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Evaluation of antibacterial property and phytochemical composition of the crude ethanolic extract from Kalingag (*Cinnamomum mercadoi*) barks

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Abstract

Cinnamomum mercadoi, locally known as Kalingag, is a native plant of the Philippines that has limited studies despite its long use in traditional Philippine medicine due to its indigenous origin. This study examined the phytochemical composition and antibacterial activities of a crude ethanolic extract derived from Kalingag barks. The plant bark gathered from Davao Oriental was thoroughly rinsed with distilled water to eliminate any attached dirt, oven-dried for 6 hours, and then pulverized using a Wiley mill. The bark was then subjected to Soxhlet extraction, and the pooled extracts were concentrated for 5 hours at 40°C to produce a green, syrupy substance. The extract was tested qualitatively, followed by FTIR analysis to confirm the results, before being subjected to the agar well diffusion method to evaluate its antibacterial properties against *Staphylococcus aureus* and *Escherichia coli*. The phytochemical evaluation and subsequent FTIR analysis revealed that the extract contained various phytochemicals, including glycosides, tannins, phytosterols, and terpenoids. The antibacterial testing revealed concentration-dependent inhibition that could be improved by purifying or isolating specific compounds from the extract. Additional research into other plant parts is suggested to enhance its biomedical and therapeutic applications.

Keywords: Antibacterial; Bark; *Cinnamomum mercadoi*; Agar well

1. Introduction

The emergence of antimicrobial resistance (AMR) necessitates the rapid development of new antimicrobial drugs. Plants contain several bioactive compounds with potential medicinal applications [1]. *Cinnamomum mercadoi*, commonly known locally as Kalingag, a lesser-known plant that belongs to the Lauraceae family, is endemic to the Philippines and has long been used medicinally [2]. The researchers focused on *Cinnamomum mercadoi* for antimicrobial studies due to the abundance of indigenous plants in the Philippines and the scarcity of research on these

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plants. Despite its conventional medical applications, the difficulty of obtaining samples has impeded scientific investigation [3]. The purpose of this research is to investigate the phytochemical profile of the crude ethanolic extract from Kalingag barks through qualitative testing and Fourier Transform Infrared (FTIR) spectroscopic analysis, followed by an assessment of its antibacterial activity against clinically relevant bacterial strains.

2. Material and methods

Herbanext Laboratories, Inc. (Bago City, Negros Occidental, Philippines) employed materials and reagents of analytical grade unless otherwise stated.

2.1. Plant Authentication and Collection

Barks of *Cinnamomum mercadoi* were harvested from Davao Oriental between December 2023 and January 2024 with permission from the Department of Environment and Natural Resources.

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

The plant barks were thoroughly rinsed with distilled water to eliminate any attached dirt. The barks were brought to Herbanext Laboratories, Inc. (Bago City, Negros Occidental, Philippines) and oven-dried for 6 hours at 50°C before being pulverized with a Wiley Mill. 15.4791 grams (g) of ground bark were subjected to Soxhlet extraction for 6 hours using 150.0 milliliters (mL) of 95% ethanol. The pooled extracts were concentrated in a rotary evaporator for 5 hours at 40 degrees Celsius (°C) to produce a green, syrupy substance. The crude ethanolic extract was stored in a tightly sealed amber bottle at a cold temperature until used. The calculation of the percentage (%) yield was performed with the following formula:

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

2.3. Qualitative Phytochemical Analysis

The plant extract was divided into labeled test tubes and qualitatively analyzed for various plant metabolites, including the following:

2.3.1. Alkaloids

Two (2) drops of Wagner's reagent were carefully added to the extract through its test tube's side. A red-brown to yellow precipitate suggests that indicates are present.

2.3.2. Flavonoids

The extract was treated with ten (10) drops of 10% sodium hydroxide (NaOH), followed by a few drops of diluted hydrochloric acid (HCl), and then tested for a deep yellow color that appeared initially but faded upon adding the diluted acid.

2.3.3. Glycosides

To the dried extract, one (1) mL of glacial acetic acid, a few drops of concentrated sulfuric acid (H_2SO_4), and neutral 1% ferric chloride ($FeCl_3$) were introduced and then observed for a reddish-brown color.

2.3.4. Phytosterols

The extract was combined with 2 drops of concentrated H_2SO_4, and 1 mL of both chloroform and acetic anhydride, then observed for a pink color that changed to green or blue-green.

2.3.5. Protein

Five (5) drops of Biuret reagent were introduced to the dried extract and then observed for a pink-to-purple color.

2.3.6. Reducing Sugars

The extract was treated with 5 mL of Benedict's reagent and then observed for a brick-red precipitate.

2.3.7. Saponins

The extract was briskly shaken after adding 2 mL of distilled water before being observed for frothing or persistent foam.

2.3.8. Tannins

Five (5) drops of neutral 10% $FeCl_3$ were added to the extract and then observed for the presence of a gray-to-black color.

2.3.9. Terpenoids

The extract was treated with 3 mL and 1 mL of concentrated H_2SO_4 and chloroform, respectively, and then observed for a reddish-brown junction.

2.4. Confirmation of Phytochemical Analysis using FTIR Analysis

The IRSpirit-T™ FTIR spectrometer (Shimadzu Philippines Corporation, Taguig City, Metro Manila, Philippines) was used to record IR spectra. It was interfaced with a single-bounce ATR (Attenuated Total Reflectance) sampling attachment with a diamond crystal. A small drop of the sample was placed on the surface of the ATR crystal, and the IR spectrum was analyzed. The spectrum was measured by accumulating 20 scans from a wavenumber region of 4000 cm^{-1} to 500 cm^{-1}, with the resolution set to 4 cm^{-1}. The spectrum was processed using LabSolutions IR software (Shimadzu Philippines Corporation, Taguig City, Metro Manila, Philippines), and the findings were presented in percentage (%) transmittance.

2.5. Testing for Antibacterial Activity

The procedure used in this test was based on the work of Chalo et al [4], with some modifications. The crude extract was evaluated against *Escherichia coli* and *Staphylococcus aureus*, using reference strains ATCC-25922 and ATCC-23235, respectively.

2.5.1. Preparation of Culture Media

Sterile plates containing nutrient agar (NA) were used to assess the antibacterial properties of the crude ethanolic extract. Approximately twenty-eight (28) g of the culture media was placed in a 1000 mL Erlenmeyer flask, with distilled water at a volume of 1 liter (L) was gradually added to mix and partially dissolve it. The Erlenmeyer flask was covered and then sterilized with moist heat in an autoclave set to fifteen (15) pounds per square inch (PSI), one-hundred twenty-one (121) degrees Celsius, and an operating time of 15 minutes.

2.5.2. Preparation of Inoculum

The test microorganisms were cultured on NA for twenty-four (24) hours before being transferred to a sterile screw-capped test tube containing a standard saline solution with turbidity adjusted to 0.5 MacFarland units, which corresponds to 1.5 x 10^{-8} colony forming unit (CFU)/mL. The suspension was mixed with a vortex mixer and stored at a cold temperature before use.

2.5.3. Agar Well Diffusion Method

Twenty (20) mL of sterile, freshly prepared NA was allowed to solidify after pouring into separate sterile Petri dishes. A sterile swab stick was used to evenly distribute the test inoculum over the NA plates, which were subsequently dried for 5 minutes. To prepare the dried extract for testing, it was dissolved in distilled water at concentrations of twenty-five (25), fifty (50), and one hundred (100) milligrams (mg)/mL. Gentamicin and tetracycline were used as positive controls at concentrations of ten (10) and thirty (30) micrograms (µg), respectively. Each NA plate was drilled using a sterile cork borer to create six (6) wells with a diameter of 10 millimeters (mm). Using a microtiter pipette, 100 µL of plant extract (25, 50, and 100 µg/mL), gentamicin, tetracycline, and distilled water as negative control were introduced to separate wells. Afterward, the plates were incubated at 37°C in a DRP-9082 electro-thermostatic incubator (Senxin®, Shanghai, China) for twenty-four (24) hours before being checked for a clear zone around the well, known as the zone of inhibition (ZOI), indicating that no microbial growth had occurred. Following the recommendations of the Clinical and Laboratory Standards Institute (CLSI), the ZOI was measured with the use of a digital Vernier caliper, and then expressed in millimeters. Such information is described in Table 1.

Table 1 CLSI guidelines for assessing antimicrobial susceptibility with tetracycline and gentamicin as positive controls

Agent	Code	Concentration (µg)	Microbe	Zone of Inhibition (mm)		
				Resistant	Intermediate	Susceptible
Gentamicin	CN	10	*E coli*	≤ 12	13 – 14	≥ 15
			S aureus			
Tetracycline	TE	30	*E coli*	≤ 11	12 – 14	≥ 15
			S aureus	≤ 14	15 – 18	≥ 19

2.6. Statistical Analysis

The antibacterial activity was evaluated three times, and the findings were expressed as mean ± standard deviation (SD) of the mean. A *t-test* was employed to determine if the samples and the positive controls exhibited a significant difference in their activity ($p < 0.05$).

3. Results and discussion

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

Approximately 5.358 g of dried extract was recovered from 15.4791 g of ground bark, yielding 34.61% of crude ethanolic extract.

3.2. Qualitative Phytochemical Analysis

Table 2 Displays the results of the phytochemical analysis for the dried extract.

Table 2 Phytochemical analysis of the dried ethanolic extract from *Cinnamomum mercadoi* barks

Phytochemical	Result
Glycosides	Present
Phytosterols	Present
Tannins	Present
Terpenoids	Present
Protein	Present
Reducing Sugars	Present
Alkaloids	Absent
Flavonoids	Absent
Saponins	Absent

According to the results of the qualitative analysis, phytochemicals such as terpenoids, glycosides, phytosterols, and tannins are present in the dried extract. The presence of these compounds is due to solvent polarity, which is thought to influence the amount of phytochemicals present [5]. The extraction of such compounds was relatively convenient due to the polar nature of ethanol. These secondary metabolites are important in medicine and pharmacy due to their complex chemical structure and subsequent variety of pharmacophores.

3.3. FTIR Analysis

Figure 1 shows the FTIR spectra of the crude ethanolic bark extract of *Cinnamomum mercadoi*, which are interpreted in **Table 3**.

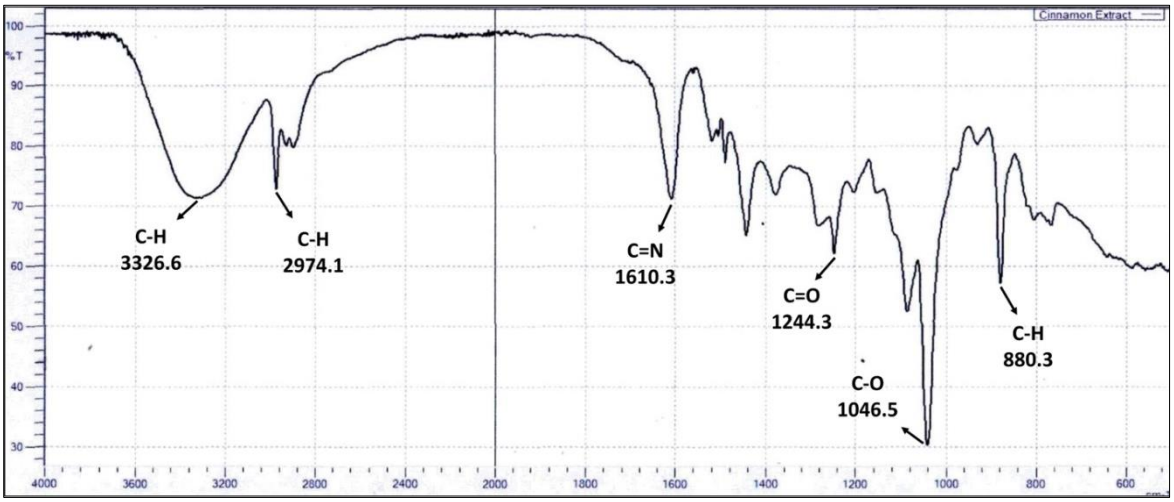

Figure 1 FTIR spectra of the dried ethanolic bark extract of *Cinnamomum mercadoi*

Table 3 Interpretation of FTIR analysis of the dried ethanolic bark extract of *Cinnamomum mercadoi*

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

The results of the qualitative phytochemical analysis were confirmed by the six distinct peaks in the spectra that correspond to the functional groups that make up the structures of the compounds found in the dried extract. The presence of substituted aromatic molecules, as revealed by the C-H bending at 880.3 cm^{-1}, represented the polyphenolic composition of tannins [6]. Alcohol/phenol and alkene groups are present in the structures of all secondary metabolites discovered in the dried extract, as indicated by C-O and -CH stretching at 1046.5 cm^{-1} and 3326.6 cm^{-1}, respectively [6, 7, 8]. 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 is indicated by the C=O stretching at 1244.3 cm^{-1} [6, 7]. Finally, C=N stretching at 1610.3 cm^{-1} indicates the existence of a nitrile group, which makes up the structure of cyanogenic glycosides [9].

3.4. Testing for Antibacterial Activity

As stated in **Table 4** and illustrated in **Figures 2 and 3**, the extract displayed antibacterial activity only against *Staphylococcus aureus*, implying that it may be more effective against Gram-positive pathogens. The extract showed concentration-dependent inhibition, with 100 mg/mL resulting in the greatest activity with a ZOI of 13.40 mm. Although the ZOI of the extract is generally interpreted as "resistant" and statistical analysis shows a significant difference in activity against the two positive controls, it is worth noting that the extract exhibited antibacterial activity despite its impure nature, highlighting that the activity of this extract can even increase when it is purified or upon search and subsequent isolation of the compounds present in the extract.

Table 4 Comparison of antibacterial activity between the crude ethanolic extract from *Cinnamomum mercadoi* and the positive controls

Microbe	Sample		ZOI*	Interpretation	*p*
S aureus	Extract	25 mg/mL	10.65	Resistant	< 0.05
		50 mg/mL	12.41		< 0.05
		100 mg/mL	13.40		< 0.05
	Gentamicin		21.94	Susceptible	
	Tetracycline		25.99		
	Distilled Water		-	-	
E coli	Extract	25 mg/mL	-	-	
		50 mg/mL	-	-	
		100 mg/mL	-	-	
	Gentamicin		22.88	Susceptible	
	Tetracycline		16.94		
	Distilled Water		-	-	

* mean (n = 3) ± SD

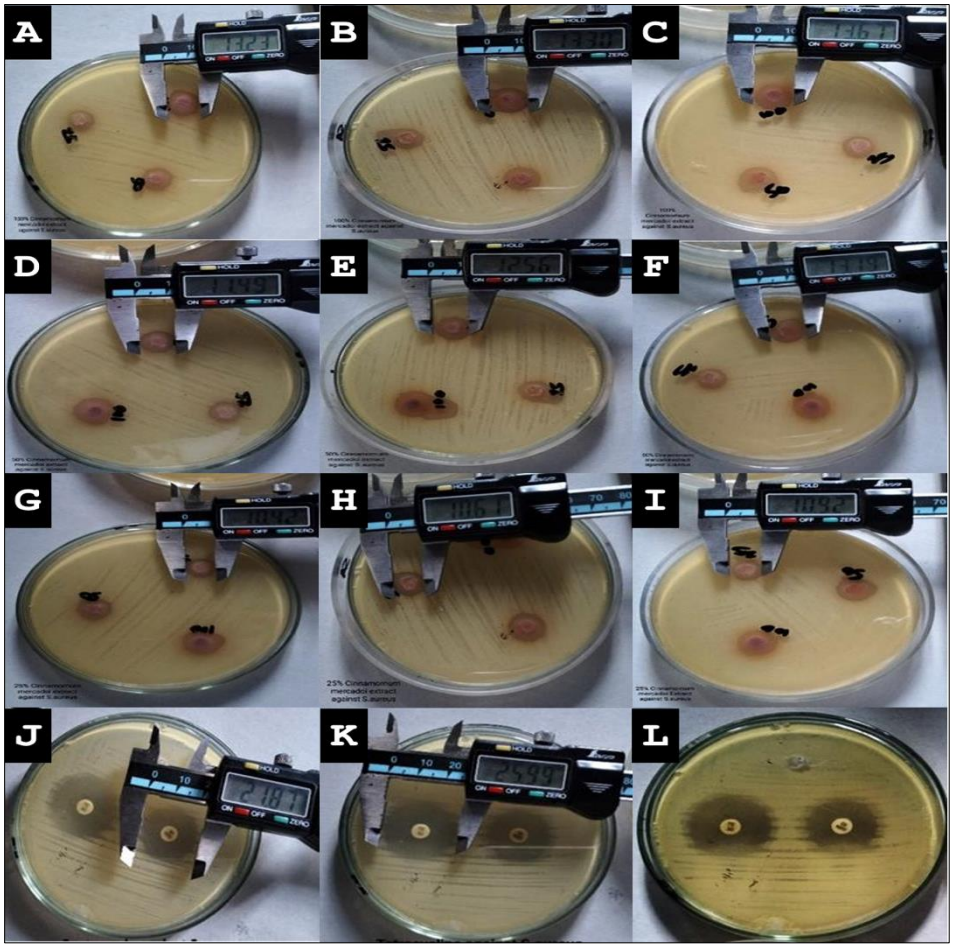

Figure 2 The antibacterial testing of *Cinnamomum mercadoi* crude ethanolic bark extract against *S aureus*. (A-C) 100 mg/mL extract. (D-F) 50 mg/mL extract. (G-I) 25 mg/mL. (J) 10 µg Gentamicin. (K) 30 µg Tetracycline. (L) Distilled water

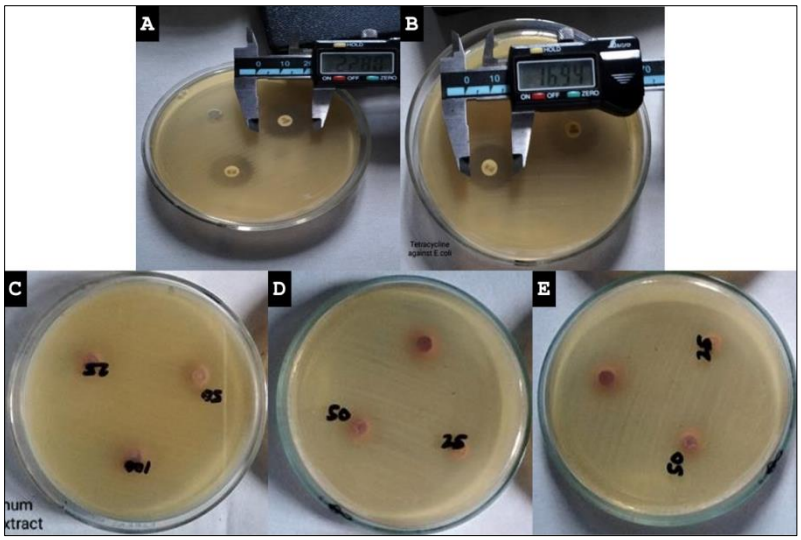

Figure 3 The antibacterial testing of *Cinnamomum mercadoi* crude ethanolic bark extract against *E coli*. (A) 10 µg Gentamicin. (B) 30 µg Tetracycline. (C-E) The three concentrations of the crude bark extract

4. Conclusion

The researchers focused on a native plant from the Philippines that has received less attention due to its indigenous nature. The discovery of its antibacterial activity against the known pathogen, *Staphylococcus aureus*, which is known to produce drug-resistant strains, paved the way for further research into the capacity of local indigenous plants to yield novel compounds with antimicrobial activity, which addresses the issue of antimicrobial resistance to commercially available agents. It is also worth mentioning that the extract displayed intermediate activity against known pathogens despite its impure state, hinting that its therapeutic potential will become higher upon purification or even isolation of specific components. Furthermore, additional research into other plant parts is suggested to enhance its biomedical and therapeutic applications.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors disclose no direct or indirect conflict of interest.

References

[1] Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. Metabolites. 2019; 9(11): 258.

[2] Torres RC, Sison FM, Ysrael MC. Phytochemical screening and biological studies on the crude methanol extract of *Cinnamomum mercadoi*, Vidal. Philipp J Sci. 2003; 132(1): 27-32.

[3] Alemu M, Lulekal E, Asfaw Z, Warkineh B, Debella A, Abebe A, Degu S, Debebe E. Antibacterial activity and phytochemical screening of traditional medicinal plants most preferred for treating infectious diseases in Habru District, North Wollo Zone, Amhara Region, Ethiopia. PLoS One. 2024; 19(3): e0300060.

[4] Chalo, DM, Lukhoba C, Fidahussein DS, Nguta JM. Antimicrobial activity, toxicity and phytochemical screening of selected medicinal plants of Losho, Narok County, Kenya. Asian J Nat Prod Biochem. 2017; 15(1): 29-43.

[5] Khanal LN, Sharma KR, Pokharel YR, Kalauni SK. Phytochemical analysis and in vitro antioxidant and antibacterial activity of different solvent extracts of *Beilschmiedia roxburghiana* nees stem barks. Sci World J. 2022; 2022:6717012.

[6] Okuda T, Ito H. tannins of constant structure in medicinal and food plants—hydrolyzable tannins and polyphenols related to tannins. Molecules. 2011; 16(3):2191–217.

[7] Kytidou K, Artola M, Overkleeft HS, Aerts JMFG. Plant glycosides and glycosidases: a treasure-trove for therapeutics. Front Plant Sci. 2020; 11:357.

[8] Masyita A, Mustika Sari R, Dwi Astuti A, Yasir B, Rahma Rumata N, Emran TB, Nainu F, Simal-Gandara J. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. Food Chem X. 2022; 13:100217.

[9] Yulvianti M, Zidorn C. Chemical diversity of plant cyanogenic glycosides: an overview of reported natural products. Molecules. 2021; 26(3):719.