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Radical Scavenging Activity, Total Phenol Content and Antifungal Activity of *Cinnamomum Iners* Wood

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Abstract: The study was done to investigate the antioxidant, total phenol content and antifungal characteristics of phenolics compounds of extracts from *Cinnamomum iners* (Reinw. ex Blume-Lauraceae) wood. Radical scavenging activity method of DPPH was used to determine antioxidant activity of the extracts. Four fungus, namely white fungi (*Pycnoporus sanguineus*, *Trametes versicolor*, *Fomitopsis palustris*) and brown fungi (*Gleophyllum trabeum*) were used to determine the antifungal activity of the *Cinnamomum iners* extracts. The results showed that ethanol extract had the highest antioxidant activity with EC_{50} value of 14.96 μg/mL followed by chloroform extract with EC_{50} >30 μg/mL while water extract did not show any significant antioxidant activity. For total phenolics content, ethanol extract resulted on the highest value of 56.23%, followed by chloroform extract and water extract having total phenolic values of 51.13% and 7% respectively. On the other hand for antifungal assay, ethanol extract produce the widest inhibition zone followed by chloroform extract and water extract respectively.

Key words: Antifungal assay; Antioxidant activity; *Cinnamomum iners*; Ethanol extract.

INTRODUCTION

Cinnamomum iners plants were originated from Sri Lanka, and commonly found distributed in Myanmar, Malaysia, Thailand, Indonesian and Southern Philippines. *Cinnamomum iners* is commonly used as a spice and aromatic. Besides that, the *Cinnamomum iners* has been used traditionally to combat microorganisms, diarrhea, and dysmenorrhea, although there is lack of information to support these claims. In medicine field, *Cinnamomum iners* is used to treat illnesses cause by bacterial such as fevers, digestive ailments and coughs since it is high in antioxidant activity [1] and has excellent antimicrobial properties [2], which is used to preserve certain foods.

The composition of *Cinnamomum iners* includes a few major bioactive compounds that include saponins, terpenes, cinnamic aldehyde and eugenol [3]. However, the impact of intended medicinal and experimental effects may be altered by varying sources of material and extraction techniques [4, 5].

An antioxidant is a substance which is capable of slowing down the rate of oxidation in an autoxidizable material. Originally, the term antioxidant was specifically referring to a chemical which prevents the consumption of oxygen. Antioxidant compound plays a major role in protecting health.

Chronic diseases that include cancer and heart disease can be reduced by antioxidants which have been proved by scientific data [6]. Natural antioxidants can be found in whole grains, fruits and vegetables [7]. Natural antioxidants that exist in medicinal plants have gained a lot of attention from researchers [8] since the synthetic antioxidants that have been used previously have been proved to cause various health problems [9].

The ability to scavenge free radicals was the important characteristic of an antioxidant. The existence of oxygen species and highly reactive free radicals in the environment were originated from various sources. Degenerative disease may be induced by these free radicals as they are able to oxidize nucleic acids, lipids, proteins or DNA. Oxidative mechanisms that caused the degenerative diseases can be inhibited by scavenging of free radicals like hydroperoxide, peroxide or lipid peroxyl by antioxidant compounds such as polyphenols, phenolic acids and flavonoids [10].

Monitoring radical scavenging activity by using free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is one of the method which is rapid, simple and require less cost to measure antioxidant capacity [11]. The DPPH is used to measure the ability of plant extracts to act as hydrogen donors and free radical scavengers, thus evaluating antioxidant activity of the extracts.

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It has also been used to quantify antioxidants in complex biological systems in recent years. Generally, the total phenolics compounds in extracts are measured by using Folin-Ciocalteu reagent [12]. This method gives a good idea of the total phenolic content [13]. The total phenolics value in the hydrolysate indicates the amount of phenolics released from the extractives as well as from lignin fraction during treatment [14].

Phenolic compound can be found widely in the plant kingdom. It contributes to variety of biological effects, such as antioxidant, anti-inflammatory, anti-allergic and anti-carcinogenic activities. Most of these functions have correlation with the antioxidant activity of phenolic compound. Phenolic antioxidants act primarily by donating a hydrogen atom to a radical, such as scavenging of free radicals. The number of hydrogen-donating hydroxyl groups and structural features that stabilize the radical formed such by resonance and hyper conjugation influence the efficiency of phenolic antioxidants. This can be achieved by an extended double bond system, and by the arrangement of functional groups, such as the presence of electron-donating groups in the aromatic rings ortho and/or para to the hydroxyl function [15]. A variety of different chemical and synthetic compounds have been used as antimicrobial agents to inhibit the plant pathogenic fungi for many years. There has been a growing interest on finding of possible use of the essential oil and plant extracts which cause less harm for pest and disease control in agriculture [16]. Also, the plants have long been recognized to provide a potential source of phytochemicals or chemical compounds including essential oil and plant extracts [17]. Recent research has demonstrated that *cinnamomum* plants is excellent in antimicrobial properties that 100 mL of refrigerated carrot broth inhibited food borne pathogenic *Bacillus cereus* growth for about 60 days [18].

EXPERIMENTAL

Antioxidant Activity (DPPH Radical Scavenging Activity): The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity method was used to measure the antioxidant activities of the extracts. Similar method was recorded to be used by Brand-Williams [19]. The DPPH is a free radical which gives a blue-violet colour when dissolved in ethanol. The loss of blue-violet colour to yellowish colour indicates radical scavenging activity. With extracts, the colour loss is inhibited. Catechin was used as a positive control and comparison purpose since it is widely known for its high antioxidant activity. Meanwhile, ethanol was used as control sample.

Sample Preparation: Three types of samples were prepared; control, catechins and test samples. Two test tubes were used for control samples. Both the test tubes were filled with 500µL of ethanol. A catechin solution of concentration 5mg/mL was first prepared using methanol. Three test tubes for concentration of 200, 40 and 8 (μg/μL) were used for catechin samples. For the test samples four test tubes of concentration 1000, 200, 40 and 8 (μg/μL) were prepared. Ethanol was also used to prepare a solution of DPPH. After 2 hours, 3000µl of the DPPH solution dissolved with ethanol was first filled into the 1st test tube of control sample with 1 minute time interval. This step was repeated for the 2nd test tube of control sample as well as for all catechin samples and continued for all other test specimens.

The UV-Vis spectrophotometer was calibrated with ethanol prior any measurement. The blank samples were first measured then followed by catechin samples and the test samples. The absorbance values of all the control, catechin, and test samples were recorded. The percentage of DPPH scavenging activity is expressed by equation (1).

$$Q = [(A_o - A_c) / A_o]\ 100 \quad (1)$$

where, Q is the reduction percentage of DPPH, A_o is the absorbance value of initial or blank sample and A_c is the absorbance value for added sample concentration in the absence of DPPH solution [19]. This activity is also expressed as the inhibition concentration at 50% (EC_{50}) which is defined as the concentration (in μg/μL) of sample extract required for 50% of maximum scavenging activity. The experiment was carried out in triplicates.

Determination of Total Phenol: Folin-Ciocalteu method was used to analyze simple phenols of samples. A solution of 10% Folin-Ciocalteu was prepared by mixing evenly 3 mL of Folin-Ciocalteu with 27 mL of distilled water. Meanwhile, a solution of 7.5% sodium carbonate (Na_2CO_3) was prepared by mixing evenly with 92.5 mL of distilled water. Three types of sample were prepared, namely control, catechin, and test samples of concentration of 5, 20, and 40 (μg/μL), respectively. All the samples of control, catechin and test samples were measured by UV-Vis spectrophotometer at 740 nm. The concentration of total phenolics was determined as percentage of equivalent by using equation obtained from catechin calibration curve, calculated based on linear regression using equation (2):

$$TP = \frac{(A - y) / z}{w} \times 100 \quad (2)$$

where, TP is the total phenolic (%), A is absorbance, y and z are the equivalent found from calibration curve and w is the sample's weight (μg) [12].

Antimicrobial activity: Antifungal activities of all extracts were tested against white fungi (*Pycnoporus sanguineus*, *Trametes versicolor*, *Fomitopsis palustris*) and brown fungi (*Gleophyllum trabeum*). For determining the antifungal activity of the *Cinnamomum iners* extract, the fungal isolates were recultured on Potato Dextrose Agar (PDA) medium. Glycyrrhizic acid dipotassium salt (GADS) was used as the positive control.

Antifungal Assay: The Potato Dextrose Agar (PDA) medium was prepared by mixing 0.58g PDA in 12 mL

distilled water and was put into 4 bottles. The experiment was done by arranging 7 paper discs on a Petri dish (1 for positive control (GADS), 6 for testing samples). The test samples were observed after 3rd days. At the end of the incubation period, inhibition zones formed on the medium were evaluated in mm. Daily evaluations were carried out by measurement of the colony diameter, starting at 24 hour after the experiment began and finishing when two-thirds of the plate surface of the control treatment was covered by the fungus [20]. The appearance of zones of inhibition was regarded as positive for the presence of antimicrobial action in the test substance. Determination of minimum inhibitory concentration (MIC) of the extract was defined as the lowest concentration that completely inhibited the growth for 24 hour. The MIC for the *cinnamomon iners* extract was determined by the agar well diffusion method [21].

RESULT AND DISCUSSION

Antioxidant Activity: Antioxidant activity is determined by using radical scavenging activity as the parameter. It is calculated as the concentration of extracts being dissolved in ethanol causing 50% inhibition of the DPPH radical. This indicates that the higher the antioxidant activity, the lower the value of EC_{50}. Hence, catechin which usually contains a great amount of antioxidant shows a low value of EC_{50} was used as standard in comparing the antioxidant activity of the samples.

Each of the samples was measured three times in order to confirm the result. Based on the spectroscopy result, graph of EC_{50} was plotted. The results of EC_{50} for the first, second and third trials of all the samples are displayed in Table 1.

Positive results in antioxidant activity can be observed for all of the samples except for those of the water extract. For the control sample as well as for comparison purpose the experiment was done by using catechin since it is widely known for its high antioxidant activity having the lowest value of 4.28 (μg/mL) EC_{50} among all of the samples. The lower the value of EC_{50} indicate the higher the antioxidant activity. However samples which are having the value of EC_{50} >30 are considered not having any significant antioxidant activity. From the Table 1 we can see that ethanol extracts had the highest antioxidant activity among the *Cinnamomum iners* extract samples with EC_{50} value around 14.96 (μg/mL) followed by chloroform extract and water extracts respectively whose having EC_{50} >30. Water extracts is not showing any significant antioxidant activity as there is no color changes when DPPH solution is mixed with the extracts while chloroform extracts is only giving a pale yellow color when it is mixed with DPPH solution.

The antioxidant activity shown by *Cinnamomum iners* extract may probably due to the extracts from the *Cinnamomum iners* samples have strong biological activities, such as enzyme inhibition antioxidant activity, and antifungal activity. It is widely known that polyphenolic compounds, such as flavonoids, anthraquinones, anthocyanidins and xanthones, possess remarkable antioxidant activities which are present quite commonly in the plant family. In the case of *Cinnamomum iners*, the presence of those established antioxidant compounds could be the reason for its reasonably good activity in the extracts [22].

Water extract are not showing any significant antioxidant activity. Such relatively low effects might be attributed by the presence of pro-oxidants generated from the higher amounts of sugars, particularly mono such as glucose, fructose, sucrose and disaccharides sugars such as pentoses, hexoses reducing disaccharides which have been reported to be strong pro-oxidants [22].

Based on the results of this study the antioxidant activity of ethanol extract had the highest values due to ability of ethanol solvent to eliminate some of the cellulose and hemicelluloses from the *Cinnamomum iners* extract. Cellulose and hemicelluloses are the sources polysaccharides and sugars in plants. The antioxidant potential of compounds present in the extract might have been deactivated by the presence of considerable quantities of the sugars, thus ultimately have reduced the antioxidant capacity [22].

Total Phenol: In order to obtain a more precise and accurate result, each sample was ran triplicates for total phenol assay and an average is obtained as the final result. By using catechin samples as standard, calibration graph was plot as shown in Fig. 1. From the calibration graph, the percentage of the total phenol for the samples was then determined and tabulated in Table 2.

Based on the result in Table 2, ethanol extract had the greatest amount of total phenol among the three samples. It contained an average 56.23% of the total phenol then followed by the chloroform extract sample with an average 51.13% and the less is water extract with an average of 7%.

Generally, the results of the total phenol were similar to that of antioxidant activity. Ethanol extracts which is high in antioxidant activity and has high total phenol percentage followed by chloroform extract. Water extract which showed low antioxidant activities have low total phenol content. This result may be attributed by the combined activities of various antioxidants, including phenolics that result in total antioxidant capacity. Previous study has shown that various flavones and flavonal compounds and some other polyphenols also contribute to antioxidative activities even at high temperature [23].

The Folin-Ciocalteau test can be used to compare “antioxidants” when the source of the antioxidant is not taken as an issue as the molecule behaves as a reducer. However it has been suggested that the amount of phenolic compounds was not the only factor in determining anti-oxidative activity as the molecular structures of different phenolic compounds also play a major role in the anti-oxidative activity [24].

Based on the results we found that there is a relationship between antioxidant efficiency and the chemical composition of phenolic compounds and the same results are also achieved by the Nsimba et al., in year 2008 [23]. The antioxidant activity has been correlated and compared to their content of phenolic components due to their ability of scavenging free radicals.

Antifungal Activity: An antifungal assay activity was carried out using white fungi (*Pycnoporus sanguineus*, *Trametes versicolor*, *Fomitopsis palustris*) and brown fungi (*Gleophyllum trabeum*). The inhibition zone could be seen after 3 or 4 days depends on the efficiency of the samples and after 10 days all of the samples were fully covered. Table 3 showed the efficiency of the samples when tested with different concentration.

The results presented in Table 3 showed that the *Cinnamomum iners* extract under investigation exhibited marked antifungal activity, as evidenced by their zones of inhibition. The area of the inhibition zones decreased with decreasing amount of sample used for antifungal assay. A subtle decrease in the inhibitory effect was observed with decrease in concentration of the *Cinnamomum iners* extract from 1000 to 50 μg. However, all of the samples showed no inhibition zones by 10 μg and 5 μg. The presence of antifungal action in the test substance was taken as positive by the appearance of inhibition zones. The results were normally expressed in terms of diameter of the inhibition zone: <9 mm, inactive; 9–12 mm, partially active; 13–18 mm, active; >18 mm, very active [25].

Generally we can see that ethanol extract give the most clear and wider inhibition zone among the samples by using *Pycnoporous sanguineus* followed by chloroform extract and GADS while water extract is not showing any antifungal activity. This shows that ethanol extract exhibited stronger antifungal activity compared to other extracts. The result was the same when using the brown rot fungi *Gleophyllum trabeum* and *Trametes versicolor* but the inhibition zone is slightly smaller. Less fungitoxic activities is observed when the extracts are tested against *Fomitopsis palustris*. The negative control did not show any inhibiting activity for all the samples.

In overall, *Cinnamomum iners* extract yielded a good microbial activity. Based on the previous study, it was found that *Cinnamomum iners* yielded good antimicrobial activity against the different strains under analysis. The result is comparable to that lemon grass oils and thyme. Antimicrobial activity of the *Cinnamomum* plants is also greater than other essential oils such as rosemary and oregano oils, whose MIC values were of 700 μg/mL and 250 to 450 μg/mL respectively [26].

CONCLUSION

Based on the results ethanol extract had shown the highest antioxidant activity with EC_{50} value 14.96 (μg/mL) followed by chloroform extract with EC_{50} >30 (μg/mL) and water extract has the least antioxidant activity with EC_{50} value also >30 (μg/mL). Ethanol extract with the highest total phenol content 56.23% showed highest antioxidant activity. Chloroform extract was found to have total phenol content of 51.13% while for water extracts was 7%. Ethanol extract with the highest antioxidant activity showed the most clear inhibition zone. Based on the results, the antifungal activities are related to antioxidant activity and total phenol content.

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