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Phytochemical Screening, Antioxidant, and Antibacterial Activities from Leaf and Stem Extracts of *Strobilanthes crispus* (L.) Collected in Sarawak and Melaka

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ABSTRACT

Strobilanthes crispus (L.), commonly known as Pokok Pecah Beling, is a medicinal plant from Southeast Asia used for treating illnesses like diabetes, cancer, and hypertension. The study aimed to screen the phytochemicals and determine the antioxidant and antibacterial activities of the leaf and stem extracts from Sarawak and Melaka. The maceration method was used to prepare extracts of hexane, ethyl acetate, and methanol. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, terpenoids, and phenols in methanol extracts. The Sarawak stem methanol extract exhibited the most potent antioxidant activity (94.14% DPPH scavenging, IC_{50} = 115.13 ppm). However, none of the extracts showed antibacterial activity against the tested bacteria. These findings highlight *S. crispus* (L.), particularly the Sarawak stem methanol extract, as a potential source of natural antioxidants.

INTRODUCTION

The term "natural products" (NPs) typically refers to chemicals generated by living organisms through metabolic processes, often arising as by-products of secondary or non-essential metabolism [1]. Secondary metabolites are complex organic compounds synthesised by living organisms, such as plants, animals, and microorganisms and are reported to exhibit diverse biological activities. On the other hand, secondary metabolites are non-essential compounds that are not directly involved in the primary metabolic pathways [2]. Significant research efforts have focused on investigating the beneficial active compounds found in medicinal plants. Although many medicinal plants have been discovered, only a few have been thoroughly studied, and many remain unexplored.

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Strobilanthes crispus (L.) is a bush-like plant that grows on riverbanks or vacant fields in tropical nations like Madagascar and Indonesia [3]. This perennial herb, a member of the Acanthaceae family, is known as "daun pecah beling" in Jakarta, "enyoh kilo," "kecibeling" or "kejibeling" in Java, and "Hei Mian Jiang Jun" in Chinese [4]. According to Arbianti et al. [5], *S. crispus* (L.) is a well-known folk medicine in Malaysia and Indonesia. For example, the leaves and stems of this plant can be used to make a nutritious beverage or cooked to treat various illnesses, including promoting wound healing and treating snakebites [6]. Pharmacologically, *S. crispus* (L.) leaf and stem are harnessed for their antioxidant, antibacterial, and wound-healing properties [7].

Phytochemicals, vital constituents found in medicinal flora, are non-essential for normal human physiological functions but exhibit bioactivity, contributing to health enhancement and disease mitigation. Recent findings by Sukendi et al. [8] provide an updated overview of *S. crispus* (L.). This plant was reported to contain various secondary metabolites, including flavonoids (kaempferol, luteolin, rutin), phenolic acids (caffeic, ferulic), and terpenoids (β-sitosterol, stigmasterol). It has also stated stigmasterol and kaempferol as potential chemical markers frequently detected in this genus, offering consistency across species. *S. crispus* (L.) has also been proven scientifically to exert antimicrobial properties and wound-healing abilities since the extracts have shown inhibition towards opportunistic Gram-negative *P. aeruginosa* [4]. The leaves and stems of the native *S. crispus* (L.) plant require more studies, despite their numerous potential applications.

EXPERIMENTAL

Plant Sampling

Fresh samples (leaf and stem) of *S. crispus* (L.) were collected in two different locations: Kampung Uma Juman, Belaga, Bintulu, Sarawak (East), and Duyong, Melaka (South). The leaf and stem samples were deposited at Universiti Kebangsaan Malaysia (UKM) for identification. The botanist (Dr. Shamsul bin Khamis) from the Universiti Kebangsaan Malaysia's Herbarium (UKMB) successfully verified them with the specimen voucher ID095/2023.

Preparation of Extracts

The samples were cleaned with tap water and air-dried under direct sunlight for five days. The dried samples were ground into a fine powder using a mixer grinder. Extraction was prepared by the maceration method, and powdered samples (100 g) were soaked in hexane (1 L) (Merck, Germany), ethyl acetate (1 L) (Merck, Germany), and methanol (1 L) (Merck, Germany) with constant stirring using a shaker [9]. The extraction process took three days and was repeated twice for each solvent to get more crude extracts for analysis. Then, the extracts were filtered using vacuum filtration and concentrated using a rotary evaporator (IKA, Germany), leaving the crude extracts.

Phytochemical Screening

The extracts were tested to identify the presence of alkaloids, flavonoids, tannins, terpenoids, and phenols.

Test for Alkaloids (Wagner's Test)

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The ethanolic extract (2 mL) was treated with three drops of Wagner's reagent, a mixture of potassium iodide (Sigma-Aldrich, USA), iodine (Sigma-Aldrich, USA), and distilled water. The formation of a reddish-brown precipitate indicates the presence of alkaloids [10, 11].

Test for flavonoids (Ferric Chloride test)

The ethanolic extract (2 mL) was added to three drops of ferric chloride (Sigma-Aldrich, USA) solution. The solution's intense green colour showed the presence of flavonoids [10, 12].

Test for tannins (Ferric chloride test)

A small amount of crude extract (2.5 mg) was boiled in distilled water (5 mL), followed by filtration. Then, the filtrate (2 mL) was treated with three drops of ferric chloride solution (5% w/v). The appearance of brownish-green, blue-black, black, blue, green, or blue-green colouration or precipitate indicated the presence of tannins [13, 14, 15].

Test for terpenoids (Salkowski test)

Each crude extract (10 mg) was dissolved in chloroform (2 mL) (Merck, Germany), followed by the addition of concentrated sulphuric acid (3 mL) along the side of the test tube. The emergence of a reddish-brown layer at the interface showed a positive result for terpenoids [16, 17].

Test for phenols (Ferric chloride)

About 10 mg of crude extract was dissolved separately in ethanol (1 mL) (Sigma-Aldrich, USA). Then, the ethanolic extract (2 mL) was further mixed with distilled water (1 mL), followed by the addition of 1-2 drops of ferric chloride solution (5% w/v). The appearance of blue, green, red, bluish-black, red, or purple colour shows the presence of phenols [10, 18, 11].

Antioxidant assay

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA) radical scavenging assay as described by Tordzagla et al. [10]. To prepare the 1000 µg/mL sample solution, 1 mg of the sample was dissolved in 1 mL of MeOH. Further dilutions of the stock solution resulted in a final concentration of 500, 250, 125, 62.5, 31.3, 15.63, and 7.81 µg/mL. DPPH solution with a concentration of 50 µM was freshly prepared by dissolving 1.9716 mg of DPPH powder in 100 mL of MeOH. Then, 3.8 mL of DPPH solution was added to 0.2 mL of the sample solution of different concentrations. The reaction mixtures were then incubated in the dark at room temperature for 30 minutes. Subsequently, the absorbance was measured at 517 nm using a UV-vis spectrophotometer (Agilent, USA). Based on the absorbance value obtained, percentage inhibition (%) was calculated using the following formula:

$$\text{Percentage inhibition (\%)} = \frac{\text{ADPPH}_{\text{Blank}} - (\text{A}_{\text{Sample}} - \text{A}_{\text{Blank sample}})}{\text{ADPPH}_{\text{Blank}}} \times 100 \quad (1)$$

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The A_{Sample} was the absorbance of the sample solution with DPPH reagent, while the $A_{Blank\ sample}$ was the absorbance of the sample solution with methanol. $ADPPH_{Blank}$ was the absorbance of the DPPH reagent and methanol. The IC_{50} value test was conducted using a sample with a percentage inhibition exceeding 50% during the screening process. GraphPad Prism 5 was used to calculate the IC_{50} value, which is defined as the concentration of each sample necessary to scavenge 50% of the DPPH radical. The results of this assay were presented as means ± standard deviation. The Independent t-test was used to compare the samples with the positive control using SPSS for Windows (version 22) software. A difference of $p<0.05$ was deemed statistically significant.

Antibacterial assay

The bacterial strains were used from the American Type Culture Collection (ATCC). Two Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus aureus* (ATCC 43300) (MRSA) and four Gram-negative bacteria: *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 25922), *Enterobacter aerogenes* (ATCC 13048), and *Shigella flexneri* (ATCC 12022) were used to determine the antibacterial activity of *S. crispus* (L.). Antibacterial screening was performed for selected bacteria using a modified Kirby-Bauer disc diffusion method [19]. Ciprofloxacin (5 µg/disc) (Sigma-Aldrich, USA) was used as the positive control, while 5% dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA) was used as the negative control.

Each bacterium was initially cultured in Mueller-Hinton Broth (MHB) (Merck, Germany) and incubated overnight in a rotary shaker at 170 rpm and 37°C for 18 hours. The sample was prepared by weighing 100 mg of each crude extract into a 1.5 mL microcentrifuge tube. A 200 mg/mL stock concentration was prepared by adding 0.5 mL of sterile 100% DMSO to each sample. A 10 mg/mL working concentration was prepared from the stock solution using the formula $M_1V_1 = M_2V_2$. This working solution contained 5% DMSO. Subsequently, 1 mL of 5% DMSO was prepared in sterile MHB using the same $M_1V_1 = M_2V_2$ formula. Following that, the turbidity of the cultures was adjusted to an OD_{625} nm of 0.08-0.1, equivalent to the 0.5 McFarland standard (10^8cells/ mL). A sterile cotton swab was used to spread the adjusted culture onto a Petri dish containing Mueller-Hinton Agar (MHA) (Merck, Germany). Next, sterile paper discs impregnated with 0.03 mL of each crude extract (10 mg/mL), sterile paper discs impregnated with 0.03 mL of 5% DMSO and ciprofloxacin (5 µg/disc) were placed onto the media, and the plates were incubated at 37°C for 24 hours. The zone of inhibition was measured and recorded. The screening was conducted in triplicate.

RESULTS AND DISCUSSION

The polarity of a solvent and its ability to extract various active compounds, both polar and non-polar, are vital factors [20]. Solvent selection is typically guided by polarity, with methanol being more polar than ethyl acetate, and ethyl acetate more polar than hexane. As shown in Table 1, the leaves and stems of *S. crispus* (L.) were collected from two locations, and the extracted yields were obtained using three solvents of differing polarities.

The crude leaf and stem extracts from Sarawak showed the highest extraction yield with methanol (5.789%), followed by ethyl acetate (2.033%) and hexane (1.315%). Similarly, methanol also produced the highest yield from another sample at 7.207%, compared to ethyl acetate (0.547%) and hexane (0.398%). In extracts from Melaka, the methanol leaf extract again exhibited the highest yield at 10.220%, followed by ethyl acetate (1.790%) and hexane (1.206%). For stem extracts, methanol yielded the highest percentage (0.587%), followed by ethyl acetate

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(0.563%) and hexane (0.202%). Although the methanol stem extract from Melaka had a slightly lower yield compared to that from Sarawak, it still exhibited the highest yield among the three solvents (methanol, ethyl acetate, and hexane). These results suggest that extraction efficiency is greater with highly polar solvents like methanol.

Table 1: The percentage yield of crude extracts of *S. crispus* (L.) leaf and stem.

Solvent	Sampling location			
	Sarawak		Melaka	
	Percentage yield (%)			
	Leaf	Stem	Leaf	Stem
Hexane	1.315	0.398	1.206	0.202
Ethyl acetate	2.033	0.547	1.790	0.563
Methanol	5.789	7.207	10.220	0.587

This outcome aligns with the findings of Huang et al. [21], who reported that polar compounds are generally easier to extract than nonpolar ones. Yield differences between Sarawak and Melaka may also be attributed to factors highlighted by Ara et al. [22], who found that origin and post-harvest handling significantly influence extract yields due to compound stability and environmental fluctuations. Furthermore, harvesting time and plant maturity play a crucial role, as younger plants collected at the optimal growth stage tend to produce higher yields [23]. These factors suggest that ecological and seasonal variations likely contributed to the higher methanol leaf yield observed in Melaka, while Sarawak demonstrated superior extraction performance in stem samples.

Phytochemical screening of *S. crispus* (L.)

The extraction of compounds from leaf and stem using various solvents underwent comprehensive phytochemical screening to identify alkaloids, flavonoids, tannins, terpenoids, and phenols. The results of phytochemical screening of leaf and stem crude extracts were tabulated as shown in Table 2.

Table 2: Phytochemical screening of *S. crispus* (L.) leaf and stem extracts.

Sampling location												
Solvent	Sarawak						Melaka					
	Hexane		Ethyl acetate		Methanol		Hexane		Ethyl acetate		Methanol	
	L	S	L	S	L	S	L	S	L	S	L	S
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	-	-	+	+	+	+	-	-	-	-	+	+
Tannins	-	-	-	-	+	+	-	-	-	-	+	+
Terpenoids	+	+	-	+	+	+	-	-	-	-	+	+

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Phenols	-	-	+	-	+	+	-	-	-	+	+	+

L: Leaf, S: Stem, '+': present, '-': absent

The findings indicate that a polar solvent exhibits suitable extraction properties for extracting a wider range of phytochemical groups. This statement reflects the results in Table 2, in which all tested phytochemical groups were detected in both extracts for the locations. In contrast, leaf extract from Sarawak for ethyl acetate exhibited a substantial concentration of alkaloids, flavonoids, and phenols, while the hexane crude extract showed the presence of terpenoids and alkaloids. The ethyl acetate of stems in the exact location was positive in Wagner's, ferric chloride, and Salkowski tests, indicating the presence of alkaloids, flavonoids, and terpenoids. Additionally, alkaloids and terpenoids were detected in the hexane extract. Several variables have influenced these differences in phytochemical presence in the extracts. The employed extraction process influences the recovery of certain chemicals. A study by Azzahra et al. [24] has demonstrated that ultrasonic-assisted extraction produced greater terpenoids and flavonoids than conventional maceration. Likewise, the drying technique before extraction, such as oven-drying versus freeze-drying, affects the stability and availability of compounds. Another study by Tourabi et al. [25] mentioned that moderate hot-air drying enhanced phenolic compound recovery in plant tissues.

Furthermore, the leaf extract from Melaka displayed the presence of alkaloids only in both ethyl acetate and hexane extracts. Meanwhile, the stem extracts detected only alkaloids in hexane, while ethyl acetate contained alkaloids and phenols. For qualitative phytochemical analysis, it can be concluded that a polar solvent such as methanol has a higher affinity for polar compounds. Hence, methanol's ability to dissolve a wide range of polar phytochemicals contributes to the extraction of more compounds than hexane and ethyl acetate, which are more specialised in extracting non-polar compounds [26].

Antioxidant assay

The free radical scavenging capabilities of all crude extracts from *S. crispus* (L.) were quantitatively assessed using the DPPH assay, and their IC_{50} values were determined. The IC_{50} values represent the specific concentration of the test extracts that inhibits activity by 50%. Table 3 presents data on the DPPH radical scavenging activity of different extracts of *S. crispus* (L.) leaf and stem, along with the standard antioxidant, ascorbic acid.

The data indicate that ethyl acetate and methanol extracts of *S. crispus* (L.) leaves from Sarawak contain bioactive compounds with notable radical scavenging activity, recording percentage inhibitions of 94.65% and 87.08%, respectively. The methanol leaf extract showed a moderate DPPH radical scavenging potential with an IC_{50} value of 126.50 ppm. In contrast, the ethyl acetate extract had the highest IC_{50} value (217.97 ppm), indicating lower antioxidant activity. Ascorbic acid, used as a positive control, exhibited the most potent antioxidant activity with the lowest IC_{50} value of 57.73 ppm. For the stem extracts from Sarawak, only the methanol crude extract of *S. crispus* (L.) showed more than 50% inhibition at 1000 ppm. As a result, its IC_{50} value was further determined, revealing a per cent inhibition of 94.18% and an IC_{50} of 115.13 ppm, confirming its moderate radical scavenging potential.

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Table 3: DPPH radical scavenging activity of *S. crispus* (L.) leaf and stem extracts.

Crude extracts	Sampling location				
	Sarawak			Melaka	
	Solvent	Inhibition at 1000 ppm (%)	IC_{50} ppm	Solvent	Inhibition at 1000 ppm (%)
Leaf	Hexane	10.23±1.99	ND	Hexane	11.59±0.79
	Ethyl acetate	94.65± 0.57	217.97 ± 11.89	Ethyl acetate	38.55±0.47
	Methanol	87.08 ± 0.47	126.50 ± 13.34	Methanol	47.31±0.72
	Ascorbic acid	89.62 ± 0.17	57.73 ± 1.01	Ascorbic acid	98.56±0.13
Stem	Hexane	ND	ND	Hexane	26.5 ± 11.03
	Ethyl acetate	ND	ND	Ethyl acetate	14.43 ± 0.88
	Methanol	94.18 ± 0.13	115.13 ±12.15	Methanol	36.3 ± 0.28
	Ascorbic acid	89.62 ± 0.17	57.73 ± 1.01	Ascorbic acid	96.4 ± 0.10

ND: Not determined

Meanwhile, the crude extract samples (both leaves and stems) from all solvents of Melaka exhibited no antioxidant properties, as the percentage of inhibition was below 50%, except for the control (ascorbic acid), which showed higher inhibition with 98.56% for the leaves and 96.40% for the stems, respectively. From the visual observation, the intensity colour of all extracts in the sample solution did not show significant changes after mixing with the DPPH solution. The enhanced antioxidant activity observed in the methanol extract may be attributed to the presence of flavonoids and phenolic compounds, which are known for their ability to donate hydrogen atoms [27]. This observation supports the presence of phenols, alkaloids, terpenoids, tannins, and flavonoids during phytochemical screening.

The antioxidant activity is expected to increase with a rise in hydroxyl group content and a decrease in the presence of glycoside groups. In addition to these structural features, recent studies have identified other influential factors that may affect antioxidant performance in DPPH assays. The type of solvent used in the assay can shift the dominant reaction mechanism. The study from Diment et al. [28] found that protic solvents like methanol tend to favour electron transfer, enhancing the activity of phenolic compounds, while aprotic solvents are more prone to hydrogen transfer. The hydroxyl group in the extract of *S. crispus* (L.) demonstrates potent antioxidant activity by effectively donating hydrogen atoms to free radicals.

Antibacterial Assay

The disc diffusion assay was used to evaluate the antibacterial activity of crude leaf and stem extracts of *S. crispus* (L.) against both Gram-positive and Gram-negative bacteria. Inhibition was assessed by measuring the diameter of the clear zones around the discs using a ruler after a 24-hour incubation period. Antibacterial efficacy was determined based on the presence and size of these inhibition zones. As shown in Table 4.3, none of the crude extracts contained potential antibacterial compounds since no observable inhibition zones were observed against any of the six tested bacterial strains.

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Table 4: The inhibition zone of each *S. crispus* (L.) leaf and stem crude extracts against bacteria.

	Sampling location																			
Bacteria	**Sarawak**										**Melaka**									
	H		**EA**		**M**		**C**		**DMSO**		**H**		**EA**		**M**		**C**		**DMSO**	
	L	**S**	**L**	**S**	**L**	**S**	**L**	**S**	**L**	**S**	**L**	**S**	**L**	**S**	**L**	**S**	**L**	**S**	**L**	**S**
S. aureus	-	-	-	-	-	-	25.0 ± 0.00	27.0 ± 0.10	-	-	-	-	-	-	-	-	26.0 ± 0.05	30.0 ± 1.00	-	-
S. aureus (MRSA)	-	-	-	-	-	-	30.3 ± 0.06	31.3 ± 0.06	-	-	-	-	-	-	-	-	35.30 ± 0.60	31.0 ± 0.10	-	-
E. coli	-	-	-	-	-	-	22.3 ± 0.06	27.3 ± 0.12	-	-	-	-	-	-	-	-	29.30 ± 0.60	26.0 ± 0.00	-	-
S. typhimurium	-	-	-	-	-	-	22.3 ± 0.06	27.3 ± 0.06	-	-	-	-	-	-	-	-	28.30 ± 0.60	26.0 ± 0.12	-	-
S. flexneri	-	-	-	-	-	-	34.3 ± 0.12	30.7 ± 0.15	-	-	-	-	-	-	-	-	35.70 ± 1.20	29.0 ± 0.25	-	-
E. aerogenes	-	-	-	-	-	-	21.0 ± 0.10	25.0 ± 0.00	-	-	-	-	-	-	-	-	25.70 ± 1.20	22.0 ± 0.10	-	-

Values were performed in triplicate and represented as mean±SD. HE: Hexane, EA: Ethyl acetate, ME: Methanol, CI: Ciprofloxacin (positive control), DMSO (negative control), -: no inhibition zone, L: Leaf, S: Stem.

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However, positive and negative controls for all the samples worked well. Positive controls for the three crudes had an inhibition zone in this antibacterial assay, and negative controls did not elicit any effect on the bacteria.

Several factors may contribute to the absence of inhibition zones observed in the antibacterial assay of *S. crispus* (L.) leaf and stem crude extracts prepared with various solvents. One possible explanation is the use of the maceration extraction method and crude extracts, which may limit the concentration or availability of active antibacterial compounds. Additionally, abiotic factors can influence the quality of the extract and, consequently, antibacterial activity [29]. These include environmental conditions such as climate, soil composition, and altitude, all of which can affect the plant's growth, maturation, and the chemical profile of its leaves and stems. This may explain the variation in antibacterial activity between samples collected from different locations, Sarawak and Melaka.

CONCLUSION

In conclusion, the extracts of *S. crispus* (L.) demonstrated that methanol was the most effective solvent for extracting bioactive compounds from the leaf and stem across two Malaysian locations. Methanol extracts yielded the highest percentage and constantly contained alkaloids, flavonoids, tannins, terpenoids, and phenols. Among the tested extracts, only those obtained using methanol demonstrated antioxidant activity, with the stem extract showing the highest DPPH radical scavenging effect (94.18% inhibition, IC_{50} = 115.13 ppm). None of the crude extracts exhibited antibacterial activity.

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AUTHOR'S CONTRIBUTIONS

Cassy Lugak and Dayang Nurliyana Abang Rosli conducted experiments on a sample collected from Sarawak. At the same time, Muhammad Amirul Asyraf Azlan and Aina Nadia Sapar performed experiments on a sample from Melaka. Suhaidi Ariffin supervised the research and revised the article written by Cassy Lugak.

CONFLICT OF INTEREST STATEMENT

The authors confirm that they have no conflicts of interest related to the publication of this manuscript.

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