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Evaluation of antioxidant activity of the crude ethanolic extract from the bark of *Cinnamomum mercadoi*

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Abstract

Kalingag (*Cinnamomum mercadoi)* is a native plant of the Philippines that has long been used in traditional medicine. Because of its indigenous nature, research on this plant remains limited. This research evaluated the phytochemical content and antioxidant properties of a crude ethanolic extract from *Cinnamomum mercadoi* bark. The plant bark collected from Davao Occidental was thoroughly cleaned with distilled water to remove any adhering dirt, air-dried for a week in the shade, and then pulverized with a Wiley mill. The bark was then subjected to Soxhlet extraction for 6 hours, and the pooled extracts were concentrated for 5 hours at 60°C in a rotary evaporator to yield a green, syrupy substance. The extract was tested for phytochemical content, followed by Fourier Transform Infrared Spectroscopy (FTIR) for confirmation of the subsequent results by determining the functional groups that comprise their structure, before being assessed using the DPPH assay for free radical scavenging. The phytochemical screening and subsequent FTIR analysis revealed important metabolites such as tannins, glycosides, phytosterols, and terpenoids, all of which contribute to the antioxidant activity of the crude ethanolic extract. In the DPPH assay, the extract demonstrated concentration-dependent activity (IC_{50} = 85.7627 µg/mL) compared to the gallic acid (IC_{50} = 4.1818 µg/mL). Such findings highlight the importance of purifying and isolating specific compounds to boost the plant's antioxidant activity. To maximize its value, further study into other parts of the plant is recommended.

Keywords: Antioxidant; Bark; *Cinnamomum mercadoi*; DPPH

1. Introduction

Oxidative stress occurs when free radicals accumulate and compromise the body's natural defenses. Such a condition can induce cellular damage, which can result in a range of diseases, including metabolic and neurodegenerative disorders. Antioxidants, which scavenge free radicals and thereby prevent oxidative damage to a specific molecule, are used to reduce oxidative stress and consequent damage [1]. Antioxidants are classified into two groups according to their source: natural and synthetic. Butylated hydroxytoluene (BHT) and hydroxyanisole (BHA) are well-known

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antioxidants of synthetic origin that have long been employed in various formulations due to their low cost and capacity to be manufactured on a large scale [2]. However, numerous studies have highlighted health problems linked to the long-term use of synthetic antioxidants, such as premature aging and various types of cancer [3]. This demanded the replacement of these antioxidants with natural alternatives. *Cinnamomum mercadoi*, locally known as Kalingag, is an indigenous tree that has long been used to treat various ailments, including digestive issues, headaches, and arthritis. A prior study found that the bark extracts outperformed the leaf extract in terms of antioxidant activity [4]. This study assessed the phytochemical content of a crude ethanolic extract from *Cinnamomum mercadoi* bark using qualitative analysis and subsequent FTIR analysis, as well as the antioxidant properties using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay for scavenging free radicals,

2. Material and methods

The materials and reagents used by Herbanext Laboratories, Inc. (Bago City, Negros Occidental, Philippines) in this study are of analytical grade unless otherwise specified.

2.1. Plant Authentication and Collection

Cinnamomum mercadoi barks were gathered from Davao Occidental between December 2023 and January 2024, with authorization from the Department of Environment and Natural Resources.

2.2. Preparation of Crude Ethanolic Extract from *Cinnamomum mercadoi* Barks

Cinnamomum mercadoi barks were thoroughly cleaned with distilled water to remove any adhered dirt. The barks were air-dried for a week in the shade before being pulverized with a Wiley mill. 15.4791 grams (g) of ground bark were subjected to 6 hours of Soxhlet extraction using 150.0 milliliters (mL) of 95% ethanol. The pooled extracts were then concentrated for 5 hours at 60°C in a rotary evaporator, yielding a green, syrupy substance. The crude ethanolic extract was stored in a tightly sealed amber bottle at a cold temperature until used. Using the following formula, the percentage (%) yield was computed:

$$\%\ yield = \left(\frac{weight\ of\ crude\ ethanolic\ extract}{weight\ of\ ground\ bark}\right) x\ 100$$

2.3. Preliminary Phytochemical Screening

The crude ethanolic extract from *Cinnamomum mercadoi* barks was divided into labeled test tubes and qualitatively tested for various plant metabolites, such as the following:

2.3.1. Protein

Five (5) drops of Biuret reagent were incorporated into the crude ethanolic extract. A pink-to-purple color suggests that proteins are present.

2.3.2. Reducing Sugars

The crude ethanolic extract was treated with five (5) mL of Benedict's reagent. Brick-red precipitation suggests that reducing sugars are present.

2.3.3. Alkaloids

The crude ethanolic extract was treated with two (2) drops of Wagner's reagent through the test tube's side. A red-brown to yellow precipitate suggests that alkaloids are present.

2.3.4. Flavonoids

Five (5) drops of 10% sodium hydroxide (NaOH), followed by diluted hydrochloric acid (HCl), were incorporated into the crude ethanolic extract. A deep yellow color appears when 10% NaOH is added, but fades upon adding the diluted acid. Such a result suggests that flavonoids are present.

2.3.5. Glycosides

Initially, 1 mL of glacial acetic acid was added to the crude ethanolic extract. Concentrated sulfuric acid (H_2SO_4) and neutral 1% ferric chloride ($FeCl_3$) were then carefully added dropwise. A reddish-brown color suggests that glycosides are present.

2.3.6. Phytosterols

Initially, 1 mL of chloroform was incorporated into the crude ethanolic extract. Afterward, 2 drops of concentrated H_2SO_4 and 1 mL of acetic anhydride were carefully dropped into the test tube from the side. A green or blue-green color suggests that phytosterols are present.

2.3.7. Saponins

The crude ethanolic extract was well-shaken after adding 2 mL of distilled water. A persistent foam suggests that saponins are present.

2.3.8. Tannins

Three (3) drops of neutral 10% $FeCl_3$ were added to the crude ethanolic extract. A gray-to-black color suggests that tannins are present.

2.3.9. Terpenoids

The crude ethanolic extract was treated with 3 mL and 1 mL of concentrated H_2SO_4 and chloroform, respectively. A reddish-brown junction suggests that terpenoids are present.

2.4. Confirmation of Phytochemical Analysis using Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectrum of the extract was analyzed using the IRSpirit-T™ FTIR spectrometer. The equipment contains a single-bounce ATR (Attenuated Total Reflectance) sampling accessory with a diamond crystal. A small drop of the material was examined and analyzed using 20 scans with a 4 cm^{-1} spectral resolution and a 4000 cm^{-1} to 500 cm^{-1} wavenumber range. The LabSolutions IR software was used to process the spectrum, and the results were displayed in percentage (%) transmittance.

2.5. DPPH Assay for Radical Scavenging Activity

A 0.1 millimolar (mM) DPPH stock solution was first prepared by weighing 39.43 milligrams (mg) of DPPH powder and diluting it with 100 mL of methanol in an aluminum foil-wrapped volumetric flask, which was then incubated for an hour in a cool, dark environment. The crude ethanolic extract was diluted in methanol six times at two-fold, resulting in concentrations ranging from 5000 µg/mL to 39.06 µg/mL. In separate aluminum-wrapped test tubes, 1 mL of each concentration was placed, followed by 5 mL of DPPH stock solution, which was incubated for an hour in a cool, dark area. The absorbance of the extract was measured with a UV-Vis spectrophotometer at a wavelength of 517 nanometers (nm). The same procedure was used to test the positive control, gallic acid. A blank solution containing 1 mL of methanol and 5 mL of DPPH stock solution was also prepared. The scavenging effect was calculated using the following formula:

$$\%\ radical\ scavenging\ activity\ (RSA) = \left(\frac{A_{blank} - A_{extract}}{A_{blank}}\right) x\ 100$$

where $A_{extract}$ and A_{blank} represent the absorbances of the extract and blank solution, respectively. % RSA was plotted against the concentration to compute the IC_{50} values [5].

3. Results and discussion

3.1. Percentage Yield of Crude Ethanolic Extract from *Cinnamomum mercadoi* Barks

Using 15.4791 g of pulverized bark, 5.358 g of crude ethanolic extract was recovered, resulting in a 34.61% yield.

3.2. Results of the Preliminary Phytochemical Screening

Table 1 shows the phytochemical analysis results for the crude ethanolic extract from *Cinnamomum mercadoi* barks.

Table 1 Phytochemical analysis results for crude ethanolic extract from *Cinnamomum mercadoi* barks

Phytochemical	Result
Protein	+
Reducing Sugars	+
Alkaloids	-
Flavonoids	-
Glycosides	+
Phytosterols	+
Saponins	-
Tannins	+
Terpenoids	+

(+) present; (-) absent

The phytochemical screening revealed the presence of various plant metabolites, including terpenoids, glycosides, phytosterols, and tannins. According to Khanal et al [6], the presence of these compounds in the extract is due to the polarity of the solvent, which affects the amount of phytochemicals present. Because ethanol is a polar solvent, it was relatively convenient to extract such phytochemicals. Secondary metabolites are compounds mainly produced by plants and microbes that are required for survival and growth in adverse environments. The variety of pharmacophores in these compounds explains the diversity of their chemical structure and the resulting therapeutic effect.

3.3. Results of the FTIR Analysis

The infrared spectra of the crude ethanolic extract from 4000 cm−1 to 500 cm−1 are illustrated in Figure 1. Table 2 summarizes the subsequent interpretation.

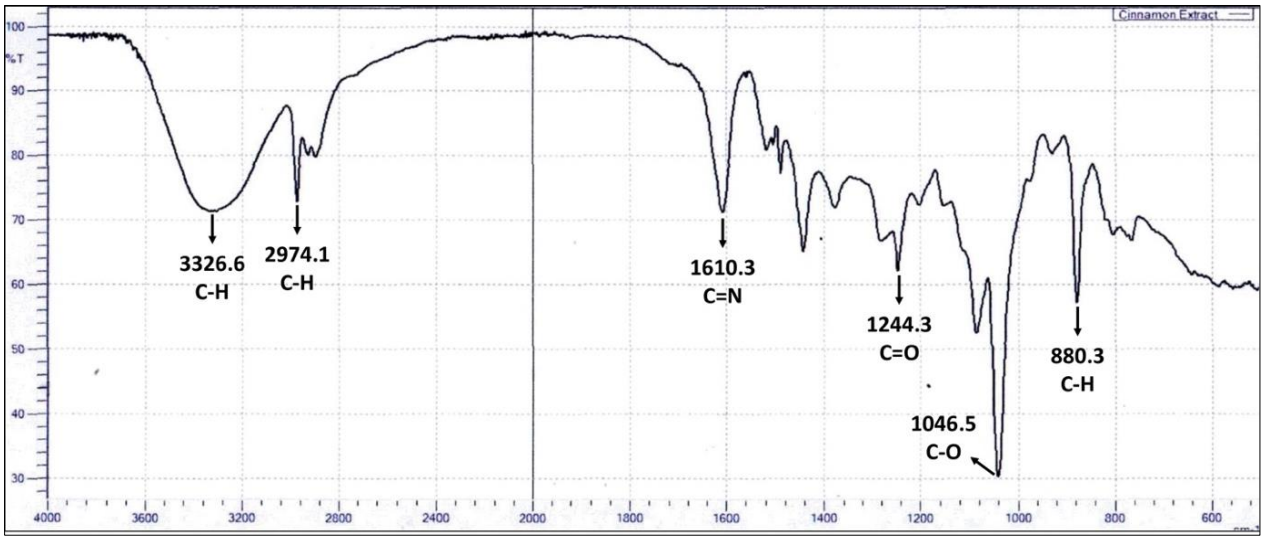

Figure 1 FTIR spectra of the crude ethanolic extract from *Cinnamomum mercadoi* barks

Table 2 Interpretation of FTIR analysis of the crude ethanolic extract from *Cinnamomum mercadoi* barks

Peak Number	Wavenumber (cm^{-1})	Chemical Bond	Functional Group
1	3326.6	C-H stretching)	Alkene (sp^2)
2	2974.1	C-H (stretching)	Alkyne (sp)
3	1610.3	C=N (stretching)	Nitrile
4	1244.3	C=O (stretching)	Ester
5	1046.5	C-O (stretching)	Alcohol (sp^3)
6	880.3	C-H (bending)	Aromatic-Para

The six distinct peaks in the spectra correspond to the functional groups that make up the structures of the different phytochemicals found in the crude extract, thereby validating the findings of the preliminary phytochemical screening [7, 8, 9]. The –CH stretching at 3326.6 cm^{-1} suggests that an alkene group is present, which is common in the structures of all secondary metabolites detected during phytochemical screening. According to Yulvianti and Zidorn [10], the basic structure of cyanogenic glycosides contains a nitrile group, which was shown to be present in the extract based on the C=N stretching at 1610.3 cm^{-1}. At 1244.3 cm^{-1}, the C=O stretching shows the presence of an ester group, which includes the unsaturated lactone ring in cardiac glycosides and the bonds that connect aromatic rings and carbohydrates in cyanogenic glycosides [7, 8]. C-O stretching at 1046.5 cm^{-1} reveals the presence of alcohols and phenols in the structures of all secondary metabolites identified in the crude ethanolic extract [7, 8, 9]. Lastly, the C–H bending at 880.3 cm^{-1} indicates the existence of substituted aromatic compounds, reflecting the polyphenolic structure of tannins [7].

3.4. Results of the DPPH Assay

Figure 2 displays the percentage RSA for the crude ethanolic extract and gallic acid.

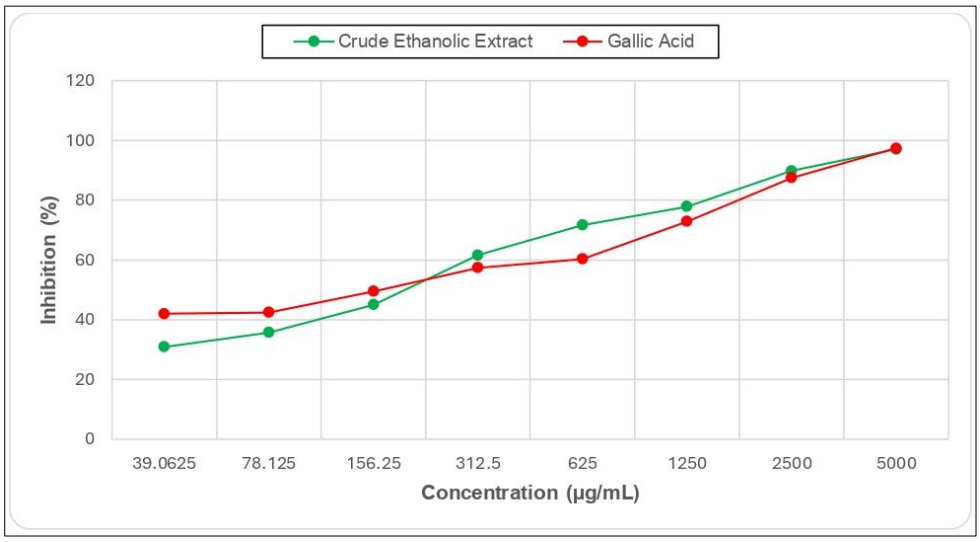

Figure 2 Percentage (%) inhibition of DPPH radical between the crude ethanolic extract and gallic acid

The DPPH assay is an established method to quickly and easily assess the antioxidant activity of a sample [11]. Gallic acid, a well-known antioxidant, exhibited potent activity. The crude ethanolic extract also showed a concentration-dependent activity. The IC_{50} values of both the crude extract and gallic acid are shown in **Table 3**. The extract's activity is due to its phytochemicals, such as polyphenols, which have been shown to donate hydrogen. However, the crude extract has a relatively lower activity than gallic acid. This is due to the impure origin and complex composition of the extract. As a result, various methods for purifying or isolating certain compounds in the extract are suggested to increase its antioxidant activity.

Table 3 IC_{50} values of the crude ethanolic extract and gallic acid

	IC_{50} (µg/mL)
Crude Ethanolic Extract	85.7627
Gallic Acid	4.1818

4. Conclusion

This study focused on a native plant from the Philippines that has received limited attention due to its indigenous origin. The discovery of antioxidant activity paves the way for more research into the various therapeutic properties of other local indigenous plants. It is also worth noting that the extract demonstrated antioxidant activity despite its impure form, indicating that its pharmacological activities will be more apparent after the extract is purified or specific components are isolated. Furthermore, additional research on other parts of the plant is recommended to maximize its biomedical benefits.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors disclose no direct or indirect conflict of interest.

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