrobial Activity of the Essential Oils of Two Aromatic Plants Cultivated in Morocco (Cinnamomum cassia and Origanum compactum). *Journal of Chemistry*, *2020*. https://doi.org/10.1155/2020/1628710
6. Cosentino, S.; Tuberoso, C.; Pisano, B; Satta, M; Mascia, V; Arzedi, E. & Palmas, F. 1999. In-vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils. *Letters in Applied Microbiology. 29,* 130-135.
7. El Atki, Y., Aouam, I., El Kamari, F., Taroq, A., Nayme, K., Timinouni, M., Lyoussi, B., & Abdellaoui, A. (2019). Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics.

Journal of advanced pharmaceutical technology & research, *10*(2), 63–67. https://doi.org/10.4103/japtr.JAPTR_366_18

8. Gucwa, K., Milewski, S., Dymerski, T., & Szweda, P. (2018). Investigation of the antifungal activity and mode of action of thymus vulgaris, citrus limonum, pelargonium graveolens, cinnamomum cassia, ocimum basilicum, and eugenia caryophyllus essential oils. *Molecules*, *23*(5). https://doi.org/10.3390/molecules23051116
9. Han, Y., Yu, M., & Wang, L. (2018). Physical and antimicrobial properties of sodium alginate/carboxymethyl cellulose films incorporated with cinnamon essential oil. *Food Packaging and Shelf Life*, *15*(October), 35–42. https://doi.org/10.1016/j.fpsl.2017.11.001
10. Hend, A. H. 2015. Activity of *Cinnmomum zeylanicum* essential oil and ethanolic extract against extended-spectrum ß-lactamase-producing bacteria. *African Journal of Biotechnology, 14,* 292-297
11. Perdones, Á., Vargas, M., Atarés, L., & Chiralt, A. (2014). Physical, antioxidant and antimicrobial properties of chitosan-cinnamon leaf oil films as affected by oleic acid. *Food Hydrocolloids*, *36*, 256–264. https://doi.org/10.1016/j.foodhyd.2013.10.003
12. Srinivasan, D; Nathan, S; Suresh, T. & Perumalsamy, P. L. 2001. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J. Ethnopharmacol. 74,* 217-220.
13. Tomar, R. S & Shrivastava, V. In-vitro efficacy evaluation of ethanolic extract of *Cinnamomum Zeylanicum (Cinnamomom)* as potential antimicrobial agent against clinical isolates. *International Journal of Pharmaceutical Sciences and Research, 6,* 2201-2204.
14. Wang, G. S., Deng, J. H., Ma, Y. H., Shi, M., & Li, B. (2012). Mechanisms, clinically curative effects, and antifungal activities of cinnamon oil and pogostemon oil complex against three species of Candida. *Journal of Traditional Chinese Medicine*, *32*(1), 19–24. https://doi.org/10.1016/s0254-6272(12)60026-0
15. Yu, H. S; Lee, S. Y. & Jang, C. G. 2007. Involvement of 5-HT1A and GABAA receptors in
16. the anxiolytic-like effects of Cinnamomum cassia in mice. *Pharmacology Biochemistry*
17. *and Behavior, 87,* 164−170.
18. Zhang, Y., Liu, X., Wang, Y., Jiang, P., & Quek, S. Y. (2016). Antibacterial activity and mechanism of cinnamon essential oil against Escherichia coli and Staphylococcus aureus. *Food Control*, *59*, 282–289. https://doi.org/10.1016/j.foodcont.2015.05.032

