Potential of *Cinnamomum iners* wood as antimicrobial agent

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Abstract. The wood of *Cinnamomum iners* (Medang Teja) believe in giving multiple beneficial, especially in traditional usage. However, due to the lack of studies regarding this species made this species unable to express its full potential being commercialised by the industry. Thus, this study is prosecuted with the objectives to evaluate the chemical composition and analyse the biological activity of extractive and essential oil derived from *Cinnamomum iners* wood. The samples that evaluated comprises of 100%, 1%, 2%, 3% essential oils and extractive of chemical composition are conducted through Fourier Transform Infrared Spectroscopy (FTIR) and Gas-Chromatography Mass Spectrometry (GCMS) analysis. Meanwhile analysing of its biological activity is made through antifungal and antimicrobial activity. The results found that the functional group presents in all the samples shared one similar functional groups which are aliphatic hydrocarbons. For 100% essential oil, the functional groups are aliphatic propionate ester, aliphatic hydrocarbons and tertiary aliphatic alcohols. 1%, 2%, and 3% essential oil consist of the same functional groups which are olefins, aliphatic hydrocarbons and aliphatic acetate esters. Extractives have aliphatic hydrocarbons and primary aliphatic alcohols. Through GCMS, the major compounds found in the samples are 2(1H)-Naphthalenone, octahydro-, trans-Linalool, beta. Fenchyl alcohol, Camphor, Benzene, ethyl- (CAS) EB. The similar compound found in all samples are Linalool, terpinene-4-ol, Terpineol, Copaene, Cadinene. From the antifungal analysis, it is proved that all of the samples can hold the antifungal traits, as all the samples show the effects of inhibitory on both brown-rot, *Coniophora puteana* and white-rot fungi, *Pycnoporus sanguineus*. The most susceptible organisms are the *Coniophora puteana* which showed a lower average diameter of inhibition zone for all the sample. Lastly, the samples shown a positive result in being antimicrobial agents for both gram negative, *Escherichia coli* and gram positive, *Staphylococcus aureus*.

1. Introduction

*Cinnamomum iners* (Medang Teja) is an evergreen medium height tree with reddish brown and smooth branchlets [1]. It belongs to the family Lauraceae. As stated by Agroforestry Tree Database, *Cinnamomum iners* (Medang Teja) are typically found in India, Myanmar, Thailand, Malaysia, Indonesia and Southern Philippines. Based on the book entitled Tumbuhan liar: khasiat ubatan & kegunaan lain published on the year 2004, there are several bioactive components present in *Cinnamomum iners* (Medang Teja). Some major components are saponins, terpenes, cinnamic aldehyde

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and eugenol. A study shows an information regarding to the medicinal properties of *Cinnamomum iners* (Medang Teja) stating that it contains antiplasmodial, cytotoxicity, amylase inhibitor, antinociceptive and anti-inflammatory activity [2].

The wood of *Cinnamomum iners* (Medang Teja) believe to give multiple beneficial especially in traditional usage and this species also contains some essential oils. Traditionally, the wood was made as herbal tea by drinking the juice and used as a detoxifying agent. The wood is sold in Malaysia by the terms called “mesni” and it has been widely used as medicinal tea and flavorings for curry. The wood is also being drunk as a post-partum medicine after child-birth by decoction [3].

Thus, it is a vital requirement to execute a research in order to know any other further potential and benefits as it will broaden the perspective regarding this species. Moreover, by executing this research, researchers can be an opportunist to exploit this species so it can continuously contribute to many other benefits.

### 2. Material and Methods

#### 2.1 Steam distillation of *Cinnamomum iners* wood

Steam distillation method was used to obtain the essential oil from the wood of the *Cinnamomum iners* (Medang Teja). The *Cinnamomum iners* (Medang Teja) Wood was crushed to reduce the sample particle size becoming powder. Then, the steam distillation with 100g to 150g of sample put into the distillation flask (1L). From this steam distillation, the essential oil was obtained and collected using a separatory funnel [4] [5].

#### 2.2 Dilution of Essential oil

The essential oil obtained from the steam distillation was diluted into 3 different dilutions. These dilutions composed of 1%, 2% and 3%. This percentage was done based on the dilution rate aligned from Denise Brown’s website recommendations [6]. The essential oil was diluted with the coconut oil as a carrier.

#### 2.3 Extraction of *Cinnamomum iners* wood

Crude extract of the *Cinnamomum iners* wood was obtained using Soxhlet Extraction method [7] [8]. The sample was filled in the thimble ethanol and toluene was used with the ratio of 2:1 for almost 10 hours. This procedure was done under the fume hood, to avoid the ethanol gas escaping in the air. This operation also was being fully supervision as the process required a continuous supply of water and heat source. The obtained extracts were kept in the stock bottle for further testing [9].

#### 2.4 Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR is to analyse the functional group based on the volatile oil obtained through the steam distillation. This analysis was done to investigate the bonding structures and any possible changes to the chemical compositions of the oil with the respect to the dilution rate [10]. This analysis was conducted with the help of the FTIR spectrometer, fitted with OMNIC software. In obtaining the IR-spectra, the standard KBr technique are executed with the scanning wavelength range between 500-4000 cm⁻¹ [11].

#### 2.5 Gas chromatographic-mass spectrometry (GC-MS) analysis

The GC-MS determined the chemical composition of the essential oil and extractive of the *Cinnamomum iners* wood. The GC-MS was performed with GC-2010 Plus Capillary GC equipped with GCMS-QP2010 Ultra High-End GC-MS and AOC-20s auto-injector. The GC-MS that was used will be specially built-in with a flame ionisation detection (FID) detector. The samples of 1.0µL were injected into the column with a split ratio of 100:1. The separation of the component was achieving accordance to linear temperature programme which was set at 80-220°C at 4°C/min and then held for about 3 and 10 min, the total of the entire run time was 47 min. Through this, the calculation was made based on the chemical composition using the peak normalisation method [12]. An analysis was carried out regarding
to the peak identification which by making comparison between the mass spectra with the mass spectra available based on guidelines from database of National Institute of Standards and Technology (NIST12 or NIST62) and Wiley 229 mass spectrometry libraries [13] [14].

2.6 Antifungal assay of Cinnamomum iners wood

Antifungal activity was executed using white rot fungi *Pycnoporus sanguineus* and brown rot fungi *Coniophora puteana*. Potato Dextrose Agar (PDA) were prepared through a mix of 15.6g of PDA with 400 mL distilled water. Then, the mixture was autoclave before pouring it onto the sterilised petri dish. A sterile cork borer was used to transfer the cultured fungi into the petri dish. Each paper disc contains different concentrations of the essential oil, which were 1%, 2%, 3% and 100%. Before the incubation, the plates were left for 30 min at room temperature in order for the oil to completely diffuse into the agar. Incubations were done under 26 ± 1 °C for 7 for 20 days [15]. Then, the incubation process was made based on the medium in the terms of measuring the diameter of inhibition zones in millimeter.

2.7 Antimicrobial assay of Cinnamomum iners wood

Two types of microorganisms were tested in this study which are *Escheria coli* (E. coli) and *Staphylococcus aureus* (S. aureus). *Escheria coli* is a gram negative bacteria, meanwhile the *Staphylococcus aureus* is a gram positive bacteria. Both of the bacteria was streaked on the nutrient agar prepared and incubated at 45°C for 24 hours by disk diffusion method [16]. This method was exerted by applying the concentration of essential oil onto a filter paper disc that had been sterile through autoclave. Then the filter paper discs are set onto the agar. The agar has been previously inoculated with the tested microorganism by streaking on the surface. After the incubation, the zone of inhibition was measured in millimeter for the interpretation of antimicrobial activity. The criteria were followed as stated, the weak activity are determined if the inhibition zone is less than 12 mm. Meanwhile as for the moderate activity, the inhibition zone in between 12 mm to 20 mm. Lastly, the strong activity is determined if the inhibition zone is above 20 mm [17] [16] [18].

3. Results and Discussions

3.1. FTIR Spectral Analysis of Cinnamomum iners wood

The spectrum divided into 2 zones and fingerprint region as shown in the Figure 1. The first zone to two is cover by 4000-2500cm⁻¹, 2000-1600cm⁻¹, respectively while the fingerprint region is 1600-500 cm⁻¹.

![Figure 1. FTIR spectra of Cinnamomum iners wood](image)

From the figure, the functional groups were identified for each sample with 1%, 2%, 3% of essential oil and extractive. Aliphatic hydrocarbon appear in all sample between 4000-2500cm⁻¹ and 2000-1600cm⁻¹. The functional group for the 100% essential oil consist of aliphatic propionate esters, aliphatic hydrocarbons and tertiary aliphatic alcohols. For 1%, 2% and 3%, the functional groups consist of olefins (general), aliphatic acetate esters and aliphatic hydrocarbons. Lastly, for the extractive, it
contains aliphatic hydrocarbons and primary aliphatic alcohols. The details spectrums are shown in Table 1.

### Table 1. The measurement of peaks of *Cinnamomum iners* wood

| Stretching frequency (cm⁻¹) | Essential oil (100%) | Essential oil (1%) | Essential oil (2%) | Essential oil (3%) | Extractive |
|-----------------------------|----------------------|-------------------|-------------------|-------------------|------------|
| **Zone 1:** 4000-2500       |                      |                   |                   |                   |            |
| 3467.01                     | 2921.59              | 2921.58           | 2921.64 2852.62   | 3248.98           |
| 2959.92                     | 2852.60              | 2852.59           |                   | 2972.53           |
| **Zone 2:** 2000-1600       |                      |                   |                   |                   |            |
| 1740.79                     | 1742.21              | 1742.19           | 1742.19           | 1604.64           |
| **Fingerprint region:** 1600-500 | 1447.06 1416.56 1390.10 1372.49 | 1465.36           | 1465.27           | 1466.33 1377.24   | 1496.08    |
| 1323.65 1277.06 1245.90 1198.00 | 1377.23           | 1377.27           | 1151.03 1110.81   | 1454.89           |
| 1165.95 1093.52 1045.92 1021.23 | 1151.10           | 1151.00           | 721.67            | 1379.56           |
| 995.18 916.24 853.44 836.55 | 1110.64           | 1110.69           |                   | 1087.30           |
| 750.09 648.29 553.56 520.96 | 721.72            | 721.63            |                   | 1046.32           |
|                              |                      |                   |                   | 879.83            |
|                              |                      |                   |                   | 729.13            |
|                              |                      |                   |                   | 794.69            |

3.2. Major compound in *Cinnamomum iners* wood by GCMS analysis

Major compound in *Cinnamomum iners* wood is Camphor and Linalool as shown in Table 2, which the peak height percentage of 69.23% and 7.46% respectively (Figure 2). Camphor is a waxy, flammable, transparent solid with a strong aroma. It is a terpenoid with the chemical formula C₁₀H₁₆O. Linalool is a monoterpenoid that is octa-1,6-diene substituted by methyl groups at positions 3 and 7 and a hydroxy group at position 3. It has a role as a plant metabolite, a volatile oil component, an antimicrobial agent and a fragrance. These two compounds show the potential of *Cinnamomum iners* wood.

### Table 2. Major compound in *Cinnamomum iners* wood

| No. | Compound name | Essential Oil (100%) | Essential Oil (1%) | Essential Oil (2%) | Essential Oil (3%) | Extractive |
|-----|---------------|----------------------|-------------------|-------------------|-------------------|------------|
| 1   | 2(1H)-Naphthalenone, octahydro-, trans- | ✓ | ✓ | ✓ | ✓ | ✓ |
| 2   | Linalool | ✓ | ✓ | ✓ | ✓ | ✓ |
| 3   | beta-Fenchyl alcohol | ✓ | ✓ | ✓ | ✓ | ✓ |
| 4   | Camphor | ✓ | ✓ | ✓ | ✓ | ✓ |
| 5   | Benzene, ethyl- (CAS) EB | ✓ | ✓ | ✓ | ✓ | ✓ |

✓ show the availability of compound in the respected sample

![Figure 2](a) GC-MS analysis of *Cinnamomum iners* wood; a: extract; b: essential oil

3.3. Antifungal activities of *Cinnamomum iners* wood

From the Table 3, it is proven that all of the samples can hold the antifungal traits, as all the samples show the effects of inhibitory on brown rot, *Coniophora puteana* and white rot fungi, *Pycnoporus sanguineus*. The most susceptible organisms are the *Coniophora puteana* which showed a lower average diameter of inhibition zone for all the sample.
Table 3. Antifungal activity of *Cinnamomum iners* wood

| Fungi species        | 100% Essential Oil | 1% Essential Oil | 2% Essential Oil | 3% Essential Oil | Extractive |
|----------------------|---------------------|------------------|------------------|------------------|------------|
| *Pycnoporus sanguineus* | ++++                | ++               | ++               | +++              | +          |
| *Coniophora puteana*  | +++                 | ++               | ++               | ++               | +          |

*, No or poor activity, +, Weak activity (inhibition zone 1-4 mm), ++, Moderate activity (inhibition zone 5-8 mm), ++++, Good activity (inhibition zone 9-15 mm), ++++, Strong activity (16-25 mm)

The inhibition effects may occur due to the presence of linalool and camphor, which are the major compounds in the samples. One of the studies conducted by Mastura et al., (1999), due to the presence of linalool it contributed to the antifungal inhibition in a moderate capacity from the oil of the *Cinnamomum iners*. From the GCMS analysis linalool was found in all the samples which are in the range of 24-27%. Meanwhile, for the extractive, it contains the least percentage of linalool compound, which are 7.46%.

3.4. Antibacterial activities of *Cinnamomum iners* wood

From the Table 4, it shows that all the samples had shown a positive result in holding of antimicrobial agents for both gram negative, *Escherichia coli* and gram positive, *Staphylococcus aureus*. 100% essential oil was extremely effective in combating bacteria from growing with an average of 13mm for *E. coli* and 17mm for *S. aureus*.

![Image](image_url)

Table 4. Antibacterial activity of *Cinnamomum iners* wood

| Bacteria species               | 100% Essential Oil | 1% Essential Oil | 2% Essential Oil | 3% Essential Oil | Extractive |
|--------------------------------|---------------------|------------------|------------------|------------------|------------|
| *Escherichia coli* (E.coli)    | +++                 | ++               | +++              | +++              | ++         |
| *Staphylococcus aureus* (S. aureus) | ++++               | +++              | +++              | +++              | ++         |

*, No or poor activity, +, Weak activity (inhibition zone 1-4 mm), ++, Moderate activity (inhibition zone 5-8 mm), ++++, Good activity (inhibition zone 9-15 mm), ++++, Strong activity (16-25 mm)

Furthermore, all the essential oils expressed a lower bacterial growth than the extractive, as the lower dilutions of essential oils, which is 1% essential oil able to inhibit the growth of bacteria by showing greater inhibition holes than the extractive. Not only that, from the results it shows that gram-positive, *Staphylococcus aureus* shows a greater average diameter of the inhibition zone than the gram-negative, *Escherichia coli*.
4. Conclusion

*Cinnamomum iners* has shown potential in various aspects, especially as antifungal and antibacterial agents. The compound of this species can be analysed using FTIR and GCMS. These samples shared similar functional groups which are aliphatic hydrocarbons. For 100% essential oil, the functional groups are aliphatic propionate ester, aliphatic hydrocarbons and tertiary aliphatic alcohols. 1%, 2%, and 3% essential oil consist of the same functional groups, which are olefins, aliphatic hydrocarbons and aliphatic acetate esters. Meanwhile, extractives have aliphatic hydrocarbons and primary aliphatic alcohols. Through GCMS, the major compounds found in the samples are 2(1H)-Naphthalenone, octahydro-, Trans-Linalool, beta-Fenchyl alcohol, Camphor, Benzene, ethyl- (CAS) EB. The similar compound found in all samples is Linalool, terpinene-4-ol, Terpineol, Copaene, Cadinen. From the antifungal analysis, it is proved that all of the samples can hold the antifungal traits, as all the samples show the effects of inhibitory on brown rot, *Coniophora puteana* and white-rot fungi, *Pycnoporus sanguineus*. The most susceptible organisms are the *Coniophora puteana* which showed a lower average diameter of inhibition zone for all the sample. This *Cinnamomum iners* also shown a good result as antimicrobial agents for *Escherichia coli* and *Staphylococcus aureus*.

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