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A Phytochemical Profiling and *in vitro* Antimicrobial Evaluation of Methanolic Extract and Fractions of *Dicranopteris linearis* Leaves

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Abstract

Introduction: *Dicranopteris linearis*, locally known as resam, has been recognized for its potential health benefits, primarily due to its rich phytochemical content. Traditionally used for medicinal purposes, the leaves are known to possess antioxidant and antimicrobial properties. This study aimed to screen the phytochemical composition and evaluate the antimicrobial activity of the methanolic extract of *D. linearis* leaves, with potential applications in the medicinal industry. **Materials and Methods:** The dried and ground leaves of *D. linearis* were macerated in 100% methanol to extract the phytochemicals. The extract was subjected to qualitative phytochemical profiling. Total Phenolic Content (TPC) was determined by the Folin-Ciocalteu method and Total Flavonoid Content (TFC) was measured using the $AlCl_3$ method. The methanol extract was fractionated by Vacuum Liquid Chromatography (VLC) with ethyl acetate (100%), ethyl acetate: methanol (5:5) and methanol (100%). Antimicrobial activity of the crude extract and fractions was assessed against *Escherichia coli* and *Staphylococcus aureus* using the disc diffusion assay and broth microdilution techniques. **Results:** Phytochemical profiling of the methanol extract revealed the presence of phenolic compounds, flavonoids, tannins, and saponins. The TPC and TFC assays showed that the methanolic extract contained 225.43 ± 4.16 mg GAE/g of phenolic compounds and 50.20 ± 4.78 mg QE/g of flavonoids. Fractionation of methanol extract was afforded three fractions, F1, F2 and F3. Antimicrobial testing demonstrated that the extract exhibited stronger activity against *S. aureus* (MIC = 1.563 mg/mL) compared to *E. coli* (MIC > 50 mg/mL). For the fraction, F1 exhibited both microbes with promising activity. **Conclusions:** The methanolic extract from *D. linearis* leaves contain bioactive phytochemicals with significant antioxidant and antimicrobial properties. These findings suggest that *D. linearis* may serve as a valuable source for the development of natural antimicrobial agents in the pharmaceutical industry

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Introduction

Dicranopteris linearis, commonly known as resam, is abundant in Southeast Asia and has been used traditionally for its medicinal benefits. The plant contains various bioactive compounds, including terpenoids, tannins, saponins, flavonoids, alkaloids, steroids, phenols, and glycosides (Rajesh et al., 2016). Previous studies have demonstrated that *D. linearis* leaves, rich in these phytochemicals, offer various potential applications, including antioxidant, anti-inflammatory, and hepatoprotective properties (Kamisan et al., 2014). One particularly promising application is its antimicrobial activity.

Wound pathogens are critically important in antimicrobial studies because they are key contributors to infection, delayed healing, and complications in clinical settings. They can be caused by bacteria such as *Staphylococcus aureus* (including MRSA), *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella* spp. Studying them helps in the development, testing, and evaluation of antimicrobial agents, especially in the context of antibiotic resistance and wound management. Studying wound pathogens also helps in testing new antimicrobials or alternative therapies e.g. herbal extracts and phage therapy. With the increasing prevalence of antibiotic-resistant pathogens, the need for natural, safe antimicrobial agents is urgent. Lai et al. (2021) investigated the antimicrobial properties of *D. linearis* methanolic and acetonic extracts, finding activity against pathogens like *M. luteus*, *E. coli*, *P. aeruginosa*, and *S. aureus*. However, these studies used multiple organic solvents and a broad range of bacterial strains.

Our study aims to provide more focused results on the phytochemical composition and antimicrobial activity of *D. linearis* leaves. (**Fig. 1**)., particularly against common wound pathogens. By narrowing our investigation to clinically relevant bacteria which are *S. aureus* and *E. coli*, we intend to evaluate the specific potential of *D. linearis* as a natural antimicrobial agent for wound management. In contrast to previous broad-spectrum studies, we will utilize a single extraction method to ensure consistency and better isolate the correlation between the extract's phytochemical content and its antibacterial efficacy. This targeted approach aims to contribute valuable insights toward the development of plant-based alternatives in combating wound infections, especially in light of increasing antibiotic resistance

Fig. 1: *D. linearis* leaves (picture taken on 18 October 2022)

Phytochemical Profiling Methods

Phytochemicals from plant material, usually extracts of plant, need identification to predict the potential and provide evidence that support medical claims against various ailments. Gas Chromatography (GC), Liquid Chromatography (LC), and High-Performance Liquid Chromatography (HPLC) are advanced techniques that can be helpful for identification and characterizing phytochemicals both qualitatively and quantitatively. However, conventional methods are still utilized to do preliminary profiling of phytochemicals when these methods are unavailable or unaffordable. The qualitative profiling of the phenolic compounds can be done with iodine test as simplest test. The procedure is to add a few drops of iodine solution to plant extract. The result will indicate positive test if it shows a transient red color. The qualitative profiling of tannins can be done using various tests, namely Gelatin test, Braymer's test, and another tests. It is done in a simple procedure, mostly using specific reagents. The test will be observed as positive when the color changes into a specific color that indicates positive test (Shaikh & Patil, 2020). The qualitative test for saponin can be done with foam high test. The procedure of this test is to use distilled water and a few drops of extract dissolved in solvent to a test tube and shake vigorously. Forming a foam means it contains saponin and no foam means absence of saponin (María et al., 2018).

Antimicrobial Activity Methods

The Kirby-Bauer method is an antimicrobial susceptibility test developed in 1966 by Alfred Bauer, William Kirby, and other associates. This method is suitable to test the antimicrobial activity of aqueous suspensions of plant extract. Several disks containing plant extracts would be placed in an agar plate that has been inoculated with the target organism. The plates are incubated to permit bacterial growth and diffusion of the antimicrobial agent into the agar. As the drug is diffused into the agar, a concentration gradient is formed. If the organism is susceptible to the agent, a clear area, also called an inhibition zone, will form around the disc, showing that the concentration is sufficient to inhibit growth. The advantages of this method are simple, low cost, and easy to interpret the results. However, this method lacks automation and is not suitable for fastidious bacteria, it also not the appropriate methods to determine the Minimum Inhibitory Concentration (MIC) because it's impossible to quantify the antimicrobial agent amount diffused in agar medium (Salem et al., 2016).

The serial dilution methods are the most appropriate methods to determine the Minimum Inhibitory Concentration (MIC) of certain antimicrobial agents. MIC is defined as the minimum concentration of antimicrobial agents to inhibit the growth of microorganisms. Either broth or agar dilution method may be used to quantitatively measure the *in vitro* antimicrobial activity against bacteria and fungi. In this test, microtiter plates containing serial dilutions of antimicrobial agents are inoculated with the target microorganisms and incubated to allow microbial growth. The clear tubes or lowest MIC result indicated that the agents successfully inhibit microbial growth. The advantages of this test are the simple determination of MIC, the convenience of having prepared panels, and the miniaturization of the test. The disadvantages of this test are some inflexibilities of drug selection available in standard panels (Balouiri, Sadiki, & Ibnsouda, 2016).

Bacteria strain for Antimicrobial Test

Staphylococcus aureus is a Gram-positive bacterium known to be an aerobic pathogen involved in various diseases affecting both humans and animals. Its growth depends on temperature, typically ranging between 18°C and 40°C. Remarkably, it can survive in freezing conditions below –20°C. The optimal pH for *S. aureus* growth falls between 4.0 and 10.0 (Rasheed & Hussein, 2021). Additionally, it has been reported that 21.8% of *S. aureus* strains are resistant to oxacillin (Bessa, Fazii, Di Giulio, & Cellini, 2015).

Escherichia coli is a Gram-negative, coccobacillus-shaped microorganism that naturally resides in the digestive tracts of humans and animals, as well as in their feces, where it acts as a decomposer. However, *E. coli* contamination in food can lead to serious health issues, including haemolytic uremic syndrome, haemorrhagic colitis, food poisoning, and diarrhea. The bacterium grows best at temperatures between 35°C and 37°C and within a pH range of 7.0 to 7.5 (Romadhon, 2016).

Materials and methods

Extract Preparation

D. linearis leaves (voucher specimen no: UKMB-PP 01248) as seen in **Fig. 1** were collected from the forest area in Kulliyyah of Pharmacy, Kuantan, Pahang. The leaves were separated from the stem and air-dried for several days. Subsequently, the leaves were oven-dried at 50-60°C for 48 hours to remove moisture completely. The dried leaves were ground into powder and macerated in 100% methanol for 48 hours (Azwanida, 2015). The mixture was then filtered using Whatman No.1 filter paper, and the solvent was evaporated using a vacuum rotary evaporator (Buchi). The crude extract was stored in a chiller until further use.

Qualitative Phytochemical Profiling

Phytochemical profiling was conducted to identify the presence of various phytochemicals in *D. linearis* leaves extract. The $FeCl_3$ test (Roghini & Vijayalakshmi, 2018) was used to detect phenolic compounds, while the alkaline test (Pant et al., 2017) was used for flavonoid detection. Tannins were identified using the Braymer's test (Saswade, 2019), and saponins were detected using the foam test (Shaikh & Patil, 2020).

Total Phenolic Content (TPC)

TPC of the methanol extract was determined using the Folin-Ciocalteu method (Zain & Omar, 2018)

with minor modifications. To each well, 10 µL of sample (2 mg/mL) was added, followed by 50 µL of Folin-Ciocalteu reagent (50% v/v). After a 5-minute incubation at room temperature, 40 µL of Na_2CO_3 solution was added, and the mixture was incubated in the dark for 1 hour. Absorbance was measured at 765 nm using a microplate reader. The TPC was calculated from a gallic acid standard curve, and the results were expressed as gallic acid equivalents per gram dry weight (mg GAE/g). The assay was performed in triplicate.

Total Flavonoid Content (TFC)

TFC of methanol extract was measured using the $AlCl_3$ colorimetric method (Zain & Omar, 2018). In this assay, 100 µL of sample (2 mg/mL) was mixed with 100 µL of 2% $AlCl_3$. The mixture was incubated in the dark for 30 minutes. Absorbance was recorded at 415 nm using a microplate reader. The TFC was determined using a quercetin standard curve, and results were expressed as quercetin equivalents per gram dry weight (mg QE/g). The assay was conducted in triplicate.

Fractionation by Vacuum Liquid Chromatography (VLC)

Silica gel 60 PF254 was activated overnight at 80°C. The activated silica gel was mixed with the sample solution (diluted crude extract in methanol) and heated on a hotplate at 70°C until the solvent evaporated. A VLC column was packed with silica gel to a height of 7 cm, then compressed to about 5 cm. Filter paper was placed on top of the silica gel to prevent direct force. Hexane was poured through the silica gel and eluted with the assistance of a vacuum pump. The process was monitored to ensure that no cracks or bubbles appeared in the packed silica gel. If any crack occurred, the packing process was repeated. After eluting with hexane, the column was left overnight to stabilize. Solvent systems, which are ethyl acetate, ethyl acetate: methanol (5:5) and methanol were used to separate fractions, F1, F2 and F3, accordingly. Each fraction was collected based on observed separation. Once separation was complete, the fractions were evaporated using a vacuum rotary evaporator at 40°C.

Kirby-Bauer Disc Diffusion Method

The disc diffusion assay was performed according to the Clinical and Laboratory Standards Institute (2019), with minor modifications. A 400 mg/mL stock solution was prepared by dissolving 200 mg of crude extract in 0.5 mL of distilled water. The mixture was stirred vigorously until the crude was completely dissolved. This stock solution was then serially diluted to obtained concentrations ranging from 400-50 mg/mL. A 20 µL aliquot of each sample was pipetted onto sterile 5 mm paper discs (Whatman AA, USA). The discs were dried and stored in a chiller.

E. coli and *S. aureus* strains, obtained from the BMS Laboratory, International Islamic University Malaysia, were used for testing. The bacterial suspension was adjusted to 0.5 McFarland (approximately 10^8 CFU/mL) and swabbed onto sterile Mueller-Hinton Agar (Oxoid, UK). After drying for 5 minutes, the sample discs were placed onto the agar. 10µg gentamycin discs (Oxoid, UK) were known to have broad-spectrum antimicrobial for testing both Gram-negative and Gram-positive organisms used as the positive control that will show inhibition activity in the assay. Distilled water served as the negative control with no inhibition activity in the assay, any inhibition indicates contamination or error. The plates were incubated at 35°C for 18 hours. The inhibition zone around the discs was measured to assess antimicrobial activity. This assay was performed in triplicate.

Broth Microdilution Assay

The broth microdilution assay was performed according to Veiga et al. (2019), with minor modifications. In a 96-well microtiter plate, 100 µL of a mixture containing inoculum, microbes, and Mueller-Hinton Broth was added to each well. Gentamicin was used as the positive control, and distilled water as the negative control. The plate was sealed and incubated at 35°C for 18 hours. The Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration at which no microbial growth was observed, as indicated by the absence of turbidity in the well. This assay was conducted in triplicate.

Minimum Bactericidal Concentration (MBC)

The MBC was determined according to Senhaji et al. (2020), with slight modifications. After identifying the MIC, the samples were streaked onto sterile

Mueller-Hinton Agar and incubated at 35°C for 18 hours. The MBC was defined as the lowest concentration where fewer than 3 colonies were present, indicating a 99.99% reduction in microbial growth.

Results and Discussion

Results

Extraction Yield

Out of 78.363 gram of dried leaves powder, 15.961 gram of extract were obtained after solvent separation. Therefore, the extraction yield of *D. linearis* leaves were 20.36 %.

Qualitative Phytochemical Profiling

The qualitative phytochemical profiling result as seen in **Table 1** showed that *D. linearis* leaves methanolic extract to contain phenolic compounds, flavonoid compounds, tannin, and saponin.

Table 1. Qualitative Profiling Result

Phytochemical	Result
Phenolic	+
Flavonoid	+
Tannin	+
Saponin	+

Quantitative Phytochemical Profiling-TPC and TFC

The TPC of the methanol extract was measured against the gallic acid standard curve with the equation y = 0.0488x +0.4593 (R2 = 0.9901) and the result was 225.43 ± 4.16 mg GAE/g. The TFC was measured against the quercetin standard curve with the equation y = 0.0548x + 0.5473 (R2 = 0.9809) and the result was 50.20 ± 4.78 QE/g.

Kirby-Bauer Disc Diffusion Agar

The obtained inhibition zones from Kirby-Bauer disc diffusion agar was shown in **Table 2**. The extract and its fractions demonstrated antimicrobial activity against both tested microorganisms, with *S. aureus* showing greater susceptibility than *E. coli*. The largest inhibition zone against *S. aureus* was observed with the F1 400 mg/mL disc, yielding an inhibition zone of 10.40 mm. Similarly, for *E. coli*, the highest inhibition zone was also produced by the F1 400 mg/mL disc, although the zone was slightly smaller at 9.80 mm. The results indicated that some of the lower concentration inhibition zones exceeded 6 mm, suggesting potential bacteriostatic effects. As a result, the Minimum Inhibitory Concentration (MIC) test was conducted. The F1 50 mg/mL disc produced a 6.40 mm inhibition zone against *S. aureus*, while the CE 50 mg/mL F3 50 mg/mL discs produced inhibition zones of 6.80 mm and 6.60 mm, respectively, against *E. coli*.

MIC and MBC Results

The broth microdilution assay of the extract showed that the MIC for *E. coli* was undetermined while for *S. aureus*, the MIC was 1.56 mg/mL. This showed that *S. aureus* was more susceptible than *E. coli*. As for the MBC assay, all of the tested concentrations still have more than three colonies. Therefore, it can be concluded that the MBC was more than 50 mg/mL and the extract is a bacteriostatic antimicrobial agent.

Table 2. D. *linearis* Leaves Extract and VLC Fractions Antimicrobial Result

Sample Name	Concentration (mg/mL)	Inhibition Zone (mm)	
		E. coli	*S. aureus*
Methanol Crude	400	8.2 ± 0.84	9.2 ± 0.84
	200	7.2 ± 0.84	-
	100	7.0 ± 0.71	-
	50	6.8 ± 0.45	-
F1 (Ethyl acetate (EA))	400	9.8 ± 1.30	10.4 ± 0.55
	200	7.4 ± 0.55	8.2 ± 0.45
	100	4.2 ± 3.90	7.2 ± 0.84
	50	2.6 ± 3.58	6.4 ± 0.55
F2 (EA: Methanol, 5:5)	400	6.8 ± 0.45	4.0 ± 3.67
	200	6.4 ± 0.55	2.0 ± 2.83
	100	2.8 ± 3.83	-
	50	1.4 ± 3.13	-
F3 (Methanol)	400	7.6 ± 0.55	7.6 ± 0.55
	200	7.4 ± 0.55	6.6 ± 0.55
	100	6.8 ± 0.45	3.8 ± 3.49
	50	6.6 ± 0.55	2.2 ± 3.03
Controls			
Gentamycin (+)		23.0	27.4
Distilled water (-)		-	-

(-) means no inhibition zone was formed; Inhibition zone are depicted in four categories: Sensitive (≥15 mm), Intermediate (10-14), Potential Inhibition (6-10), Resistant (≤5 mm). Results are depicted as mean of five replicates ± standard deviation.

Discussion

Extraction is a crucial step in isolating bioactive compounds from plant materials. Solvent extraction is the most common method used, though alternative techniques such as distillation, pressing, and sublimation are also employed. Factors such as solvent choice, temperature stability, and extraction duration must be considered (Abubakar & Haque, 2020). The clinical significance of using ethyl acetate and methanol as extracting solvents lies in their distinct physicochemical properties, which influence the types of bioactive compounds they can extract. This is particularly important in phytochemical research, drug discovery, and clinical pharmacology. The profiling result of the phytochemical concedes with the study conducted by Aboshoufa & Elgubbi (2019), who reported that *D. linearis* leaves methanolic extract contains various phytochemicals such as tannins, saponins, flavonoids, steroids, phenols, steroids, terpenoids, glycosides, and reducing sugars. These metabolites were the cause of *D. linearis* leaves possessing significant antioxidative properties and other medicinal benefits, such as antimicrobial activity, gastroprotective, anti-inflammatory, and antipyretic effects (Rajesh et al., 2016).

In terms of TPC and TFC, the results from this study align with previous research by Aboshoufa & Elgubbi (2019), which reported high TPC and TFC levels in *D. linearis* methanolic extracts. The variation in results may be due to differences in extraction solvents. The TPC value of *D. linearis* leaves, roots, and stems ranging from 193.50 – 266.39 mg GAE/g. The most notable difference is between TPC obtained from extraction with different solvent selection, as shown by Zakaria et al., (2019) that used aqueous as solvent obtained less TPC than this study that used methanol as solvent, with TPC value of 193.50 ± 14.80 mg GAE/g and 239.63 ± 3.91mg GAE/g, respectively.

The plant part and solvent that were used in all three studies vary among each other, indicating these factors that influence the number of phenolic compounds that can be extracted from the plant. Compound extraction can be done if the solvent can separate the target compound from its matrix. Solvent polarity plays a crucial role in the extraction process, as solvents with polarity similar to the target compounds are more effective in extracting them. Phenolic compounds, for instance, are generally polar and are more efficiently extracted with high-polarity solvents like methanol (Gil-Martín et al., 2022). This study showed a higher extraction yield compared to the study by Ismail et al. (2014), which used distilled water as the solvent. As noted by Rasul (2018), polyphenols are best extracted with higher-polarity solvents like methanol, which may explain the difference in yield between the two studies. For future studies, non-conventional extraction methods, such as those utilizing additional energy sources, could enhance extraction efficiency (Farooq et al., 2022).

Fractionation was done with 100% ethyl acetate (F1), 50% ethyl acetate and 50% methanol (F2), and 100% methanol (F3). Ethyl acetate is suitable solvent to be used for fractionating the medium-polarity compounds from the crude extract. Meanwhile, methanol suitable to be used for fraction high-polarity compounds, such as phenols, flavonoids, tannins, and saponins. F1 shows that medium-polarity compounds inhibit *S. aureus* better than *E. coli* and effective on Gram-positive bacteria. F2 as a combination of two solvents, acts as a flush for medium polar compounds that were left behind. It didn't show any significance inhibition activity to *S. aureus* and *E. coli.* F3 shows that high-polarity compounds inhibit *E. coli* better than *S. aureus* with more consistent inhibition zone and effective on Gram-negative bacteria.

The antimicrobial activity test demonstrated that the methanolic extract and fractions of *D. linearis* leaves possess antimicrobial properties against the tested microorganisms, with a more pronounced inhibition zone observed against *S. aureus*. This finding is consistent with the study by Breijyeh et al., (2020), which suggested that the difference in inhibition zones can be attributed to the structural differences between *S. aureus* and *E. coli*. Gram-negative bacteria are generally more resistant to antimicrobial agents due to their outer membrane, composed of phospholipids and lipopolysaccharides, which acts as a barrier to certain antimicrobial compounds, especially those that disrupt peptidoglycan structure.

The antimicrobial activity of the crude extract and its fractions can be attributed to the phytochemicals present, including phenolic compounds, flavonoids, saponins, and tannins. The varied inhibition zones observed against *E. coli* and

S. aureus, with smaller zones at lower concentrations, suggest that higher extract concentrations contain more active phytochemicals. As noted by Behbahani et al. (2019), higher concentrations of extracts typically result in greater antimicrobial effects due to the higher content of bioactive compounds.

Phenolic compounds, present in the extract, are known to protect plants from pathogens by altering microbial cell membrane permeability, leading to irreversible damage (Bouarab-Chibane et al., 2019). Flavonoids, another class of compounds found in the extract, can penetrate microbial cell membranes, disrupting their integrity and leading to bacterial inhibition or even death (Kumar & Pandey, 2013). Tannins inhibit microbial growth by several mechanisms, including metal chelation, membrane and protein interaction, destabilization, and enzyme inhibition (Molino et al., 2020). Saponins, identified in this study, possess surfactant properties that interact with cell membranes, reducing surface tension and leading to microbial death (Dong et al., 2020).

A notable limitation of this study is the lack of minimum inhibitory concentration (MIC) data for *E. coli*. While the extract showed inhibitory effects in diffusion assays, the absence of a defined MIC restricts the ability to characterize whether the activity is bacteriostatic or bactericidal for this organism. The data for *S. aureus* suggest a bacteriostatic activity, as evidenced by the minimum bactericidal concentration (MBC) being higher than the MIC. This distinction is crucial because bacteriostatic agents inhibit bacterial growth without killing the organism (Loree & Lappin, 2023), necessitating an intact immune system to clear the infection. Therefore, the potential therapeutic application of *D. linearis* extract may be limited in immunocompromised individuals or in systemic infections without co-treatment. Moreover, the lack of MIC data against *E. coli* precludes meaningful comparisons between the antimicrobial potency of the extract across different bacterial species.

While the findings support the antimicrobial potential of *D. linearis* extracts, especially against Gram-positive bacteria, further research is needed to detail the phytochemical profile from the fractions, establish MIC values for Gram-negative organisms, assess the extract's bactericidal efficacy, and exploring the full antimicrobial potential of the extract.

Conclusion

The methanolic extract of *D. linearis* leaves was found to contain several phytochemicals with known antimicrobial properties, including phenols, flavonoids, tannins, and saponins. The TPC and TFC assays indicated that the extract contains 225.43 ± 4.16 mg GAE/g of phenolic compounds and 50.20 ± 4.78 mg QE/g of flavonoids. The antimicrobial activity of the extract was confirmed through disc diffusion and broth microdilution assays, where it showed greater activity against *S. aureus*, with an inhibition zone of 10.4 mm. The inhibition zone increased with higher extract concentrations, suggesting a dose-dependent effect. The MIC for *S. aureus* was 1.563 mg/mL for F1, while no MIC was determined for *E. coli* due to inconsistent results (growth observed in all wells). The MBC was not determined for *S. aureus*, as no bactericidal activity was observed at concentrations of 50 mg/mL or lower. These results indicate bacteriostatic potential, but further research is needed to determine the bactericidal properties of the extract.

For future studies, non-conventional extraction methods and factors such as agitation could be explored to improve extraction efficiency and yield while preserving bioactive compounds. Additionally, isolating and identifying the specific bioactive compounds responsible for antimicrobial activity is essential for understanding the mechanism of action and assessing the toxicity of these compounds. MIC and MBC determinations for a broader spectrum of pathogens to better understand the extract's full antimicrobial profile and potential clinical relevance must be prioritized.

Authors contribution

Study design, S.Z.M.S and G.R.C.R. Direction and Coordination, S.Z.M.S. Investigation, G.R.C.R. Resources, S.Z.M.S. Writing-Original Draft, G.R.C.R. Writing-Review, S.Z.M.S. Writing-Editing, G.R.C.R. and S.Z.M.S. Supervision, S.Z.M.S., and E.Z. Project Administration, S.Z.M.S. and G.R.C.R., Botanical identification, S. K.

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Ethical statement

This study did not involve any research with human participants or animals. No ethical approval was required for this work, as it did not include experiments, data collection, or procedures involving humans or animals in any form.

Conflict of interest

The authors declare there were no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author declared the use of ChatGPT in order to improve readability and language.

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