Antioxidant activity of Camphor leaves extract based on variation solvent

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Abstract. Since the ancient time, camphor’s tree has been used as one of the traditional medicines to cure wounds. It is believed to have active component that can heal the wounds. Therefore, the present study was undertaken to prepared crude extracts of camphor leaves with different polarity organic solvents using a hot extraction (Soxhlet) and cold extraction (maceration) method and assessing the antioxidant activity by the spectroscopic. The dried camphor leaves were crushed into powder form (size - 250 µm) and subjected to extraction using different polarity organic solvents such as hexane, chloroform and ethanol to extract chemical compounds from the leaves. DPPH (2,2-diphenyl-1-picrylhydrazyl) method was used to determine the antioxidant activity due to its characteristics as a stable free radical and acts as an indicator of scavenging activity. Quantitative analysis found that all extract shown a significant antioxidant activity. The ethanol extracts resulting highest antioxidant activities than other extracts. The highest inhibition percentage (87%) was found in the hot extraction method extracts compared to chloroform (40.4%) and hexane (12%) extracts, meanwhile cold extraction resulting to lower inhibition percentage. The yield obtained from cold extraction is higher than hot extraction. However, it contains higher impurities as compared to hot extraction. These finding is proven that camphor leaves with high antioxidant properties is suitable for medicine composition, especially related to cure wounds.

1. Introduction
Camphor tree (Dryobalanops aromatic) is locally known as ‘kapur peringgi’. The wood of camphor tree also known as Cinnamomum camphora. Dryobalanops aromatic is a genus tree from Dipterocarpaceae that consist of seven species which are widely distributed in Sumatra, Peninsular Malaysia and Borneo. There are four species found in Brunei Darussalam, Dryobalanops aromatic Gaertn, D. beccari Dyeri, D. lanceolataxBurck and D. rappa Becc [1]. Camphor tree also grew natively in the valley sand and mountain slopes of Japan, China, Taiwan and Vietnam. Camphor tree can be found in almost all tropical forest around the world. It is a large tree that almost reaching up to 65 m in height and it was valuable timber tree. The tree has camphor odour and was sought out for medical purposes [1].

Camphor leaf is a herb plant which is family of Lauraceae. Folk from China use it for treatment such as inflammation-related diseases which are rheumatic, arthritis, muscular strains, abdominal pain, rheumatism, cough and bronchitis [2]. Generally, camphor is suitable for developing new anti-
inflammatory drugs and to explore more potential drugs. According to Chinese tradition, camphor leaves can be used as medicine for mentally problem such as hysteria, nervousness, neuralgia and stomach problem such as diarrhea [3]. Previous study found the crude extract and purified constituents of camphor possessed antioxidant, anti-inflammatory, anthelmintic, anti-fungal and antibacterial activities which were verified with various experiment and can be used for medical purposes [2-3].

Evaluation of antioxidant activity found that hexane extract of camphor bark and oil of camphor wood showed low activity of antioxidant. However, ethanol and chloroform extract revealed high antioxidant activity and free radical activity was deactivated [4]. Camphor fruits also possess antioxidant and microbial activities. According to [5], water extract of camphor leaf, contains the most antioxidant and phenolic content compared to ethanol and acetone. The order for the antioxidant activity of extract was water > ethanol > acetone [5]. Camphor extracts also showed the presence of radical scavenging activities in butanol and ethyl acetate extract with IC50 values 14.0 and 15.0 µg/mL, respectively [3]. It is found that the yield was varied from 1-10% for camphor extracts.

Based on the above, there was an antioxidant’s component in camphor leaf with extraction analysis using various organic solvent namely as hexane, chloroform and ethanol. Antioxidant activity was evaluated by using DPPH method while the presence of components in the camphor leaves was study by using different extraction method; hot extraction (Soxhlet) and cold extraction (maceration) to identity the best extraction method.

2. Methodology
2.1 Preparation for sample
Camphor leaves samples were ground using grinder machine. The powdered leaves then were stored in a container. The grounding process was repeated for the remaining leaves until it become powder. The fine powder was sieved to obtain 250-micron in size. This process was repeated until the powdered leaves sample reach 300 g in weight and stored in a container [6].

2.2 Hot Extraction (Soxhlet)
The dried powdered leaves were extracted using Soxhlet extractor. Camphor leaves powdered (50 g) were heated at 50%-60% heat in 600 mL of n-hexane. The sample was extracted for 16-hours. After extraction complete, the extract was left to evaporate until crude extract was obtained. The crude extract was collected and weighted for yield calculation. The process was repeated by using different polarity organic solvent such as chloroform and ethanol [7].

2.3 Cold Extraction (Maceration)
The powdered (50 g) of camphor leaf were put into a beaker. Then, 600 mL of n-hexane were poured until the powder were soaked and submerged completely. The beaker was covered with aluminum foil to prevent volatile component to evaporate. The extraction method was carried out for 72 hours while stirring occasionally. After 72 hours, the crude extract was filtered using filter paper. The filtered extract was collected and left to evaporate until crude extract was formed. This process was repeated by using different polarity of solvent such as chloroform and ethanol [8].

2.4 Percentage of Yield Calculation
The weight of dried crude extract was measured and percentage of yield was calculated per weight of sample. The data were recorded for following crude extracts along with two method of extraction [9-11]. The percentage of yield crude extracts obtain were determined by Eq. 1.

$$Yield = \frac{Weight \ of \ extracts}{weight \ of \ Sample} \times 100\%$$  \hspace{1cm} (1)

2.5 DPPH antioxidant assay
The antioxidant activity of different camphor leaves crude extracts were evaluated by using DPPH method with modification [12]. The serial concentration of 1000, 500, 250, 125, 62.5, 31.25, 15.62 and 7.81pp were prepared by using pure methanol solvent. Ascorbic acid (as reference) and sample solution
were prepared for different concentration. Each concentration contains 0.2 mL in volume and the solution was added with 3.8 mL of 500 ppm of DPPH. After adding DPPH, the mixture is then incubated in a dark place at room temperature for 30 minutes. The absorbance of the tested samples was measured using UV-Vis spectrophotometer at the wavelength 516 nm. The absorbance of pure methanol was used as a reference. The inhibition percentage was calculated using the absorbance from UV-VIS spectrophotometer [13]. The percentage of DPPH inhibition was calculated by using Eq. 2.

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Inhibition\% = \frac{Absorbance_{Blank} - Absorbance_{Sample}}{Absorbance_{Blank}} \times 100\%
\]

3. Results and Discussion
Antioxidant is a compound that can donate electron. Antioxidants work by donating electron to a compound which contain oxidant to inhibit the activity of the oxidant compound. The antioxidant used to stabilize the free radicals in an oxidant compound by supplementing the lack of electron that possess free radical and preventing the chain reaction from the free radical formation [13]. In this study, the antioxidant activity of Camphor leaf extract will be evaluated using three different solvent.

The antioxidant activity of different crude leaves extract was tested through DPPH method and the results were presented in Figure 1 and Figure 2. 2,2-diphenyl-2-picrylhydrazyl (DPPH) was used and react as the stable free radical which are deep violet in colour converted to 1,1-diphphenyl-2-picrylhydrazine with discolouration. In this present study, different concentration of hexane, chloroform and ethanol extract exhibited different percentages of free radical activity. Previous studies have reported that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (hydroquinone, pyrogallol, etc.), and aromatic amines (p-phenylene diame, p-aminophenol, decolourized the deep violet colour of DPPH to clear yellow colour through their hydrogen-donating ability [14]. This study shows that the radical scavenging activity of camphor leaf extracts is due to their electron donating ability.

The extraction yield is a measure of the solvent efficiency to extract chemical constituents from the samples. A result of different extraction method by using different polarity organic solvent such as hexane, chloroform and ethanol on the camphor leaves were obtained as shown in Table 1. The yield obtained was differ for each solvent and both methods used. The yield for hot and cold extraction using ethanol solvent has the highest yield with 8.52% and 49.56%, respectively. However, cold extraction gave high percentage than hot extraction because of the high impurities include in the extract. It shows that both extraction were efficient and gave more influence on the percentage yield using ethanol solvent compare to hexane and chloroform extract with only small amount of yield obtained. In cold and hot extraction technique, the extract yield percentage of all crude extracts was found to be in order: ethanol > chloroform > hexane.

| Table 1. Yield value of ethanol, chloroform and hexane extracts of Camphor leaf |
|-----------------|--------|--------|
| Solvent         | Extract (g) | Yield (%) |
| Hot Extraction  |         |        |
| Hexane          | 1.35    | 2.70   |
| Chloroform      | 1.86    | 3.73   |
| Ethanol 96%     | 4.26    | 8.52   |
| Cold Extraction |         |        |
| Hexane          | 0.42    | 0.84   |
| Chloroform      | 1.19    | 2.38   |
| Ethanol 96%     | 24.78   | 49.56  |

The choices of the solvent depend on the polarity and ability to attracting all kinds of active compounds such as polar and non-polar compounds [15]. Ethanol was chosen because alcohol group has high polarity than most non-polar but lower polar than water. Ethanol with 96% concentration can easily pass through and penetrate cells and obtain higher and better concentration extraction compared
to low concentration [13]. Ascorbic acid has the highest amount of antioxidant and inhibition percentage. Therefore, ascorbic acid is used as a standard and references in this study. Figure 1 and Figure 2 showed the inhibition percentage of all extracts for hot and cold extraction. According to these figures, it is clearly showed that the ethanol extract for both extraction methods gave the highest percentage inhibition compared to other solvent extract.

The results for all extracts were compared with ascorbic acid and it showed that the antioxidant activity pattern is more likely as the ascorbic acid. The most likely antioxidant activity was showed by ethanol extract. This means that ethanol extract has high radical activity as ethanol solvent attracted polar molecule in the camphor leaf while hexane and chloroform both has low inhibition percentages because of its nature of non-polar and semi-polar respectively.

The measure for expressing the radical capture test is called IC\textsubscript{50} (50% inhibition concentration). Antioxidant activity can be classified as very strong (10 ppm < IC\textsubscript{50} < 50 ppm), weak (100 ppm < IC\textsubscript{50} < 250 ppm) and inactive (IC\textsubscript{50} > 250 ppm) [13]. In Figure 1 and Figure 2, we can compare the inhibition percentage for each method using different solvent. The IC\textsubscript{50} can only be obtained from ascorbic acid and ethanol extracts and both have IC\textsubscript{50} in range of 10-50 ppm which was strong in antioxidant. There was no IC\textsubscript{50} value for hexane and chloroform extracts because their IC\textsubscript{50} cannot be calculated. According Figure 1 and Figure 2, both methods can be used for solvent extraction of the camphor leaves in terms study of antioxidant activity. The order of degree of antioxidant activity of all extracts is ethanol > chloroform > hexane. Thus, ethanol extract has the highest amount of free radical scavenging activity.

![Figure 1. Inhibition percentage of DPPH radical scavenging activity of Camphor extracts (Hot Extraction)](image-url)
Figure 2. Inhibition percentage of DPPH radical scavenging activity of Camphor extracts (Cold Extraction)

The absorbance of samples between ascorbic acid and all extracts for hot and cold extraction are shown in Figure 3 and Figure 4. Based on these results, ascorbic acid showed the lowest absorbance in most of stated concentration. It indicates that ascorbic acid has the most active antioxidant activity since ascorbic acid is a part of Vitamin C. The second lowest absorbance of overall result is ethanol extract which has the slightest increment of absorbance compare with ascorbic acid. Ethanol is a polar solvent which can attract all kinds of active substances such as antioxidant, as well as good absorbance and low reactive toxicity [13]. The absorbance level of ethanol extracts (125-1000 ppm) are below 0.1 nm and shows that ethanol is a good solvent for extracting active compound. On the other hand, both hexane and chloroform extract showed very high absorbance (> 0.3 nm) which indicating that the extracts have very low antioxidant activity. Therefore, hexane and chloroform solvents are inefficient in extracting active compounds. Methanol with DPPH gives the highest possible absorbance of light in methanol dilution samples (0.548 nm) for comparison purpose. Since there is no antioxidant added to the methanol with DPPH, no scavenge of free radical DPPH are occurred, hence it will absorb most of the light radiated by ultraviolet spectrophotometer.

Based on Figure 3 and Figure 4, absorbance of ethanol extract for hot extraction is slightly lower than cold extraction. This can be concluded that hot extraction have better efficient in extracting crude extract from the leaves in term of it antioxidant. In Figure 4, chloroform has higher absorbance than hexane because the variable of temperature. Extraction using hexane solvent is more efficient in lower temperature as the absorbance obtain in Figure 4 is lower than Figure 3. However, for lower concentration of sample which is 7.81 ppm, ethanol extract in hot extraction obtain higher absorbance than cold extraction. Therefore, lower concentration can extract more efficiently using cold extraction.
Figure 3. Absorbance of ascorbic acid and Camphor leaves extract using three different solvents (Hot Extraction)

Figure 4. Absorbance of ascorbic acid and Camphor leaves extract using three different solvents (Cold Extraction)
4. Conclusion
In this study, three types of organic solvent with two different methods were used for extraction of camphor leaves. As a result, ethanol from hot extraction method shown the best results in extracting with reference to amount of yield percentage, compounds composition and amount of antioxidant extracted. Extraction process using higher temperature is proven to higher extraction due to thermal activity that accelerates the extraction process. Higher temperature assists in loosening the leaves particle and let the extract release easily dissolve in solvent. A cold extraction method however takes time for loosening the leaves particle. Other than temperature, type of solvent is also playing an important role in the extraction processes. The study’s results favour to ethanol as it shows best results in extraction. The polar solvent properties in ethanol has better performance in attracting polar molecules in the leaves, it extracts phenolic compound such as linalool, nerolidol and borneol. Application of hexane and chloroform solvent in this study performs poor in extraction due to nonpolar and semi polar properties in nature, which can only extract the leave at small amount. In a conclusion, alcohol group which is ethanol is the best solvent to extract antioxidants and other chemical compound in camphor leaves. In the future, the researcher is planning to create bio-based product that give benefit of medication from the usage of camphor leaves. It is based on results of this study that hot extraction method by using ethanol solvent can extract many antioxidant and minerals content against camphor leaves.

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