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Evaluation of Antioxidant Activity and Toxicity of *Cinnamomum Burmannii* B. from Different Provinces of Indonesia

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Abstract: *Cinnamomum burmannii* B. is an indigenous spice plant from Indonesia that is commonly used as a folk medicine. Differences in the location of cinnamon cultivation in Indonesia can affect its bioactive compounds, which have therapeutic value. This study aims to evaluate the antioxidant activity and toxicity of *C. burmannii* extracts from five different Indonesian provinces where the location of cultivation may influence the plant bioactive compounds. Total phenolic and flavonoid contents were determined using Folin-Ciocalteu and $AlCl_3$ methods, respectively. The antioxidant activity was measured using the DPPH assay and toxicity was assessed using the BSLT method. Results showed that *C. burmannii* extracts from different locations had varying total phenolic and flavonoid content (82.42 ± 3.4—316.26 ± 20.94 mg GAE/g and 18.08 ± 0.23—96.10 ± 7.97 mg QE/g, respectively) as well as antioxidant activity and toxicity (170.54 ± 24.61—43.85 ± 1.09 µg/mL and 826.10 ± 57.79—341.95 ± 32.24 µg/mL, respectively). The presence of active secondary metabolites in these extracts including phenolic and flavonoid compounds was found to contribute to their antioxidant and toxicity properties. The study emphasizes the importance of the location of cinnamon cultivation in determining its bioactivity and the bark of *C. burmannii* products from Indonesia, particularly from the five provinces included in this study, can be considered relatively safe for consumption within reasonable limits. Further comprehensive research is necessary to assess the safety of cinnamon for human consumption due to the variability in coumarin content and the potential toxicity risks associated with its use, especially from Indonesian sources.

Keywords: antioxidants, brine shrimp lethality test, cinnamon, DPPH, Indonesia, methanol extract.

印度尼西亚不同省份阴香的抗氧化活性和毒性评价

摘要: 阴香乙.是一种来自印度尼西亚的本土香料植物，通常用作民间药物。印度尼西亚肉桂种植地点的差异会影响其具有治疗价值的生物活性化合物。本研究旨在评估来自印度尼西亚五个不同省份的 阴香提取物的抗氧化活性和毒性，这些省份的种植位置可能会影响植物生物活性化合物。总酚和类黄酮含量分别使用福林-乔卡图和氯化铝 方法测定。使用 DPPH 测定法测量抗氧化活性，使用 BSLT 方法评估毒性。结果表明，不同产地的丁香提取物具有不同的总酚和类黄酮含量（分别为 82.42±3.4—316.26±20.94 毫克通用电气工程师协会/克和

18.08±0.23—96.10±7.97 毫克量子效率/克）以及抗氧化活性和毒性（分别为 170.54±24.61—43.85±1.09 微克/毫升和 826.10±57.79—341.95±32.24 微克/毫升）。发现这些提取物中存在的活性次级代谢产物（包括酚类和类黄酮化合物）有助于它们的抗氧化和毒性特性。该研究强调了肉桂种植地点在确定其生物活性方面的重要性，并且来自印度尼西亚，特别是来自本研究中包含的五个省份的 C.布尔曼尼产品的树皮在合理范围内可以被认为是相对安全的消费。由于香豆素含量的可变性和与其使用相关的潜在毒性风险，特别是来自印度尼西亚的来源，因此需要进一步的综合研究来评估人类食用肉桂的安全性。

关键词: 抗氧化剂,丰年虾致死率试验,肉桂,DPPH,印度尼西亚,甲醇提取物.

1. Introduction

Cinnamomum burmannii B., a traditional medicinal plant from Indonesia and a member of the Lauraceae family, has been a major national commodity and widely exported globally [1]-[3]. As a leading cinnamon producer, Indonesia has cultivation centers in Jambi and West Sumatra. This cinnamon is referred to by trade names such as cassiavera, Indonesian cassia, kaneel cassia, or padang kaneel [4]. The export volume of Indonesian cassia reached 48.899 tons in 2016 [5], which equated to 37.04% of the total cinnamon exports in the world in the same year [6]. According to the latest statistics, the export volume of Indonesian cassia was 32.553 tons in 2021 [5].

The trade and popularity of cinnamon is due to its recognized health benefits in addition to its use as a spice [7]. Extensive research has been conducted on its pharmacological effects, revealing its antibacterial, antifungal, antitumor, antidiabetic, antioxidant, antirheumatic, and antithrombotic properties [8]. These pharmacological activities are attributed to the presence of various bioactive compounds, including cinnamaldehyde, epicatechin, proanthocyanidins, cinnamic acid, coumarin, and eugenol [9]-[11]. These compounds belong to the phenolic group, with phenolic and flavonoids are the most abundant components and they act as antioxidants [12]-[14]. Thus, cinnamon (*Cinnamomum burmannii*) bark extract is of significant medicinal importance and continues to be a focus of research [15]. Research into phenolic compounds as antioxidants is becoming more prevalent [16]-[17] as many diseases are believed to be caused by excessive levels of reactive oxygen species (ROS) in the body [18], making them a potentially important factor in disease prevention [19].

Although *C. burmannii* is known for its potent antioxidant properties, its phenolic content also includes coumarin, a compound that has raised concerns about the herb's safety due to its toxicity [20]. *Cinnamomum burmannii*, a member of the false cinnamon group along with *C. cassia*, *C. aromaticum* and *C. loureiroi*, has been reported to contain higher levels of coumarin compared to the true cinnamon group. Excessive consumption of coumarin can cause hepatotoxicity [14]. Since 1940, synthetic coumarin has also been prohibited as a food in the United States due to its hepatotoxic effects on animals [21]. In 2004, the European Food Safety Authority (EFSA) established the Tolerable Daily Intake (TDI) of coumarin at 0.1 mg/kg of body weight [22]. Furthermore, the European Parliament and Council established regulations in 2008 regarding the maximum limits of coumarin in food, including 50 mg/kg in traditional and/or seasonal bakery ware with cinnamon labeling, 20 mg/kg in breakfast cereals and muesli, 15 mg/kg in fine bakery ware (excluding traditional and/or seasonal bakery ware with cinnamon labeling), and 5 mg/kg in desserts [23].

It was estimated that cinnamon is the primary dietary source of coumarin [24]. Cinnamon is widely used in households and industries for food preparation and is derived from two species: true cinnamon (*Cinnamomum verum*/*C. zeylanicum*) and false cinnamon. False cinnamon, also known as cassia, is more affordable and widely used in Europe than true cinnamon, but the two have distinct tastes due to their different chemical compositions [25]. False cinnamon contains a higher amount of coumarin (1-benzopyran-2-one), an aromatic compound, compared to true cinnamon [14]. The extensive use of false cinnamon in food products has led to the evaluation of maximum coumarin limits in cinnamon-flavored food products. The prevalence of false cinnamon, including Indonesian cassia, being sold as true cinnamon raises concerns about the safety and efficacy of cinnamon products. There is growing concern about the adulteration of true cinnamon with cassia, emphasizing the need to study the toxicity and antioxidant activity of cinnamon products [20], [26].

This study aims to assess the antioxidant activity and toxicity of *Cinnamomum burmannii* methanol extract from several cross-island provinces in Indonesia, namely Jambi, West Sumatra, East Java, South Kalimantan and Maluku. Previous studies have

demonstrated that the antioxidant properties of plants can vary depending on their growing location and geographical region [13], [27]-[28]. This variation may also extend to the toxicity of *C. burmannii*, making it important to gather data on differences in antioxidant activity and toxicity of *C. burmannii* from Indonesia. Such information is essential for understanding the safety of consuming cinnamon of Indonesian origin.

The bioactivity of natural compounds can be determined by evaluating their free radical scavenging properties and toxicity [29]-[30]. Phenolic compounds act as free radical scavengers through electron transfer (ET) or hydrogen atom transfer (HAT) mechanisms [31]. The DPPH free radical method, which uses hydrogen atom transfer (HAT) and single electron transfer (SET), is considered a suitable, simple, rapid, accurate, and efficient method for evaluating the antioxidant value of compounds [29], [32]-[33]. Toxicity can be evaluated using the brine shrimp lethality test (BSLT) method, which is simple, fast and safe [34]-[35].

2. Materials and Methods

2.1. Chemicals

The chemicals used in the study were 2,2-diphenyl-1-picrylhydrazyl, gallic acid (≥ 98.5% purity), quercetin, and were supplied by Sigma-Aldrich Chemical Co. (St. Louis, USA). Methanol, Folin-Ciocalteu reagent, ascorbic acid, sodium carbonate, sodium nitrite, sodium hydroxide, aluminum chloride, and dimethyl sulfoxide were supplied by Merck (Darmstadt, Germany).

2.2. Sample Collection and Preparation

The study collected the stem barks of *Cinnamomum burmannii* from five different geographical regions in Indonesia, including Jambi, West Sumatra, East Java, South Kalimantan, and Maluku. The cinnamon samples were not obtained directly from farmers but were instead purchased through the Tokopedia marketplace with the requirement of grade A cinnamon to ensure sample quality. The samples were stored at room temperature to prevent mold and then dried in an oven until they were consistent in dryness. The dried cinnamon bark was then cut into pieces, ground into a fine powder, and passed through a 40-mesh sieve to obtain a fine powder with uniform particle size. The fine powder was stored in 300 g glass jars until used in the study.

2.3. Sample Extraction

The samples were extracted using maceration method. In brief, 10 g of cinnamon bark powder was mixed with a methanol solvent and stirred until homogeneous. The sample to solvent ratio was 1:10 (w/v) [36]. The mixture was then left for 24 hours and filtered through Whatman No. 1 filter paper. The filtrate was then evaporated using a rotary evaporator to obtain solvent free cinnamon extract and the yield was calculated.

2.4. DPPH-Scavenging Activity

The DPPH free radical scavenging activity was assessed following a modified protocol based on [37]. To begin, 50 µL of sample solutions at concentrations of 250, 200, 150, 100, and 50 µg/mL, as well as ascorbic acid solutions at concentrations of 50, 40, 30, 20, and 10 µg/mL, were added to 150 µL of DPPH (500 µg/mL) in each well of a 96-well microplate. Additionally, 200 µL of methanol was added to the wells as a blank solution. The negative control solution was a mixture of 50 µL of methanol p.a and 150 µL of DPPH 500 µg/mL. The samples were then incubated in a dark place protected from light for 30 minutes, and their absorbance was measured at a wavelength of 517 nm. The data was collected and tabulated using a microplate reader and the antioxidant activity was determined by calculating the amount of binding or inhibition of the sample and vitamin C standard solution to the free radicals (DPPH solution). The IC_{50} value was obtained by finding the intersection of the lines between inhibition and concentration percent and then using the equation y = mx + c, where y = 50 and the x value indicates the IC_{50}. The free radical scavenging activity was determined:

$$\text{DPPH Scavenging effect} = \frac{(Absorbance\ of\ control\ solution - Absorbance\ of\ extract\ solution)}{Absorbance\ of\ control\ solution} \times 100$$

2.5. Determination of Total Phenolic Content

The Folin-Ciocalteu method, as described by Baba and Malik [38], was used to determine the total phenolic content (TPC) of the extract, with minor modifications. Briefly, 200 µL of the extract solution (1000 µg/mL) or a standard solution of gallic acid (at concentrations of 20, 40, 60, 80, and 100 µg/mL) was mixed with 2800 µL of distilled water. The mixture was then combined with 0.5 mL of Folin-Ciocalteu reagent and 2 mL of Na2CO3 (20%) and then incubated at room temperature for 60 min. The absorbance was measured using UV-Vis spectrophotometer at 750 nm and a standard curve was created by plotting the absorbance values against the concentration of gallic acid. The TPC in the extract was calculated using the standard curve equation and was determined in three replicates with results expressed in mg GAE/g extract.

2.6. Determination of Total Flavonoid Contents

Total flavonoid contents were estimated using a colorimetric assay described by Baba and Malik [38] with slight modifications. The procedure involved mixing 1 mL of the extract solution (at a concentration of 1000 µg/mL) or standard quercetin (at concentrations of 5, 10, 15, 20, and 25 µg/mL) with 4000 µL of distilled water. The mixture was then combined with 30

µL of $NaNO_2$ (5%) and 30 µL of $AlCl_3$ (10%). After letting it sit for 6 minutes, 200 µL of 1 M NaOH (4%) and 10 mL of distilled water were added. The mixture was allowed to sit for 15 minutes before the absorbance was measured using UV-Vis spectrophotometer at 510 nm. A standard curve was created by plotting the absorbance values against the quercetin concentration and the TFC of the extract was determined using the standard curve equation. This TFC was determined in three replicates.

2.7. Preparation of *Artemia Salina* Leach Shrimp Larvae

The preparation of brine shrimp larvae involved incubating 0.5 g of brine shrimp eggs in 1 L of pre-filtered seawater in a brine shrimp incubator for 24 hours, with illumination from a room lamp and aeration of the solution.

2.8. Brine Shrimp Lethality Test (BSLT)

Briefly, the following steps were taken to test the toxicity of the extract on *A. salina* larvae: 500 µL, 250 µL, and 100 µL of the extract solution (5000 µg/mL) were added to a vial and dried using an oven at 40°C until all residual methanol solvent was gone.

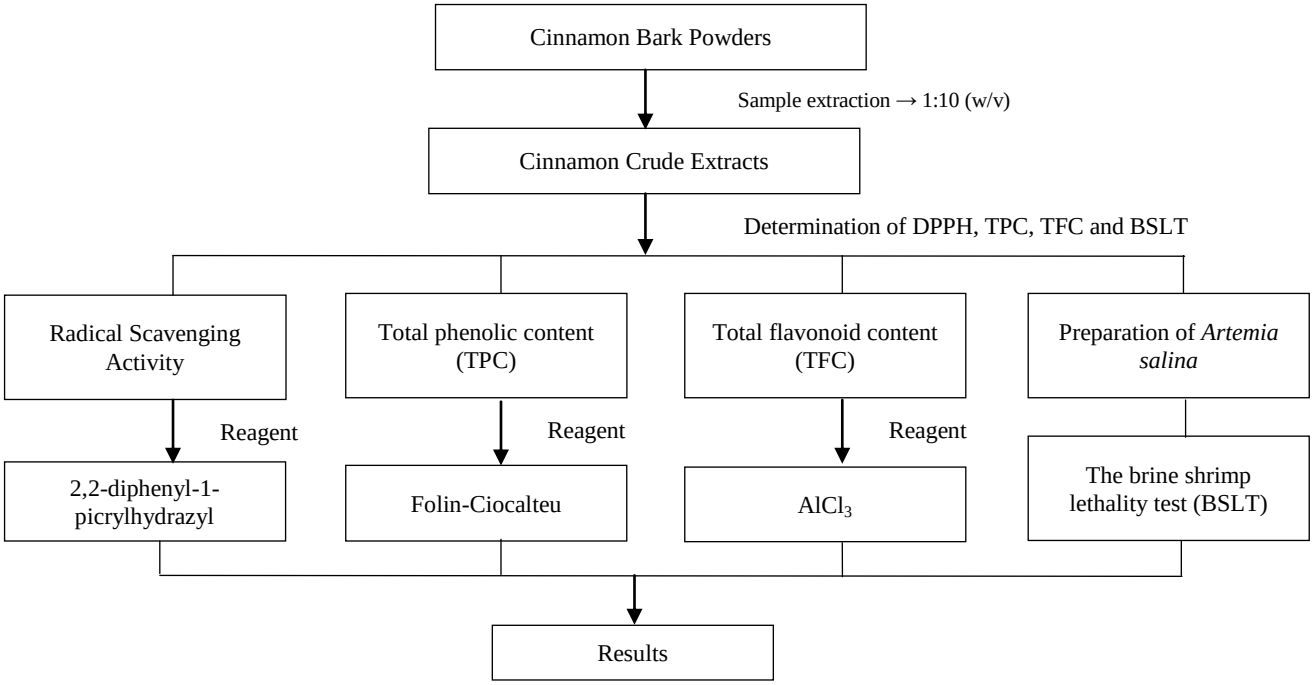


Fig. 1 Flowchart of the main steps in the research process (Developed by the authors)

A control with no extract was established by using vials that did not contain any extract (0 µg/mL). A 20 µL of DMSO was added to each vial and mixed in. After mixing, 3 mL of seawater was added to each vial and 20 *A. salina* larvae were pipetted into the vial. Additional seawater was added until the volume reached 5 mL so that the final concentrations in the solution were 500 µg/mL, 250 µg/mL, 100 µg/mL, and 0 µg/mL. After 24 hours, the live and dead larvae were observed and counted to determine the mortality rate at each concentration using the formula by Meyer et al. [39]:

$$\% \text{ of death larvae} = \frac{Number\ of\ death\ larvae}{Total\ number\ of\ initial\ larvae}$$

The extract toxicity was evaluated by observing the mortality of *A. salina* larvae after adding different concentrations of extract solution to seawater and incubating for 24 hours. The LC_{50} value, which indicates the concentration that causes 50% mortality, was calculated using the probit method with SPSS v.26 and was considered toxic if it was below 1000 µg/mL. The primary stages of the research process are outlined in Figure 1.

3. Results and Discussion

The extraction yield of *Cinnamomum burmannii* crude extracts from various locations is depicted in Figure 2-3, with the highest yield observed in South Kalimantan 3/SK-3 (44.30%), followed by Maluku 3/MU-3 (43.70%) and Maluku 2/MU-2 (34.51%) (Fig. 3). The color of all crude extracts was dark brown (Fig 2).

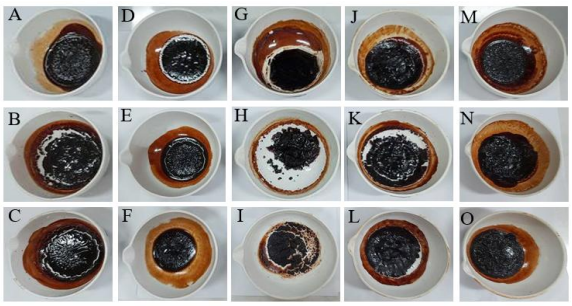


Fig. 2 (A–O) *C. burmannii* methanol extract: A - JB-1; B - JB-2; C - JB-3; D - WS-1; E - WS-2; F - WS-3; G - EJ-1; H - EJ-2; I - EJ-3; J - SK-1; K - SK-2; L - SK-3; M - MU-1; N - MU-2; O - MU-3 (Developed by the authors)

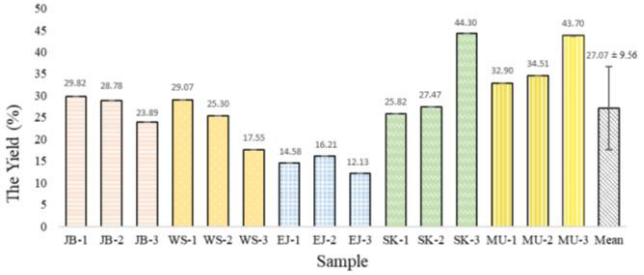


Fig. 3 The yield of cinnamon methanol extract (Developed by the authors)

The phenolic and flavonoid contents were measured as gallic acid equivalent (GAE) and quercetin equivalent (QE) respectively. The calibration curve for the absorbance of gallic acid at concentrations of 3.63, 2.91, 2.18, 1.45, and 0.73 µg/mL can be seen in Fig. 4a and the calibration curve for the absorbance of quercetin at concentrations of 3.28, 2.63, 1.97, 1.32, and 0.66 µg/mL can be seen in Fig. 4b. The total phenolic and flavonoid content of *Cinnamomum burmannii* extracts were calculated using the linear regression equation from the respective calibration curves and the absorbance values of the sample tests.

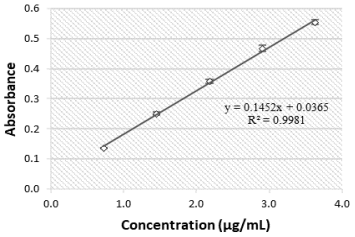


Fig. 4A Gallic acid standard curve (Developed by the authors)

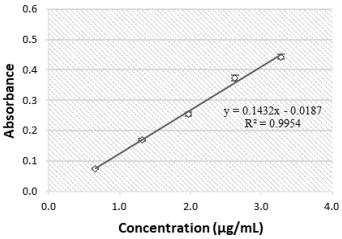


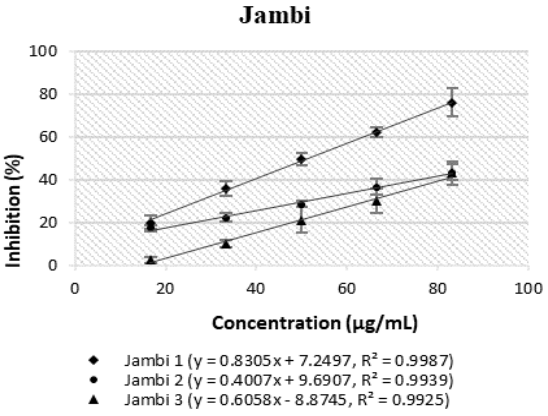
Fig. 4B Quercetin standard curve (Developed by the authors)

The results for the total phenolic and total flavonoid content of *Cinnamomum burmannii* extracts from different locations are presented in Table 1, with South Kalimantan 2/SK-2 having the highest total phenolic content (316.26 ± 20.94 mg GAE/g) and Maluku 1/MU-1 having the highest total flavonoid content (96.10 ± 7.97 mg QE/g). The lowest total phenolic and total flavonoid contents were measured in East Java 3/EJ-3 (82.42 ± 3.4 mg GAE/g and 18.08 ± 0.23 mg QE/g, respectively).

Table 1 Total phenol and flavonoid contents of cinnamon methanol extract (Developed by the authors)

Sample	**Total Phenolic content**		**Total Flavonoids**	
	Sample Absorbance	**mg GAE/gr**	**Sample Absorbance**	**mg QE/gr**
Jambi 1 (JB-1)	1.158 ± 0.080	212.41 ± 15.09	1.091 ± 0.127	58.88 ± 6.72
Jambi 2 (JB-2)	0.585 ± 0.020	103.82 ± 3.85	0.475 ± 0.009	26.20 ± 0.49
Jambi 3 (JB-3)	0.627 ± 0.047	111.90 ± 8.89	0.461 ± 0.020	25.48 ± 1.06
West Sumatra (WS-1)	0.661 ± 0.044	118.34 ± 8.42	0.489 ± 0.043	26.94 ± 2.26
West Sumatra 2 (WS-2)	0.838 ± 0.075	151.86 ± 14.22	0.590 ± 0.062	32.32 ± 3.28
West Sumatra 3 (WS-3)	1.523 ± 0.106	281.60 ± 19.99	1.163 ± 0.007	62.72 ± 0.37
East Java 1 (EJ-1)	0.698 ± 0.059	125.28 ± 11.24	0.480 ± 0.032	26.49 ± 1.69
East Java 2 (EJ-2)	0.794 ± 0.038	143.40 ± 7.16	0.576 ± 0.071	31.58 ± 3.75
East Java 3 (EJ-3)	0.472 ± 0.018	82.42 ± 3.4	0.322 ± 0.004	18.08 ± 0.23
South Kalimantan 1 (SK-1)	1.510 ± 0.058	279.14 ± 10.93	1.360 ± 0.091	73.15 ± 4.82
South Kalimantan 2 (SK-2)	1.706 ± 0.111	316.26 ± 20.94	1.427 ± 0.083	76.71 ± 4.4
South Kalimantan 3 (SK-3)	1.351 ± 0.114	249.02 ± 21.65	1.391 ± 0.087	74.83 ± 4.62
Maluku 1 (MU-1)	1.517 ± 0.129	280.40 ± 24.44	1.792 ± 0.150	96.10 ± 7.97
Maluku 2 (MU-2)	1.306 ± 0.141	240.37 ± 26.79	1.000 ± 0.036	54.05 ± 1.89
Maluku 3 (MU-3)	1.510 ± 0.113	279.07 ± 21.4	1.488 ± 0.132	79.95 ± 7.01

The results for the inhibition values and the concentration of DPPH scavenging activity are presented in Figure 5. The IC_{50} values which represent the effective concentration needed for each extract to capture 50% of DPPH radicals were used to determine the DPPH scavenging activity. Antioxidant activity was categorized as very strong (IC_{50} < 50 µg/mL), strong (IC_{50} 50-100 µg/mL), moderate (101-150 µg/mL), or weak (IC_{50} 151-200 µg/mL) based on the IC_{50} value, with a lower IC_{50} value indicating a greater antioxidant potential [40].



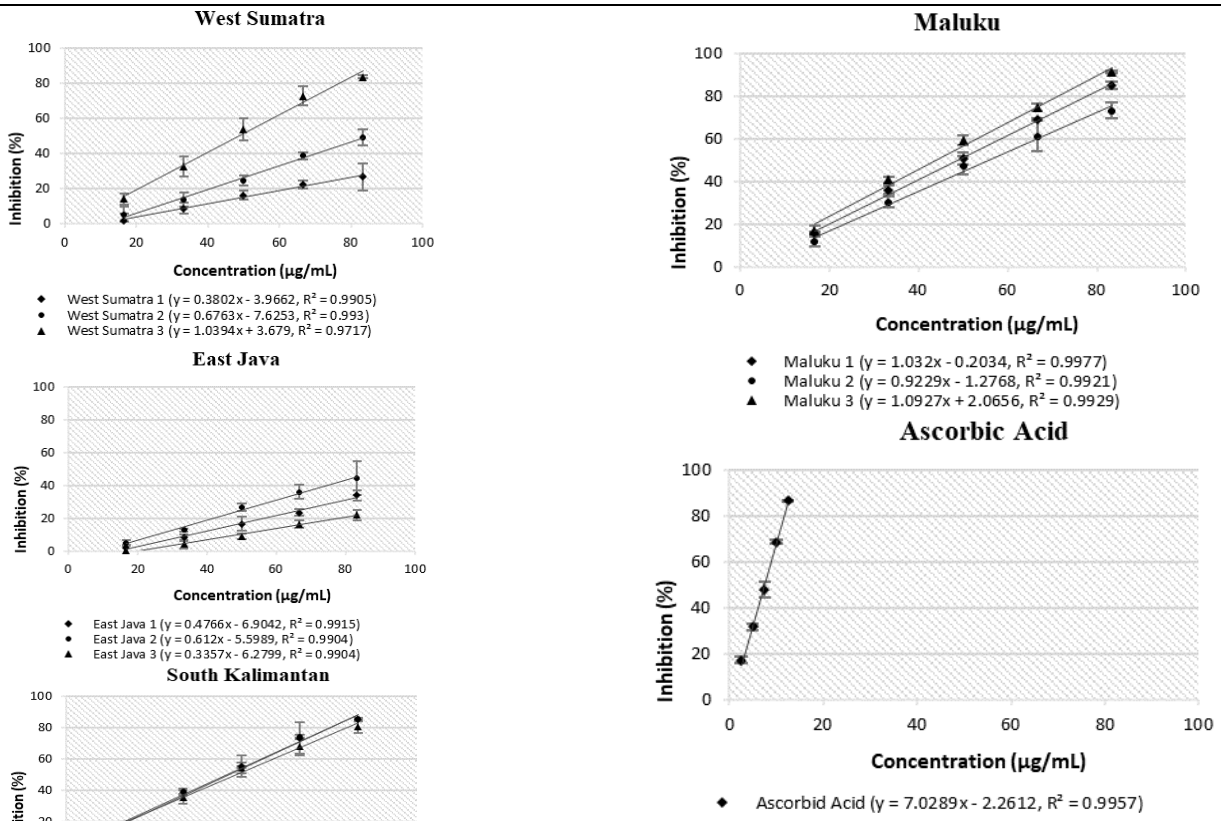


Fig. 5 The linear regression equation curve of cinnamon methanol extract (Developed by the authors)

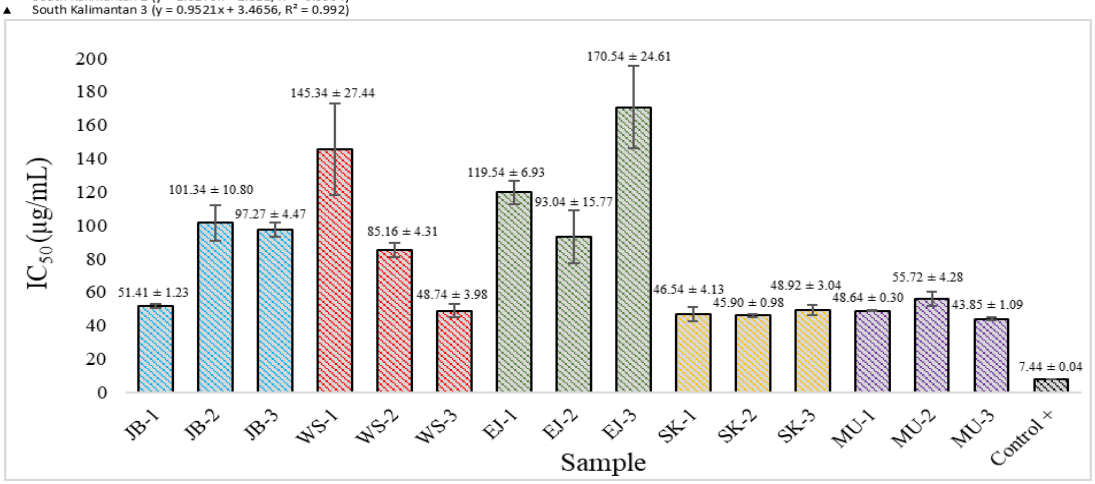


Fig. 6 IC_{50} value of cinnamon methanol extract (Developed by the authors)

Based on Fig. 6, the highest IC_{50} value, indicating the weakest scavenging activity, was found in East Java 3/EJ-3 (170.54 ± 24.61 µg/mL) with a classification of "Weak", while the lowest IC_{50} value, indicating the strongest scavenging activity, was found in Maluku 3/MU-3 (43.85 ± 1.09 µg/mL) with a classification of "Very Strong". The locations of South Kalimantan 1, 2, and 3 (SK 1-3) showed the most predominant scavenging activity with IC_{50} values of 46.54 ± 4.13 µg/mL, 45.90 ± 0.98 µg/mL, and 48.92 ± 3.04 µg/mL, respectively (Fig.6). These results confirm the in vitro scavenging activity of cinnamon extract, as reported by [41], who found an IC_{50} value of 53 µg/mL, classified as "Strong". The lowest scavenging activity was found in the East Java group, with IC_{50} values of 119.54 ± 6.93 µg/mL, 93.04 ± 15.77 µg/mL, and 170.54 ± 24.61 µg/mL, respectively.

Table 2 Spearman correlation coefficient of TPC, TFC and antioxidant activities (Developed by the authors)

	Spearman rho correlation		
	IC_{50}	**TPC**	**TFC**
IC_{50}		-0.918**	-0.929**
TPC	-0.918**		0.921**
TFC	-0.929**	0.921**	

Notes: DPPH - 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity; TFC - total flavonoid content; TPC - total phenolic content; ** Correlation is significant at 0.01 level (two-tailed).

Analysis showed significant correlations between

the antioxidant activity measured by IC_{50} values and the total phenolic content (TPC) as well as the total flavonoid content (TFC). The correlations were ρ = -0.918 for IC_{50} and TPC and ρ = -0.929 for IC_{50} and TFC. There was also a positive correlation between TPC and TFC with a value of ρ = 0.921 (Table 2). These findings suggest that phenolic and flavonoid compounds play a significant role in the antioxidant activity of *Cinnamomum burmannii*. These results agree with previous studies that have shown a correlation between DPPH scavenging activity and total phenolic and flavonoid content [42]-[43]-[44].

Table 3 LC_{50} value of cinnamon methanol extract (Developed by the authors)

Sample	% Death in 24 hours				LC_{50}
	0 µg/mL	100 µg/mL	250 µg/mL	500 µg/mL	
Jambi 1 (JB-1)	0	15.0	32.5	67.5	341.95 ± 32.24
Jambi 2 (JB-2)	0	12.5	27.5	60.0	412.77 ± 18.14
Jambi 3 (JB-3)	0	10.0	27.5	47.5	551.22 ± 80.49
West Sumatra (WS-1)	0	10.0	25.0	42.5	657.95 ± 27.84
West Sumatra 2 (WS-2)	0	12.5	27.5	55.0	460.7 ± 28.49
West Sumatra 3 (WS-3)	0	2.5	15.0	32.5	765.49 ± 48.57
East Java 1 (EJ-1)	0	0	7.5	25.0	826.10 ± 57.79
East Java 2 (EJ-2)	0	7.5	20.0	45.0	611.48 ± 80.41
East Java 3 (EJ-3)	0	0	12.5	30.0	739.65 ± 12.04
South Kalimantan 1 (SK-1)	0	10.0	27.5	52.5	479.41 ± 21.07
South Kalimantan 2 (SK-2)	0	12.5	27.5	40.0	758.92 ± 32.12
South Kalimantan 3 (SK-3)	0	12.5	27.5	47.5	573.67 ± 70.81
Maluku 1 (MU-1)	0	0	5.0	25.0	785.24 ± 0
Maluku 2 (MU-2)	0	7.5	22.5	40.0	690.27 ± 8.99
Maluku 3 (MU-3)	0	15.0	32.5	62.5	371.6 ± 9.68

The number and concentration of active secondary metabolites in the cinnamon extract, particularly flavonoids and phenolic compounds, allegedly influence its toxicity, as indicated by the varying LC_{50} values of extracts obtained from different locations (Table 3) [45]-[46]. The samples from East Java were found to be the least toxic (LC_{50} values ranging from 611.48-826.10 µg/mL), while samples from Jambi were the most toxic (LC_{50} values ranging from 341.95-551.22 µg/mL). While all sample extracts were classified as toxic (with LC_{50} < 1000 µg/ml) [39], [47] provided a more detailed classification, defining extracts with LC_{50} values of 100-500 µg/ml as medium toxic and those with LC_{50} values of 500-1000 µg/ml as low toxic.

Coumarin, a compound found in cinnamon's phenolic content, has been linked to toxicity in *A. salina* Leach and is known to have cytotoxic activities [48]. *Cinnamomum burmannii*, along with other species referred to as "false cinnamons", including C. cassia, C. *aromaticus*, and C. *loureiroi*, are known to have higher coumarin content compared to "true cinnamons", *C. verum*/*C. zeylanicum*, which are considered safer for consumption [14]. As reported in [20], coumarin content levels in *C. cassia* are high (up to 916.71 mg/kg), while [26] and [49] reported significantly higher coumarin content levels in *C. burmannii* (up to 3357 mg/kg and 16,980 mg/kg, respectively). However, it should be noted that *C. burmannii* from Bogor, West Java, has been found to have only slightly higher coumarin content than *C. zeylanicum*, with levels of 30 mg/kg and 17 mg/kg, respectively [49]. Furthermore, all samples of *C. cassia* from Kerala, India were found to have coumarin content levels below 100 mg/kg [26].

Previous studies have shown that there is a positive correlation between the LC_{50} of the BSLT test and the LD_{50} of the acute oral toxicity test in mice (r = 0.85; P < 0.05). According to Parra et al. [49], an LC_{50} brine shrimp < 10 µg/mL corresponds to an LD_{50} ranging from 100 to 1000 mg/kg; LC_{50} < 20 µg/mL corresponds to an LD_{50} ranging from 1000 to 2500 mg/kg, and LC_{50} > 25 µg/mL corresponds to an LD_{50} ranging from 2500-8000 mg/kg. Based on this correlation, we can predict that the acute oral toxicity of cinnamon methanol extract will be more than 2500 mg/kg since the LC_{50} of BSLT test in our study was 341.95 ± 32.24 - 826.10 ± 57.79 µg/mL. The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) and the United States Environmental Protection Agency (US-EPA) classify acute oral systemic toxicity as nontoxic if LD_{50} > 2000 mg/kg and "very toxic" if LD_{50} <50 mg/kg [51]-[52]. Therefore, if the LD_{50} of acute oral toxicity for cinnamon methanol extract is more than 2500 mg/kg, *C. burmannii*, especially from the five provinces of Indonesia in the present study, can be considered relatively safe for consumption within reasonable limits and with further detailed study. However, given the potential toxicity associated with varying levels of coumarin content in cinnamon, particularly from Indonesia, the results of this study are significant in highlighting the need for further research to address any doubts about the safety of Indonesian cinnamon products. Additionally, there are concerns about the adulteration of true cinnamon with cinnamon from Indonesia, making it all the more important to comprehensively evaluate the safety of cinnamon for human consumption.

4. Conclusion

In conclusion, the study demonstrates the presence

of active secondary metabolites in *Cinnamomum burmannii* extracts including phenolic and flavonoid compounds that contribute to its antioxidant and toxicity properties. The location of cinnamon plants plays a significant role in determining the level of these compounds and overall activity of the extract. The results suggest that the methanol extract of *C. burmannii*, especially from five provinces in Indonesia, exhibits varying degrees of antioxidant activity, ranging from very strong to weak, as determined by the DPPH radical scavenging activity test (43.85 ± 1.09—170.54 ± 24.61 µg/mL). Additionally, the extract demonstrated medium and low toxicity on the brine shrimp lethality test (341.95 ± 32.24—826.10 ± 57.79 µg/mL). However, given the varying levels of coumarin content and potential toxicity associated with cinnamon use, particularly from Indonesia, it is crucial to conduct further research to comprehensively evaluate the safety of cinnamon for human consumption. Furthermore, the concern over the adulteration of true cinnamon with cinnamon from Indonesia emphasizes the significance of conducting more detailed studies to address any doubts about the safety of Indonesian cinnamon products. In summary, this research enhances our understanding of *Cinnamomum burmannii* extracts and their health benefits, providing a foundation for future studies to explore their potential applications along with ensuring their safety.

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