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Phytochemical Screening and Biological Studies on the Crude Methanol Extract of *Cinnamomum mercadoi*, Vidal

Rosalinda C. Torres*, Fe M. Sison[1] and Mafel C. Ysrael[2]

*Chemicals and Minerals Division, Industrial Technology Development Institute
Depatment of Science and Technology, Bicutan, Taguig, Metro Manila
[1]Standards and Testing Division, Industrial Technology Development Institute
Depatment of Science and Technology, Bicutan, Taguig, Metro Manila
[3]Department of Biochemistry, Faculty of Pharmacy, University of Sto. Tomas
España, Manila, Philippines

***Cinnamomum mercadoi* Vidal (syn. *C. celebicum* Koord.) of the Lauraceae family is an endemic species in the Philippines. Phytochemical screening of the crude methanol extract of *C. mercadoi* indicated the presence of saponins, condensed tannins, an unsaturated lactone ring and leucoanthocyanins. The mean lethal dose (LD_{50}) of the extract administered orally in male strong A mice is 5.2723 ± 0.2218 g/kg. Toxidrome ranged from decreased motor activity and respiratory rate, ptosis, hyperemia, diarrhea and death. Using the Plantar test method for evaluation of analgesic activity, the methanol extract of *C. mercadoi* produced 84.0% protection at 500 mg/kg while the positive control (aspirin) exhibited 72.07% protection at 300 mg/kg.**

Keywords: endemic species, phytochemical screening, analgesic activity

Cinnamomum mercadoi Vidal (Lauraceae) is a large endemic tree with relatively thick aromatic bark. This plant is indigenous to the Philippines where it is named kalingag, kaningag, kalingad, kandoroma and kanilau (Fig. 1). Like other Cinnamomum species, it is variable in height and in shape, size and texture of the leaves (Santos, 1930). The plant appears to grow best in forests at low and medium altitudes, sometimes ascending to 2,000 meters.

One of the earliest chemical studies on *C. mercadoi* reported the presence of essential oil, oleoresin and resin (Bacon, 1909). The oil was investigated to contain mainly safrole (Fig. 2). Further study showed that the volatile oil and safrole were found in the leaves, bark and roots of *C. mercadoi* (Concha, 1966). Sapogenin was also identified to be present in the leaves and seeds of *C. mercadoi* (Anzaldo, 1958). The alkaloid content of the leaves was published by Willaman-Li (Santos, 1981). To this date, no other chemical studies have been undertaken on the plant. Phytochemical screening, isolation and identification of the plant's constituents were not carried out. Toxicological and pharmacological studies were also not conducted.

A number of recorded medicinal uses of the bark of *Cinnamomum mercadoi* Vidal has been reported. The bark was found to help in digestion when taken internally (Alzina, 1668). It is also used in flatulence and as expectorant. The bark has rubefacient properties and is utilized as a remedy for headaches and rheumatism (Guerrero, 1921). It is also chewed for stomach troubles, and is used in tuberculosis (Quisumbing, 1978).

The essential oil content and preliminary screening

*Corresponding author: lindactorres@yahoo.com

Figure 1. *Cinnamomum mercadoi* (a. Habit of a fruiting branch; b. Inflorescence; c. Flower; d. Stamen; e. Pistil; f. Fruit; g. Crude drug material (bark).

Safrole

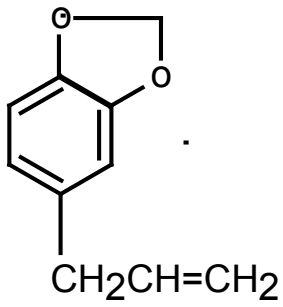

Figure 2. Safrole is one of the first chemical constituents identified to be present in the bark of *Cinnamomum mercadoi* Vidal (Bacon, 1909).

of the antibacterial activity of the oil was reported (Torres, et al., 1999). Cinnamon bark oil was found to have high antibacterial activity against *Staphylococcus aureus* ATCC 25923.

This paper presents the phytochemical screening of the crude methanol extract from the bark of *C. mercadoi* Vidal. Results of the pharmacological, toxicological and antimicrobial studies are also included.

Experimental

Sample Collection

Bark of *Cinnamomum mercadoi* Vidal was obtained in Buruanga, Aklan in the Western Visayas region of the country (Fig. 3). Herbarium specimen was prepared by Ms. Evangelina C. Monroyo (ITDI-DOST) and identified by Dr. Wilfredo Vendivil of the National Museum as *C. mercadoi* syn. *C. celebicum* Koord. Voucher specimen was deposited at the National Museum and at the Chemicals and Minerals Division of ITDI-DOST.

Extraction

Figure 3. Bark of *Cinnamomum mercadoi* Vidal obtained from Buruanga, Aklan

Air-dried bark of *C. mercadoi* with a moisture content of 10% was ground in a Wiley Mill to a mesh size of 100 and exhaustively extracted by cold percolation for 3 days with methanol. The combined methanolic extracts were concentrated under reduced pressure at a temperature maintained at 45°C – 50°C until thick and syrupy. It was then placed in water bath at a temperature not exceeding 60°C and further air-dried to reddish-brown crystals. Crude methanolic extract was subjected to phytochemical screening and toxicity test. Assay for analgesia and anti-inflammatory activities were also conducted. The extract was also evaluated for anti-microbial activity.

Phytochemical Screening of the Crude Extract

The crude methanol extract was tested for the presence of saponins, tannins and polyphenols, cardenolides and bufadienolides, flavonoids, anthraquinones, and alkaloids by test tube methods described in the reference (Acta Manilana, 1985).

Toxicity and Bio-assay Methods

Modified Acute Oral Toxicity Test (LD_{50}) in Mice

Preliminary dosing of the crude extract was conducted to determine the expected dose that will cause 50% death of the experimental animals (male strong A mice, 20-30 g). Concentration of sample suspension was 100 mg/ml with a pH of 4.8. Four increasing log doses of the test substance was given orally to the animals in 4 groups of ten including the control (10% carboxymethyl cellulose in normal saline solution). The number of deaths and other adverse/abnormal signs and manifestation were closely observed and noted for the first two hours after administration of test sample. This was continued in the next twenty-four hours to forty-eight hour, daily up to fourteen days. The median lethal dose (LD_{50}) was computed using the Probit Analysis Method by Fisher and Yates.

Assay for Analgesia (Plantar test Method)

Three (3) increasing doses of the test material (250 mg/kg, 500 mg/kg & 1000 mg/kg), the negative (NSS) and positive (aspirin) controls were given orally to the animals (female Sprague-Dawley rats 147 - 177 g) in groups of two (2), respectively. Exactly after thirty (30) minutes 0.05 ml of 0.1% carrageenin was injected into the plantar tissue of the hind paw of each rat. The initial and final paw withdrawal latency were measured using a plantar test before drug administration and two and a half (2 ½) hours after injection of carrageenin, respectively. Percent protection against thermal stimulus was calculated based on the control as follows:

$$\% \text{ inhibition} = 1 - \frac{\text{Test sample difference}}{\text{Negative Control Difference}} \times 100$$

Assay for Anti-inflammatory (Carrageenin-induced edema Method)

Three increasing doses of the test material, the negative and positive controls were given orally to the male Sprague – Dawley rats in groups of 2, respectively. The negative control was 10% Tween 80 in normal saline solution (NSS) and the positive control was aspirin. Exactly after one hour, 0.05 mL of 0.1% carrageenin was injected into the plantar tissue of the right hind paw of the second rat of each group. The initial and final foot volumes were measured by a plethysmometer before drug administration and three hours after injection of carrageenin, respectively. % inhibition was calculated based on the control.

Microbiological Assay

Against reference bacterial strains

$$\% \text{ inhibition} = [1 - \frac{\text{test sample difference}}{\text{negative control difference}}] \times 100$$

Preliminary screening for antibacterial activity used the following reference strains: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. Reference strains were provided by the Clinical Isolate Bank of Biological Sciences Department, United Laboratories, Inc.

The Kirby-Bauer Disk Diffusion Method as standardized by the National Committee on Clinical Laboratory Standards and World Health Organization, was used throughout the study. Bacterial suspensions of approximately 1.5 x 10^8 cells/ml. were streaked onto the Mueller Hinton Agar plates. Blank disks (Schleicher and Schuell Cat. No. 740E, 6.35 mm) were eluted with 20 µL each of the crude extract and were placed in an equidistant fashion on the seeded agar. The extract was allowed to diffuse for one hour and the plates were incubated at 37°C for 18 hours. The diameter of the zones of inhibition was read to the nearest 10^{th} of a millimeter using an Antibiotic Zone Reader.

Against reference fungal strains

Screening for antifungal activity used *Microsporum canis* as the fungal strain.

The same method mentioned above was followed except that Sabourauds Dextrose Media was used instead of Mueller Hinton Agar. Also, the agar plates with the extract were incubated at room temperature for 48 hours.

Results and Discussion

Phytochemical Screening

Results of the phytochemical screening of the crude methanolic extract from the bark of *C. mercadoi* Vidal are presented in Table 1. Phytochemical screening of the said extract showed the presence of saponins as indicated by the presence of a 3-cm. froth which persisted for 30 seconds. Results also showed the presence of tannins as evidenced by the positive reaction for the gelatin test, specifically condensed tannins as indicated by the ferric chloride test. The presence of unsaturated lactone ring and leuco-anthocyanins was also identified by Kedde, Bate-Smith and Metcalf test, respectively.

Previous studies on *C. mercadoi* reported the presence of sapogenin in its leaves and seeds (Anzaldo et al., 1958). An alkaloid in the leaves was reported by Willaman-Li (Santos, 1981).

Table 1. Results of the phytochemical screening of the crude methanolic extract of *Cinnamomum merca-*

TEST	RESULT	INDICATION
Froth	Persistence of a 3 cm. froth for 30 secs.	Presence of saponins
Gelatin	Formation of white precipitate.	Presence of tannins
Ferric chloride	Formation of brownish-green coloration of the solution.	Presence of condensed tannins.
Liebermann-Burchard	No color changes	Absence of steroid
Keller-Killiani	Failure to produce a reddish-brown color at the interface.	Absence of 2-deoxy-sugar
Kedde	Production of a blue-violet color.	Presence of unsaturated lactone ring.
Bate – Smith & Metcalf	Formation of a violet color.	Presence of leuco-anthocyanins.
Wilstatter "Cyanidin"	Absence of layer formation.	Absence of cyanidin
Dragendorff's	Non-formation of an orange precipitate.	Absence of alkaloids
Mayer's	Non-formation of a white precipitate.	Absence of alkaloids

Table 2. Results of the Behavioral Observation/Toxidrome after Oral Administration of Sample to Male Strong A Mice.

Dose g/kg	n	Observation
0[a]	10	No effect
4.5789	10	Twenty (20) minutes after dosing, the mice manifested decreased motor activity and respiratory rate, hyperemia, ptosis, followed by diarrhea and death of one (1) mouse within twenty four (24) hours. The remaining nine (9) mice recovered after twenty four (24).
5.2415	10	Twenty (20) minutes after dosing, the mice manifested decreased motor activity and respiratory rate, hyperemia, ptosis, followed by diarrhea and death of one (1) mouse within twenty four (24) hours. The remaining seven (7) mice recovered after twenty four (24) hours.
6.0	10	Ten (10) to fifteen (15) minutes after dosing, the mice manifested decreased motor activity and respiratory rate, hyperemia, ptosis followed by diarrhea and death of four (4) mice after twenty four (24) hours; five (5) mice died after forty eight (48) hours. The remaining mouse recovered by after twenty four (24) hours.

[a] – control, the same volume as in the highest dose

Modified Acute oral toxicity test (LD_{50}) in mice

Using the modified acute oral toxicity test (LD_{50}) in mice, the mean lethal dose (LD_{50}) of the crude methanolic extract of *C. mercadoi* administered orally in male strong A mice is 5.2723 ± 0.2218 g/kg. Toxidrome ranged from decreased motor activity and respiratory rate, ptosis, hyperemia, diarrhea and death. Four increasing doses of 4.0 g/kg, 4.5789 g/kg, 5.2415 g/kg and 6.0 g/kg were used in the experiment. Behavioral observation/toxidrome after oral administration of sample to male strong A mice is presented in Table 2, with the summary of mortality ratio of mice in Table 3.

Autopsy findings: Animals sacrificed after fourteen (14) days had grossly normal findings.

Assay for analgesia

The analgesic activity of the crude methanolic extract of *C. mercadoi* was undertaken using the plantar test (Hargreaves method). The crude methanolic extract of *C. mercadoi* produced strong protection against writhing

Table 3. Summary of Mortality Ratio of Mice Administered Orally with the Sample.

$$\text{Mortality Ratio} = \frac{\text{Number of mice with positive sign (death)}}{\text{Total Number of animals tested}}$$

Group Number	Dose g/kg	n	Mortality Ratio*				
			Day 1	Day 2	Day 3	Day 7	Day 14
I	0[a]	10	0/10	0/10	0/10	0/10	0/10
II	4.0	10	1/10	1/10	1/10	1/10	1/10
III	4.5789	10	1/10	1/10	1/10	1/10	1/10
IV	5.2415	10	3/10	3/10	3/10	3/10	3/10
V	6.0	10	4/10	9/10	9/10	9/10	9/10

[a] Control, the same volume as in highest dose.
autopsy findings: Animals sacrificed after fourteen (14) days had grossly normal findings.

at 500 mg/kg and 1,000 mg/kg as shown below:

Results showed that *C. mercadoi* exhibited an analgesic activity that is comparable with aspirin. The folkloric use of this plant in headaches and rheumatism might be attributed to this activity.

Table 4. Results of the analgesic test in rats using the plantar test method.

Group No.	Drug	Dose Mg/kg	Average Paw Withdrawal Latency (seconds)[a]	Percent Protection
I	NSS	0[b]	0.625	–
II	Aspirin	300	0.275	72%
III	Sample	250	0.275	56%
IV	-do-	500	0.1	84%
V	-do-	1000	0.1	84%

a – Average of final minus initial paw withdrawal latency per group.
b – Control, same volume as in the highest dose.

Assay for anti-inflammatory activity

The anti-inflammatory activity of the crude methanolic extract of the bark of *C. mercadoi* was conducted using the carrageenin-induced edema method. The crude methanolic extract of *C. mercadoi* administered orally to male Sprague Dawley rats did not produce protection against edema as compared to a positive control (aspirin).

Results showed that *C. mercadoi* did not exhibit an anti-inflammatory activity.

Microbiological Assay

The antimicrobial activity of cinnamon bark oil has been reported (Torres, et al., 1999). The crude methanolic extract was tested for antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*.

Results of the study showed that the crude extract has moderate activity (11.05 mm. zone of inhibition) against *S. aureus* and no activity was exhibited against *E. coli* and *P. aeruginosa*. The minimum inhibitory concentration (MIC) of the crude extract against *S. aureus* is 500 μg. However, the crude extract exhibited strong antifungal activity against *Microsporum canis* at 30 mm. zone of inhibition.

Cinnamic aldehyde has been identified as the active fungitoxic constituent of cinnamon bark oil (Singh, et al., 1995). Studies on the in-vitro activity of *Cinnamomum zeylanicum* against azole resistant and sensitive Candida species has been reported. Results showed that the MICs of the bark ranged from <0.05 – 30 mg/ml, and were found to be slightly better than the commercially available cinnamon powder. Trans-cinnamaldehyde and 0-methoxycinnamaldehyde had MICs of 0.03 – 0.5 mg/ml (Quale, et al., 1996).

A substance from *C. zeylanicum* was also found to inhibit the activity of bacterial endotoxin (LPS) (Azumi, et al., 1997).

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