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Research Article

Phytochemical analysis and antibacterial activity on methanolic extract of *Boesenbergia stenophylla* (Jerangau Merah) rhizome against waterborne bacteria

Emmanuelle Usun Stephen, and Samuel Lihan*

Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, 94300, Kota Samarahan, Sarawak, Malaysia

*Corresponding author: emma.usun@gmail.com

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Abstract

Boesenbergia stenophylla, locally known as Jerangau Merah, is a native wild ginger in Borneo Highlands, Sarawak. It is commonly used by the local as a traditional medicine for various diseases. The purposes of the current study are to investigate the phytochemicals and evaluate the antibacterial activities of methanolic *B. stenophylla* rhizome extract against *Bacillus* sp., *Staphylococcus* sp., *Citrobacter* sp. and *Enterobacter* sp. The rhizomes of *B. stenophylla* were extracted with pure methanol using Soxhlet method at 64°C for 8 h. The antibacterial properties of methanol extract were assessed using disc-diffusion assay. Phytochemical assays analysis revealed the presence of alkaloids, terpenoids, steroids, saponins, flavonoids and phenols. The antibacterial activity revealed that the extract had high to moderate inhibition against the growth of *Bacillus* sp. and *Staphylococcus* sp. at 100 mg/mL, with inhibition zones of 16.3 mm and 11.3 mm, respectively. Thus, the methanolic extract from *B. stenophylla* has potential to act as antibacterial agent.

Keywords: Antibacterial, *Boesenbergia stenophylla*, Jerangau Merah, phytochemical assays, Soxhlet apparatus

Introduction

The utilisation of medicinal plants in traditional herbal practises by indigenous communities' dates to ancient periods, intending to treat different diseases. Despite the significant developments in modern pharmaceutical businesses, herbal medication remains a crucial healthcare system in current times. The extensive historical utilisation of these substances in traditional medicine, in addition to their perceived lower risk to human health, have sparked significant attention, particularly within developing nations.

Phytochemicals play a crucial role in the pharmaceutical industry by facilitating the development of novel medications and the manufacturing of therapeutic agents. The initial stage of drug development involves the discovery of active compounds derived from natural sources. This is followed by the screening and conducting of biological assays to identify bioactive components present in medicinal plants [1]. Plants and their derived products are fundamental sources of diverse phytochemical compounds, including phenols, flavonoids, alkaloids, glycosides, tannins, terpenoids, saponins, and steroids [2].

Boesenbergia stenophylla is a highly endemic to the highlands of Borneo. The species has been well-documented from the Ba'kelalan and Bario regions [3]. The plant is locally referred to as Jerangau Merah based on its maroon colouration from the leaf sheath. It is a member of the Zingiberaceae family, which includes galangal, ginger, and turmeric, all of which have medicinal qualities due to their chemical compositions. Locals in Sabah and Sarawak highly covet the rhizome due to its reputed ability to alleviate alcohol intoxication [5, 6].

The applications of this plant are linked to native traditional knowledge and cultural beliefs. Nevertheless, it is necessary to substantiate this prevailing theory with empirical proof. Therefore, it is crucial to explore more literature that provides comprehensive information regarding *B. stenophylla*, including its chemical compositions and antibacterial activities. Therefore, it is important to comprehend the core functional principle of herbal medications to optimise their efficacy within the framework of

medical treatments. The present paper has focused on these aspects scientifically using phytochemical and gas chromatography-mass spectrometry analysis on the methanolic extract of *B. stenophylla* rhizomes.

Materials and Methods

Collection and preparation of plant material

The fresh plants of *B. stenophylla* was collected from the Bario Highlands, Sarawak in between July to October 2022. They were taken to the Institute of Biodiversity and Environmental Conservation laboratory (IBEC) at the Universiti Malaysia Sarawak (UNIMAS). A voucher specimen was deposited at the Herbarium of Universiti Malaysia Sarawak, Sarawak. The plant was identified with the help of Professor Gabriel Tonga Noweg by comparing it with the voucher specimen. The samples were washed thoroughly with distilled water and cut into several parts. Then, the samples were dried in the oven at 60 °C for three days before being ground into powders.

Extraction of plant crude

Twenty grams of the powdered samples of *B. stenophylla* was subjected to Soxhlet extraction by sequentially immersed in 300 mL of methanol at 64°C as followed by Teethaisong et al. [7] with slight modification. The extraction was carried out for 8 hours and the extraction was concentrated by evaporation in a Rota-vacuum evaporator. Furthermore, the extract was collected, weighed, and refrigerated at 4 °C until further processing.

Phytochemical analysis

Phytochemical analysis of methanolic crude extract of *B. stenophylla* rhizomes were carried out using qualitative analysis methods from established procedures [8, 9] with slight modifications. The qualitative results are shown as (+) to indicate the presence and (-) to indicate the absence of phytochemicals

Antibacterial analysis

Bacillus sp., *Staphylococcus sp.*, *Citrobacter sp.*, and *Enterobacter sp*. from overnight nutrient broth cultures were spread on Mueller-Hinton agar plates in three different directions using sterilized cotton swabs, allowed to dry for 15 minutes. Subsequently, paper discs (ADVANTEC 49005010, 8 mm thick) were placed on the agar, and 50 µL of plant extracts with adjusted concentrations (12.5, 25.0, 50.0, and 100 mg/mL) were carefully added to the discs. Meanwhile, 50 µL of 100% (v/v) DMSO was added as the negative control. The agar plates were incubated at 37 °C for 18 hours after a 15-minute acclimation period at room temperature. The diameters of the growth inhibition zones around the paper discs were measured using callipers. The antibacterial activities of the plant crude extracts were tested in triplicate, and the results were expressed as the mean and standard deviation of inhibition diameters in millimetres (mm) [10]. The inhibition levels were categorized based on the growth inhibition diameter (mm) as detailed in **Table 1**.

Results and Discussion

Phytochemical analysis

The phytochemical analysis identifies the components present in plant extracts, particularly those that are most abundant, while also supporting searching for bioactive molecules that possess the potential for manufacturing valuable pharmaceuticals. Phytochemical screening of the methanolic *Boesenbergia stenophylla* rhizome extract revealed the presence of alkaloids, steroids, terpenoids, saponins, flavonoids and phenolic as shown in **Table 2**, although tannins and glycosides were absent.

Several studies have demonstrated that *Bosenbergia* spp. exhibit chemical constituents, including flavonoids, diarylheptanoids, phenolic acids, terpenoids, and steroids. Moreover, this plant has exhibited significant pharmacological activities, such as anti-inflammatory, antibacterial, antiviral, analgesic, and antioxidant effects [11, 12].

Alkaloids possess antibacterial, antitumour, anti-inflammatory, and antimalarial activity, cytotoxicity and antispasmodic effects [13, 14, 15]. Furthermore, alkaloid-rich plants are toxic substances against insects or plant-eating animals because of their bitter taste. Thus, they are proven to have pharmacological activities, such as decreasing blood pressure, alleviating pain, and inhibiting microbial infections. They are reported to be abundant in *Zingiber officinale* [16].

Table 1. Zone of inhibition and classification

Zone of Inhibition (mm)	Classification
⩾20	Extremely sensitive
15 – 19	Sensitive
9 – 14	Intermediate
< 8	Resistant

Table 2. Phytochemical analysis of methanolic rhizome extract of *B. stenophylla*

Test	Interferences
	Rhizome
Alkaloids	
(a) Mayer's Test	+
(b) Wagner's Test	+
Steroids and terpenoids	
(a) Salkowski test	+
Tannins	-
Saponins	+
Glycosides	
(a) Anthraquinone Glycoside (Borntrager's Test)	-
(b) Cardiac Glycoside (Keller-Killiani Test)	-
Flavonoids	+
Phenols	+

Note: (+) presence, (-) absence

Saponins have demonstrated significant pharmacological properties. Several saponins exhibit many pharmacological properties, including analgesic, adaptogenic, antitumor, piscicidal, diuretic, expectorant, and galactagogue, hypoglycaemic, molluscicidal, nervine tonic, sedative, spermicidal and stimulant [17]. Moreover, Uaraksakul and Chanprapai [18] have documented the identification of saponins through the phytochemical analysis of *B. rotunda* rhizomes. According to recent studies, there is evidence suggesting that it possesses efficacy as an anti-thrombotic agent, antitumour agent, anti-diabetic agent, and antihypertensive agent as well as potential for treating reproductive diseases [19-22]. Furthermore, saponins are shown to activate mucous membrane protective factors and are also helpful for anti-inflammatory activity, they are shown to treat chronic hepatitis and cirrhosis [17, 23]. A similar report from Marlik et al. [24] reported a significant presence of saponins, which could be biolarvasida against *Culex* and *Aedes* aegypti.

The presence of flavonoid and polyphenolic compounds found in the methanolic rhizomes of *B. stenophylla* is suggestive of its antioxidant properties, which protect the body from damage caused by free radical-induced oxidative stress. Apart from antioxidant properties, flavonoids and other polyphenolics also exhibited several biological activities such as antibacterial, antifungal, anti-diabetic, antiglycation, anti-inflammatory, anti-parasitic, anti-ulceration, hepatoprotective, anticancer and anti-anthelmintic secondary metabolites since polyphenolic compounds are abundantly present in medicinal plants [25-28]. Likewise, it is found that its similar species such as *Boesenbergia* sp., is rich in flavonoids and polyphenolic compounds from its antioxidant assays, which have been extensively implicated in the bioactivity of the plant that helps to cure stomach discomfort, aphthous ulcers, dysentery, dry mouth, and leucorrhea [29].

Terpenoids are the primary, secondary metabolites produced by aromatic and medicinal plants that play a crucial role in resistance. Several studies claim that many terpenoid compounds have potent antibacterial activity [30]. It is also shown that terpenoids contained in *B. rotunda* exhibit a wide range of biological activities, including antifungal, antiviral, anti-inflammatory, anticancer, antiosteoporosis, antioxidant, and anti-obesity activities [31, 32]. Similarly, steroids derived from plants are known to have a cardiotonic effect and possess antibacterial and insecticidal properties [33].

Antibacterial activity

In this research, methanol was chosen as the solvent extraction of *B. stenophylla.* Moneim et al. [34] and Riyadi et al. [35] stated that applying plant using methanol solvent extracts both polar and non-polar chemical compounds due to its high polarity, hence exhibiting more biological capacities. The antibacterial activity possessed by extracts of increasing polarity against four waterborne bacteria is reported in **Table 3**.

It was generally observed that bacterial growth increased with the concentration of the crude extracts. The methanolic extract of *B. stenophylla* rhizomes showed significant activity against all tested waterborne bacteria. Among the tested bacteria, *Bacillus* sp. was the most susceptible, followed by *Staphylococcus* sp. According to a study by Zainin et al. [36], methanolic plant extracts demonstrated superior antibacterial activity compared to other solvents with different polarities such as hexane and water. Furthermore, phytochemical constituents are more soluble in moderately polar organic solvents

despite the presence of a mixture of volatile and non-volatile compounds.

In the methanolic extract of *B. stenophylla* rhizomes, alkaloids and saponins have been reported to exhibit antibacterial activity, and the high activity against most microorganisms may be attributed to a single or combined effect of these secondary metabolites [37]. Our study showed that the methanolic extract of *B. stenophylla* rhizomes contained alkaloids, flavonoids and phenols, which consistent with past reports. Moreover, the methanolic extract has been tested against ATCC cultures (*Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 14028, *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 25923) as described by Stephen and Lihan [38].

Additionally, the bacterial strains and hydrophobic properties of certain plant extracts that can affect the uniformity of diffusion through the growth media must be considered [61]. As supported by Breijyeh et al. [39], Gram-negative bacteria are more resistant than Gram-positive bacteria because of their distinctive structure. Thus, the methanolic *B. stenophylla* extract was more effective against Gram-positive bacteria than Gram-negative bacteria.

Table 3. Antibacterial activity rhizome extracts of *Boesenbergia stenophylla* against selected waterborne bacteria with tetracycline and chloramphenicol

Bacterial Strains	**Methanolic *B. stenophylla* Rhizome Extract (mg/mL)**					
Gram-positive bacteria	**12.5**	**25.0**	**50.0**	**100.0**	**Tetracycline, 30ug**	**DMSO**
Bacillus sp.	9.3 ± 1.2	10.9 ± 0.4	13.0 ± 0.7	16.3 ± 0.8	22.1 ± 1.3	NI
Staphylococcus sp.	8.0 ± 0.0	8.6 ± 1.1	9.8 ± 0.3	11.3 ± 0.7	32.8 ± 1.2	NI
Gram-negative bacteria					**Chloramphenicol, 30ug**	**DMSO**
Citrobacter sp.	8.0 ± 0.0	8.3 ± 0.6	9.0 ± 0.9	9.8 ± 0.8	24.5 ± 1.2	NI
Enterobacter sp.	8.3 ± 0.5	8.6 ± 0.6	9.3 ± 0.2	10.1 ± 0.6	20.6 ± 0.5	NI

Results are presented as mean ± standard deviation (mm), NI indicates no inhibition zone.

Conclusion

The results of this study revealed that *Boesenbergia stenophylla* rhizomes contain alkaloids, terpenoids, steroids, saponins, flavonoids, and phenols, while being devoid of tannins and glycosides. Concerning the antibacterial assay, the methanolic extract has been shown to inhibit the growth of Gram-positive bacteria, specifically *Bacillus* sp. and *Staphylococcus* sp., effectively (16.3 mm and 11.3 mm, respectively), compared to Gram-negative bacteria. The study results confirm that *B. stenophylla* rhizomes possess medicinal properties, making them potentially suitable as antibacterial agents in the pharmaceutical industry. This research also validates the traditional use of *Boesenbergia stenophylla* rhizome and contributes to establishing a database of medicinal plants indigenous to Sarawak, Malaysia.

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