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Traditional Uses, Phytochemistry Profile and Biological Properties of Jarum Tujuh Bilah, *Pereskia bleo*: A Review

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ABSTRACT

Pereskia bleo is a species that belongs to the family of Cactaceae and is distributed across Asia, Southeast Asia, and America. This plant grows in regions receiving high annual rainfall. Featuring their non-succulent green leaves, thorny stems, and occasionally red-orange flowers, they are very popular ornamental shrubs. They are widely used as traditional medicines to prevent cancer and treat diseases such as hypertension, muscle aches, boils, and diabetes. This species has been scantily studied so far. More attention and effort are required to draw a link between these species' traditional uses, active compounds, and pharmacological activities. This manuscript aims to critically analyze the distribution, traditional uses, factors influencing the extraction of bioactive compounds, and phytochemical contents of *P. bleo*. The numerous biological properties of *P. bleo* were also presented, including antioxidant, anticancer, anti-diabetic, anti-nociceptive, antimicrobial activities, and larvicidal studies. *P. bleo* exhibits diverse biological activities that are very useful for medical treatment. The integrated knowledge of *P. bleo* will support its potential exploration in research of pharmacology and food sectors and sanguinely proffer commercialization opportunities.

INTRODUCTION

Throughout the ages, people used nature to meet their primary needs. This also applies to the use of natural products for medicinal purposes to treat various ailments. Medicinal plants have proven their worth as a source of compounds with pharmacological activity and continue to be a key source of novel drug leads today. Traditional medicines meet the primary healthcare needs of about 80% of the world's developing population, and this past decade has witnessed a surge in public interest and acceptance of natural herbal remedies [1]. Plant medicine has also emerged as an important alternative therapy option for individuals seeking to be treated holistically and naturally following an unsatisfactory response to conventional medication [1].

Secondary metabolites are pivotal compounds of the medicinal plant's therapeutic actions, such as phenolics, alkaloids, terpenoids, carotenoids, sterols, and flavonoids. These metabolites have been showing

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a diverse variety of bioactivities, including hypoglycemic, hypolipidemic, anti-proliferative, immunomodulatory, cytotoxic, antimutagenic effects, antimicrobial, anti-inflammatory, antitumor, antioxidant, and analgesic properties [2,3]. For instance, the potent antioxidant activity of plant leaves can be ascribed to different types of phenolic compounds, the high content of flavonoids [4], and the presence of carotenoids [5]. They are the free radical scavengers that cause the human body's anti-inflammatory and oxidative stress ramifications to be concealed [6,7].

Among the potential medicinal plants, *Pereskia bleo* belongs to the family of Cactaceae and has a long ethnomedical history, mainly in Malaysia, Central America, and China, for managing various diseases. In Malaysia, it is popularly known as Jarum Tujuh Bilah in Malay society and called Cak Sing Cam or Qi Xing Zhen by the Chinese community [8]. In English, this plant is well-known as the rose cactus. It is helpful against diseases such as diabetes, cancer, hypertension, stomachache, and muscle aches; a different formulation of this plant is found in several preparations such as decoction [8] and paste form supplements [9]. In various studies, *P. bleo* is reported to exhibit excellent antioxidant properties [10, 11, 12, 13, 14, 15, 16, 17], anti-proliferative agents [18, 19, 20, 21, 22], anti-diabetic [23, 24], antihypertensive [25], anti-nociceptive agent [26, 27] and an effective antimicrobial agent [11, 16, 28, 29, 30, 31, 32].

Although this plant has been proven valuable through its numerous beneficial properties, only a few clinical part studies are available. Exploring the usefulness of *P. bleo* will broaden the botanical knowledge, the scope of plant consumption, and the applications. Therefore, the phytochemical constituent and therapeutic properties based on the published reports were reviewed. This review may serve as a useful reference for future pharmaceutical, nutraceutical, and drug discovery.

ECOLOGY, GEOGRAPHICAL DISTRIBUTION, AND BOTANICAL DESCRIPTION

Pereskia bleo, which grows in a highly precipitous forest [9, 33], is native to the regions of Columbia and Panama [8], Argentina, Korea, and Paraguay [34]. It has been widely grown in Southeast Asian countries such as Malaysia, Indonesia, Singapore, and India due to its ease of cultivation and propagation by seeds or stem cutting. In addition, *P. bleo* is the Pereskia that thrives in environments with high yearly precipitation rates above 187mm each wet month [8].

Pereskia bleo (Fig. 1) is a thorny and deciduous perennial shrub that grows to a height of approximately 0.6 to 8 meters. Distinct from other cactus species, *P. bleo* has substantial, non-succulent, green leaves and thin stems [34]. When young, the trunk grows 10 cm in diameter, is typically red, has spines, and is leafy. The spines may be up to 2 cm old and are arranged in 5-6 fascicles, yet premature shoots often bear 1 to 4 [8, 34]. Furthermore, the spines protrude from the areoles. The flowers can bloom singly or in a group, often seen as a rose. The flower's colors vary depending on the cultivar, ranging from white to yellow, fuchsia, or red. Fruits are typically glossy, round green; at ripening, they are yellow and fleshy with thick walls resembling conical berries and have seeds that are dark brown or black [8, 34].

THERAPEUTIC USES OF *P. bleo*

A deep link has existed between humans and plants since ancient times, as using plants to alleviate diseases has always played a significant role in human life. Utilizing plants to treat ailments is aligned with the trend to retreat to nature. Moreover, plant-based treatment is also becoming more popular than chemical pharmaceuticals due to their effectiveness in curing numerous ailments, availability, affordability, lesser toxicity, and ability to be used without the supervision of medical practitioners [35].

The traditional use of *P. bleo* to treat various ailments is tabulated in Table 1. The data from these studies revealed that plant parts, preparation prior to usage, and therapeutic use differ among countries. Traditionally, *P. bleo* has been used in herbal medicine to cure malignancy-related diseases in Malaysia and China despite its regular uses as a food flavoring agent and spice in some places [8, 36]. Previous

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studies on Pereskia species have also revealed that they are used as natural remedies to relieve gastric pain, ulcers, and hemorrhoids [10]; to prevent cancer, to treat diabetes, hypertension, and skin inflammation [11]. Moreover, Columbians use various parts of this plant to alleviate pain in stomachaches, mainly neutralizing snake bites and muscle aches [36].

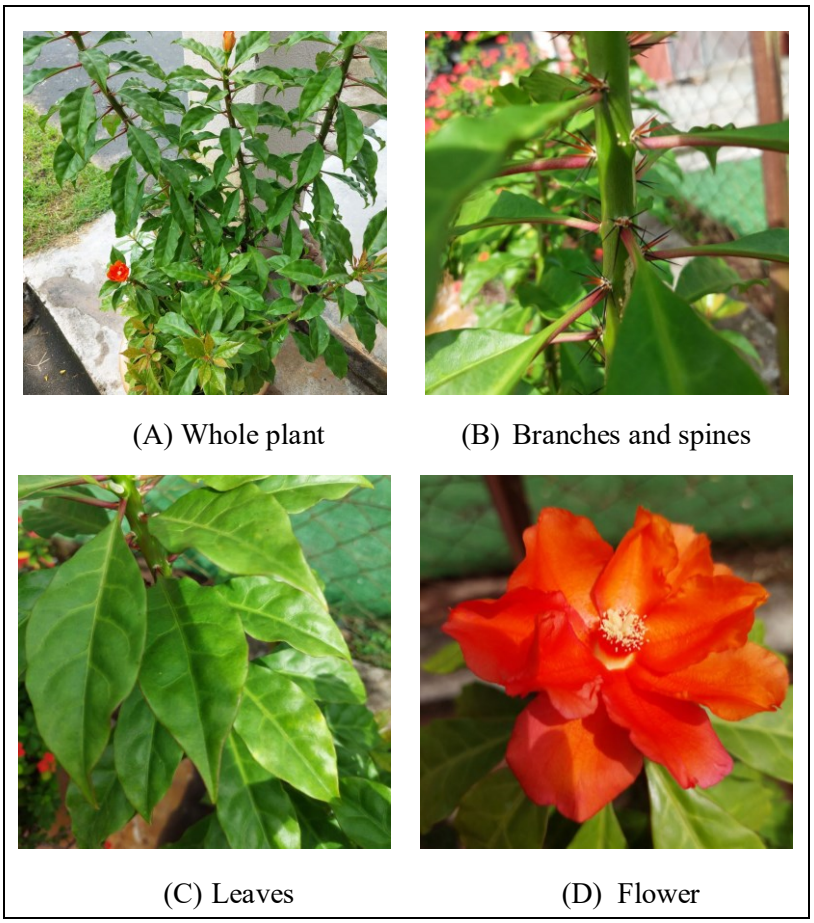

Figure 1: Different parts of *P. bleo.*

FACTORS AFFECTING THE EXTRACTION OF BIOACTIVE COMPOUNDS

Extraction methods must be best suited as the target compounds may be non-polar to polar and thermally labile. The current study extracted *P. bleo* leaves using maceration, Soxhlet, and decoction techniques, which are commonly used in the extraction process of medicinal plants. The Soxhlet method outperformed the maceration method in successive extraction, yielding the highest percentage of extract yield [38]. Extraction methods are important in discovering phytochemicals as different extraction techniques will isolate different compounds, and heating will eliminate heat-sensitive compounds. According to [39], equilibrium and mass transfer rate are key variables that govern the extraction process. Therefore, investigating the impact of extraction conditions, solvent types, solvent-sample proportion, extraction period, and temperature on the quantification of phenolic compounds and antioxidant action of

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extracts is a way to improve the recovery of secondary metabolites (Table 2). This review will discuss the factors affecting the extraction of bioactive compounds by comparing the different conditions of the phenolic extraction process.

Pure solvents, such as ethanol, methanol, ethyl acetate, chloroform, and water, are the most common solvents used in the extraction and fractionation process. The extraction solvents significantly affected the total phenolic content and antioxidant activities [40]. For instance, [10] observed that the yield of phenolic compound and antioxidant activity of *P. bleo* leaves were influenced by fractionation solvent composition. The authors obtained a 4.04% yield of phenolic compounds and 225µg/mL EC50 value for antioxidant activity with ethyl acetate as a fractionation solvent. However, when water was used, the extract yielded a 2.76% yield of phenolic compounds and a high EC50 value, 1700µg/mL of antioxidant activity.

Table 1. Traditional uses of *P. bleo* among different countries.

Location	Therapeutic use	Part	Traditional preparation	References
Columbia, Central America	Stomachache	Leaves	Prepares *ina kuamakalet*: A special mortar moistened with water is used to mix leaves with the excrements of red ants. The mixture molds into an oval shape and will be sundry. *Ina kuamakalet* rubbed with a small amount of water in a container and ingested.	[8, 36]
	Muscle ache	Leaves	A decoction from the leaves and used as a warm bath	
Kelantan, Malaysia	Regulate body health	Shoots; Stem	Young shoot with stem eaten raw	[9]
	Cancer	Leaves	(1)Seven leaves are eaten raw, daily (2) Leaves mixed with woody ingredients, eaten (3) Count the leaves from one to seven in ascending and descending order. Then, the leaves are dipped in boiled water for a few minutes before drinking.	
	Muscle ache	Shoots	Taken daily for two to three days	
	Hypertension	Shoots	Eaten raw	
		Fully bloomed flower	Eaten raw	
	Treat boils	Leaves	Grind into paste form and apply to the infected area	
Malaysia	Pain alleviation of skin injury	Leaves	Grind into paste form and apply to the infected area	[34]
Singapore	Neoplasm Malignant: breast, lung, prostate, uterus, thrombocytosis, lymphoma	Leaves	(1) Two to three fresh leaves eaten raw twice a week or daily (2) The juice of two to three fresh leaves in combination with some other herbs is drunk two to three times per week. (3) 10 to 15 leaves are boiled in water, and drink a cup daily	
	Benign: breast, thyroid, salivary gland	Leaves	Two to three leaves eaten raw daily	
		Fruit	The fruit was grilled on the fire and cut in half. The cut surface is positioned on top of the tumor for two hours twice daily. It is done daily for thyroid mass or weekly for parotid mass.	
	Anti-diabetic	Leaves	5 to 6 leaves, eaten raw, daily	

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Cold and flu	Flower	Boiled in a cup of water, drink daily
Treat Herpes Simplex-I viral infection	Leaves	Decoction, drink daily
Osteoarthritis	Leaves	Three leaves are eaten raw, weekly
Constipation and gastritis	Leaves	Three to four leaves eaten raw three times per week or daily
Renal failure	Leaves	Up to 12 leaves eaten raw, daily

Table 2. The Extraction Conditions, Total Phenolic Content, and Antioxidant Potential of *P. bleo leaf.*

Extraction protocol	Antioxidant assay	Total phenolic content	Antioxidant activity	Reference
100% methanol (1.5 L), the mixture rest for three days at room temperature and filtered. Repeated twice with 100% methanol (1.5 L each time). Filtered, the solvent from each extraction was combined and concentrated using a rotary evaporator.	(1) DPPH assay (2) Reducing power assay (3) ß-carotene bleaching method	(mg GAE/g extract) Ethyl acetate (40.12) Methanol (27.88) Hexane (23.15) Water (27.70)	(1) EC_{50} value (µg/ml) Ethyl acetate (225) Methanol (750) Hexane (210) Water (1700) (2) Reducing power assay (absorbance at 700nm) Ethyl acetate (1.155) Methanol (1.605) Hexane (2.222) Water (1.676) (3) ß-carotene (AA%) Ethyl acetate (83.68) Methanol (72.96) Hexane (73.31) Water (46.17)	[10]
The cold extraction method of 24-hour intervals was repeated twice using methanol. Filtered and concentrated using a rotary evaporator. Chlorophyll was removed prior to the fractionation process. The liquid-liquid partition of hexane, chloroform, and methanol.	DPPH scavenging assay	(mg GAE/g extract) Hexane (25.20) Chloroform (31.91) Methanol (40.82)	IC_{50} value (µg/mL) Hexane (143.55) Chloroform (379.41) Methanol (33.83)	[11]
1:30(w/v); 10 g powdered sample in 300 mL solvent, distilled water, and methanol. The mixture was sonicated for 30 minutes (40kHz and 30°C) and rested for 24 hours. Filtered, concentrated using a rotary evaporator, and kept at -4°C.	DPPH scavenging assay	(g/100 g) Methanol (6.5 ± 0.12) Water (4.6 ± 0.20)	IC_{50} value (mg/ml) Methanol 68.751 ± 1.32 Water 180.41 ± 2.50	
Extracted with ethanol, methanol, ethyl acetate, and hexane using a shaking water bath for 2 hours at 40ºC. Filtered, concentrated using a rotary evaporator, and kept in a dark bottle at 4ºC.	(1) DPPH scavenging assay (2) FRAP (3) β-carotene bleaching method	N.I	(1) IC_{50} value (µg/ml) Ethyl acetate (168.35 ±6.5) Hexane (244 ± 4.5) Methanol (277.5 ± 6.5) Ethanol (540.88 ± 9.0) (2) FRAP assay (µMTE/g)	

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			Ethyl acetate (50.48 ±1.53) Hexane (41.7 ± 2.2) Methanol (45.2 ±1.2) Ethanol (40.45 ±1.54) (3) AA% Ethyl acetate (71 ± 4.5) Hexane (66 ± 1.2) Ethanol (65 ± 2.7) Methanol (67.8 ± 4.6)
Soxhlet extraction, by using various solvents.	DPPH scavenging	N.I	% of inhibition Hexane 37.55% Ethyl acetate 16.1% (2) Dichloromethane16. 1% (3) Methanol 13.2%

DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power; GAE: Gallic acid equivalent; TE: Trolox equivalent. AA%: Antioxidant activity (A higher AA% indicates a higher antioxidant activity); ABTS: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonicacid) (A higher ABTS value indicates a higher antioxidant activity); EC50: Half maximal effective concentration (A lower EC50 value indicates a higher antioxidant activity); IC50: Half maximal inhibitory concentration (A lower IC50 value indicates a higher antioxidant activity); w/v: weight/volume (g/mL); N.I: not indicated

This is in accordance with the findings of [13], who discovered that methanol extracted the total phenolic content compared to chloroform and hexane extract. Therefore, it is conceivable that the recovery of phenolic components is affected by the extraction solvent. The fluctuation may be related to several variables, such as the degree of polarity and solubility of the targeted active compounds in the extract, for the selectivity of different phenolic compounds; according to the law of similarity and intermiscibility, solvents whose polarity values are close to those of the solute's are likely to extractable effectively, and vice-versa.

In addition, the extraction duration is also an important aspect of the recovery of antioxidant compounds. A longer extraction time within the time range will improve the extraction efficiency [41]. Interestingly, an extraction procedure took 72 hours [10], while another experiment only took 24 hours to extract the phenolic compounds [11]. The increment of extraction time will, however, be undoable once the solute-solid material equilibrium has been reached [41]. Similarly, temperature is another crucial determinant that affects the extraction of *P. bleo* bioactive components. According to [17], with the employment of 30°C during the extraction process, an aqueous extract of *P. bleo* leaves had a significant concentration of myoinositol, sugars, and fatty acids. However, a gas chromatography–mass spectrometry (GC-MS) study by [18], who carried out an extraction at 50°C, revealed that the main compound found in the aqueous extract of the leaves was phenol. Therefore, it is conceivable that different extraction temperatures release different phytochemicals; the extraction at a greater temperature releases phenolic compounds. According to [42], the plant tissues are softened under high extraction temperatures, and the interaction between the phenol-protein and phenol-polysaccharides in the plant weakens. This process increases the solubility and diffusibility of the phenolic compounds and consequently allows for the transfer of compounds into the solvent.

Unfortunately, due to variations in experimental settings, direct comparisons between studies are not feasible. Wide-ranging testing is necessary regardless of the positive influence of the extended extraction time and higher temperature for phenolic compound extraction. The extraction process needs to be strictly monitored as prolonged heating at higher temperatures accompanied by elevated extraction time may cause the oxidation and deterioration of active compounds, render their antioxidant quantification, and suppress the biological activity of the plant materials. Furthermore, it is important to consider *P. bleo*'s ecology when comparing studies, as abiotic stress and biofertilizers can influence antioxidant contents [43].

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Another critical aspect is the surface contact between the sample and solvent. Surface contact between the dried sample and solvents can enhance extraction efficiency. Grinding produced coarser, smaller samples. Conversely, powdered samples have more homogenized and smaller particle sizes, resulting in more excellent contact between target analytes and extraction solvents. Particles smaller than 0.5 mm are optimum for efficient extraction [44].

Considering the unstable nature of the compound, each phenolic source necessitates a particular approach for extraction and optimization. Therefore, greater attention ought to be exercised in developing time-efficient protocols and characterizing the influence of extraction for *P. bleo* leaves to avoid heat-induced degradation of thermolabile compounds, unnecessary wastage of time and solvents, and reduction in quantification as well as the quality of the active compounds. This finding could contribute to the body of knowledge of this plant, particularly the extractability of phytochemicals from P. bleo.

PHYTOCHEMICAL PROFILE, BIOACTIVE MINI-PROTEIN, AND MINERAL COMPOSITION OF *P. bleo*

Many phytochemicals have been isolated from *P. bleo* with separation techniques like GC-MS, high-performance liquid chromatography (HPLC), column chromatography, and thin-layer chromatography (TLC). Alkaloids, flavonoids, phenolic compounds, antimicrobial peptides (AMPs), sterols, terpenoids, fatty acids, sugars, and carotenoids are among the bioactive compounds identified in phytochemical studies of *P. bleo* extracted with different solvents of different polarity. The mixture of sterols (stigmasterol, campesterol, and β-sitosterol), flavonoid of apigenin 6-C-glucoside (vitexin), chrysin, quercetin, epicatechin, myricetin, β- carotene, α-tocopherol are worthy mentions in them. Table 3 summarizes the phytochemicals profile of *P. bleo* and the plant parts used.

Table 3. Phytochemicals and plant parts used from *P. bleo.*

Phytochemicals	Compounds	Part	References
Alkaloids	Mescaline (3,4-Dimethoxy-phenethylamine)	Leaves	[47]
	3-Methoxytyramine		
	Tyramine		
	1,2,3,4-Tetrahydro-cyclopenta(b)indole		[18]
Fatty acid	Methyl palmitate	Leaves	[33]
	Methyl linoleate		
	Methyl α-linoleate		
	1-Heptacosanol		[48]
	1-Hexadecanol		
	1-Octacosanol		
	9-Octadecenamide, (Z)-		
	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-		
	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-		
	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-		
	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester		
	Octadecanoic acid, methyl ester		
	Octacosyl acetate		
	Hexadecanoic acid, methyl ester		
	Hexadecanoic acid, 2,3-dihydroxypropyl ester		
	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester		
	n-Tetracosanol-1		
	Nonanoic acid,9-(3-hexenylidenecyclopropylidene)-2-hydroxy-1-(hydroxymethyl)		
	Methyl dihydrojasmonate		
Flavonoid	Vitexin (Apigenin 6-C-glucoside)	Leaves	[23]
	Chrysin		
Sterol	β-sitosterol	Leaves	[19]

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	Campesterol		
	Stigmasterol		[20]
	γ-sitosterol		[18]
	Stigmast-7-en-3-ol, (3β,5α)-		
	Stigmastan-3,5-diene		
Lactone	Dihydroactinidiolide	Leaves	[20]
Phenol	2,4-di-tert-butylphenol	Leaves	[20]
			[18]
	2H-1-Benzopyran-6-ol,3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-		
	2-Methoxy-4-vinylphenol		
	4-vinyl-2-methoxy-phenol		
	4-vinyl-phenol		
	β-tocopherol		
	γ-tocopherol		
Terpenoids	Phytol	Leaves	[18, 20]
			[18]
	Neophytadiene		
	Squalene		
	2-Pentadecanone,6,10,14-trimethyl-		
	(-)-Loliolide		
Vitamin E	α-tocopherol	Leaves	[8, 13, 19]
Carotenoids	Lutein	Fruits	[8, 13, 49]
	Zeaxanthin		

As the leaf is commonly used for medicinal purposes, the leaf has received more attention than other parts of the plant. Phytol is the copious isolated constituent from *P. bleo* leaves of ethyl acetate extract [18], and the result of this study was also well-matched with [20], who noted the presence of phytol in active ethyl acetate fraction. Hexane extract of the leaves contains a high concentration of sterols comprising γ-sitosterol with 17.53%, followed by phenolic compounds [18]. It has long been noted that γ-sitosterol has a hypocholesterolemic effect, which affects cholesterol synthesis in intestinal and liver cells [45]. It significantly reduces lipid levels and can be considered for developing a hypolipidemic agent [46].

Apart from the leaf, the fruit contains carotenoids called lutein (β,é-carotene-3,3′-diol) and zeaxanthin (β,β-carotene-3,3′-diol) [13, 49]. Studies have discovered that lutein and zeaxanthin play various biological processes, including slowing down the progression of eye diseases. These natural antioxidants provide eye protection primarily by quenching the free radicals, thus reducing oxidative stress in the retina [50]. They also suppress mitochondrial dysfunction, apoptosis, and inflammation in diabetes [50, 51].

The bioactive mini-protein bleogen pB1 is a cysteine-rich peptide that plays a significant role in plant stress and defense mechanisms. Initially identified as a heparin-binding antifungal from *P. bleo* (Kunth) [30], bleogen pB1 consists of only 36 residues. This positively charged mini-protein possesses a single domain stabilized by three disulfide bonds, with a structure comprising two antiparallel β-strands forming a cystine knot [52] (Loo et al., 2022). In a study by [53], the discovery of the first plant-derived and noncanonical agonist for the epidermal growth factor receptor (EGFR) is announced. This mini-protein, bleogen pB1, is notably the smallest known EGFR agonist, distinguished by its unique disulfide arrangement from all other known EGFR agonists. In vivo, similar to EGF, bleogen pB1 actively promotes skin wound healing, as reported previously [53]. Additionally, it expedites corneal wound healing while mitigating prolonged inflammation and reducing the accumulation of myofibroblasts [52]. Consequently, bleogen pB1 emerges as a potential bioactive component in P. bleo, contributing to its traditional application for wound healing.

Element content study using energy dispersive X-ray microanalysis revealed proximate mineral composition (% w/w) of *P. bleo* leaves, which 0.3% calcium, 0.4% phosphorus, and magnesium, 1.2% chlorine, 1.5% sulfur, 10.2% potassium, 35.4% oxygen and the highest percentage of 50.6 carbon [54].

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Higher potassium content makes it possible to lower blood pressure and avoid cardiovascular diseases [55], which proposes why *P. bleo* leaves are used in the treatment of hypertension. On the other hand, the crude protein, moisture, and ash content of mucilage from leaves are high [56]. Mucilage's high protein composition is renowned for its emulsifying properties [56]. Moreover, they have a far higher water-holding capacity (461.87%) than Arabic gum (7.49%), which could be responsible for facilitating bowel movement [37, 56].

The Pereskia genus appears to have outstanding potential; however, many species still need to be discovered or have just been scantily screened for their synthetic components. The immense diversity of its species merits extensive study and exploration by researchers. Numerous *P. bleo* components have remarkable pharmacological effects that should be addressed when evaluating their individual target site structure-activity correlations and other pharmaceutical applications.

PHARMACOLOGICAL PROPERTIES OF ACTIVE CONSTITUENTS

In this review, the main biological activities of *P. bleo* discussed include antioxidant, antiproliferation, and apoptotic, anti-diabetic, anti-nociceptive, and antimicrobial activities.

Antioxidant

Oxygen free radicals cause oxidative stress, which has been implicated in the initiation or progression of several chronic diseases, ranging from premature hair loss [57] to cancer [58], cardiovascular diseases [59], hypertension [60], neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's disease, and amyotrophic lateral sclerosis [60], obesity [57]. Oxidative stress is characterized by a biochemical imbalance between the production of reactive oxygen species (ROS), reactive nitrogen oxide species (RNOS), and the endogenous antioxidant defense system. The imbalance between oxidant and antioxidant species could lead to a rise in dangerous species that may alter the structure and function of biomolecules (proteins, lipids, and nucleic acids). An antioxidant system in the human body can typically scavenge free radicals, preserving the proper balance between oxidation and anti-oxidation. Even so, unhealthy lifestyles such as lack of exercise, fast-processed food intake, and exposure to various chemicals are the keys to oxidative stress induction. Therefore, an antioxidant may aid in lowering the potential harm, as the molecule is stable enough to donate and neutralize an electron to a raging free radical. These antioxidants prevent or delay cell damage in a way that reestablishs a proper and healthy cell metabolism, primarily by their ability to scavenge free radicals.

A study evaluated the antioxidant activity of two parts of P. bleo, leaves and stems [14]. The leaves of *P. bleo* had higher antioxidant properties than stem samples, based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging assay. Compared to other extractions, extraction with 100% ethyl acetate gave the highest antioxidant activity, with a moderate potential of antioxidant activities of 66.32% and 56.44% inhibition at 100 ppm of leaves and stems extract, respectively. The biphasic effects of ethyl acetate enable it to extract both polar and non-polar compounds. Moreover, this study indicates that the leaves of *P. bleo* have higher antioxidant activity than their stems because there is a correlation between total phenolic content and the inhibition's activity percentage.

On the other hand, [17] observed that the antioxidant activity of *P. bleo* ethanolic extract by Supercritical Fractioned Extraction was significantly influenced by pressure, temperature, CO2 flow rate, time, and modifier percentage. The final maximum extraction of antioxidant activity of *P. bleo* was optimized at the temperature of 52°C, time pressure of 36 MPa, and modifier amount of 31% (v/w) of the dry sample weight. To conclude, these studies indicate varied results based on different extraction methods. Therefore, all extraction factors must be considered to obtain the highest antioxidant activity and avoid the degradation of antioxidant compounds.

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ANTIPROLIFERATION AND APOPTOTIC ACTIVITIES

With more than 10 million deaths due to cancer in 2020, cancer remains the leading cause of death globally. The cancer cases with the highest incidence in 2020 were breast (2.26 million), lung (2.21 million), colon and rectum (1.93 million) and prostate (1.41 million) [61]. Cancer is characterized by the rapid development of abnormal cells that grow beyond their usual bounds. These cells then invade adjacent body parts and migrate to other organs; the latter event is known as metastasis. Widespread metastasis is the leading cause of cancer-related fatalities. Plants, on the other hand, have been documented to be a source of anticancer agents. Drugs such as Vincristine and Vinblastine, a vinca alkaloid derivative from Catharanthus roseus G. Don, and Paclitaxel, a taxane from Taxus brevifolia L, are readily available in clinical settings [62]. According to several studies, *P. bleo* has anticancer properties. Table 4 revealed the IC50 values (μg/mL) of the extracts in vitro of the different cell lines.

Table 4. IC_{50} values of *P. bleo* leaves extract on selected cancer and normal cell lines.

Cell lines	IC_{50} values (μg/mL)				Reference
	Hexane	Ethyl acetate	Methanol	Aqueous	
HeLa	278.01 ± 12.8	17.51 ± 8.6	683.47 ± 15.7	100.40 ± 2.3	[18]
MDA-MB-231	95.75 ± 27.9	19.39 ± 1.26	213.23 ± 27.7	224.31 ± 25.6	[18]
SW480	154.0 ± 2.0	31.80 ± 16.1	> 990	128.2 ± 7.5	[18]
NIH/3T3	275.0 ± 16.0	182.0 ± 23.0	631.0 ± 22.0	359.5 ± 27.5	[18]
CasKi	89.5	58	40.5	>100	[19]
KB	28	4.5	6.5	>100	[19]
HCT116	67.5	22	41	>100	[19]
MCF-7	25	28	39	>100	[19]
MRC-5	>100	>100	61	>100	[19]
4TI	N.I	N.I	>50	>50	[21]
T-47D	N.I	N.I	2	N.I	[22]

According to the National Cancer Institute (NCI), plant crude extracts should have an IC50 of ≤ 20 μg mL−1 for a potent cytotoxic effect. Hence, the study by [18] documented that *P. bleo* leaves ethyl acetate extract had the highest cytotoxic effect on HeLa and MDA-MB-231 cell lines; phytol was the most copious compound isolated from the extract. This study is in accordance with [20], which reported that phytol isolated from *P. bleo* leaves was found to have significant antitumor activity against some cancer cell lines (Table 5). Apart from the cytotoxic activities against cancer cell lines, no cytotoxic activity was recorded against the normal human fibroblast cell lines, MRC-5 [19].

IC50: 50% of maximum cell inhibition; HeLa: human cervical cancer cell lines; SW480: human colon cancer cell line; NIH/3T3: normal mouse fibroblast cell line; CasKi: human cervical cancer cell line; KB: human nasopharyngeal epidermoid cancer cell line; HCT116: human colon cancer cell line; MCF-7: hormone-dependent breast cancer cell line; MRC-5: normal human fibroblast cell lines; 4T1: mouse mammary cancer cell line; T-47D: human breast carcinoma cell line); N.I: not indicated.

The potent biological effects of this plant are associated with the phytochemical compounds present in the extract (Table 5). Phytol is believed to suppress tumor progression factor (glucose-6-phosphate dehydrogenase) in lung carcinoma cell lines and induces caspase 9 and 3 to trigger apoptosis [63]. In addition to the phytoconstituents listed in the table, [18] analysis also discovered notable substances such as loliolide, whose antioxidant and anti-proliferative properties have been noted, neophytadiene, which is well-known as an excellent antioxidant as well as γ-sitosterol. The compounds' synergistic effect may cause this extract's lethal effect on several cancer cell lines.

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Table 5. IC_{50} values of *P. bleo* cytotoxic compound on selected human cell line [20].

Cell lines	IC_{50} values (ug/ml)					
	CasKi	KB	HCT116	MCF-7	MRC-5	A549
Dihydroactinidiolide	40	6.7	5	30	91.3	97
β-sitosterol	62	>100	>100	72	>100	78
2,4-ditertbutylphenol	4.5	0.81	29	5.75	20	6
α-tocopherol	6	8	31	7.5	30.5	6
Phytol	18	7.1	100	34	74.1	31

IC_{50}: 50% of maximum cell inhibition; CasKi: human cervical cancer cell; KB: human nasopharyngeal epidermoid cancer cell; HCT116: human colon cancer cell; MCF-7: hormone-dependent breast cancer cell; MRC-5: normal human fibroblast cell; A549: human lung cancer cell.

Moreover, [22] in their study proposed that the apoptosis elicited by methanolic leaf extract on human breast carcinoma cell line (T-47D) cells was mediated via c-myc and caspase-3 pathways, as they activated death proteases, which catalyze the specific enzymatic cleavage of many cellular proteins. On the other hand, *P. bleo* may contain some prodrug, an inactive compound that, after ingested, is metabolized into a pharmacologically active drug [8]. At the same time, [64] suggested DNA intercalation as the anti-proliferative mechanism of this plant.

Apart from the report of cytotoxic effect against some cell lines, *P. bleo,* however, did not exhibit a discernible cytotoxic impact in several investigations. Differences in plant sources, extraction techniques, assay techniques, and cell lines could drive these discrepancies. To date, *P. bleo* in vivo anti-proliferative abilities have not yet been the subject of any reports. Hence, additional research is required to fully comprehend both the plant anti-proliferative action in vivo and the mechanism of cancer cell death.

Antihypertensive

Hypertension stands as a leading cause of premature mortality on a global scale. Approximately 1.28 billion adults aged 30–79 years worldwide grapple with hypertension [65]. The alarming aspect of hypertension lies in its association with increased risks of developing other chronic conditions, including cardiovascular and kidney diseases. It is crucial to emphasize evidence-based treatment for hypertension to reduce cardiovascular-related mortality significantly. Alongside this, there is a growing trend of patients incorporating herbal remedies alongside standard medication for hypertension [66, 67].

Several articles have acknowledged the use of *P. bleo* in controlling high blood pressure [8, 20]. In a study conducted by [25], a scientific evaluation was carried out to assess the effectiveness of 70% ethanol of *P. bleo* extract as an antihypertensive agent, specifically focusing on systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, and diuretic effects. The antihypertensive testing of the plant extract, administered at doses of 250, 500, and 1000 mg/kg body weight (BW), demonstrated its ability to reduce the blood pressure of hypertensive rats induced with 4% NaCl.

At a dose of 1000 mg/kg BW, the plant extract produced the most significant percentage reductions in SBP (59.38% ± 6.70%), DBP (22.67% ± 8.95%), and heart rate (24.20% ± 8.98%). Furthermore, the average urine volume, potassium, and sodium levels in the rat group treated with the dose of 1000 mg/kg were higher compared to other groups and similar to the positive control group that received captopril at 12.5 mg/kg BW. Sodium excretion in all treatment groups increased compared to the standard group, indicating a mechanism of natriuresis, which helps lower arterial blood pressure back to normal. Additionally, potassium excretion increased due to *P. bleo* extract's diuretic function, potentially reducing the risk of hyperkalemia [25].

The findings of this study indicated that *P. bleo* extract, acting as an antihypertensive agent, could effectively reduce blood pressure by influencing variables such as heart rate, diuresis, natriuresis, and vasodilation of blood vessels. However, the drawback associated with employing animal models for

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hypertension lies in the requirement for additional evidence to substantiate that the model accurately mirrors the occurrence of hypertension in humans. Currently, there is a need for more reports on the in vivo antihypertensive capabilities of P. blew. Therefore, further research is crucial to comprehensively understand both the plant's in vivo antihypertensive effects and its underlying mechanisms.

Anti-diabetic

Diabetes mellitus is a body metabolic derangement characterized by abnormally high blood glucose levels or hyperglycemia with carbohydrate, fat, and protein metabolism disturbances. Insulin resistance, insufficient insulin secretion, or excessive glucagon secretion commonly brings it about. Accompanied by several symptoms such as blurred vision, thirst, weight loss, and polyuria, long-term uncontrolled and untreated diabetes may trig damage, impairment, and failure of multiple organ systems including the blood vessels, eyes, kidneys, hearts, and nerves, resulting in disability and premature mortality [68]. Diabetes complications might be abated by a decline in the body's antioxidant defense system, increased oxidative stress, and dyslipidemia [69, 70]. These days, medicinal plants have become favored treatments for diabetes as they contain phytochemical compounds such as flavonoids, terpenoids, saponins, carotenoids, alkaloids, and glycosides that may exert anti-diabetic properties. Over the last decades, several studies have been dedicated to clarifying the mechanisms underlying the plant's anti-diabetic properties. Each active principle has distinct pharmacological actions in the biological system.

It was discovered that the flavonoid chrysin, a compound in *P. bleo,* could exert similar effects to metformin, a drug used to treat diabetes and reduce glucose and triglyceride levels [23]. They inhibited the production of pro-inflammatory cytokines associated with the development of diabetes and its possible repercussions, like atherosclerosis and other cardiovascular diseases [71]. In addition to that, [23], in their study stated that the presence of apigenin 6-C-glucoside and chrysin in aqueous extract of *P. bleo,* increasing insulin secretion by stimulating the pancreas, inhibits hormone-sensitive lipase activity in adipose tissue, and regulates the release of fatty acids during glycogen metabolism regulation. In alloxanized diabetic rats, aqueous *P. bleo* leaf, stem, and root extracts at 500 mg/kg reduced fasting plasma glucose levels by 66%, 65%, and 55%, respectively. It also reduces total cholesterol and triglyceride levels while maintaining HDL levels [23].

A recent study by [24] showed that the aqueous, chloroform, petroleum extracts and aqueous fraction of *P. bleo* leaves do not induce hypoglycemic action in non-diabetic rats and are capable of keeping the glycemic condition in streptozotocin (50 mg/kg)-induced diabetic Sprague Dawley rats. Even so, in the intraperitoneal glucose tolerance test, chloroform, petroleum ether, and aqueous extracts reduced blood sugar levels significantly ($p>0.05$); when compared to the diabetic control, aqueous extract and aqueous fraction significantly ($p>0.05$) reduced blood glucose levels in streptozotocin-induced diabetic rats as early as day six and restored the serum insulin. Furthermore, the aqueous extract and aqueous fraction reduced total cholesterol, triglycerides, and low-density lipoprotein levels. They portrayed an increase in high-density lipoprotein and improved body weight loss. Thus, the outcomes of these studies add to the body of evidence supporting P. bleo's traditional use as an anti-diabetic remedy for treating diabetes. Further research could influence the development of safe and effective plant-derived anti-diabetic medicines.

Anti-nociceptive

Antinociception blocks the body's sensory nervous system from detecting pain or potentially toxic stimuli such as mechanical injury, inimical temperature, extreme heat or cold, and harmful chemicals such as formalin and capsaicin [72]. Meanwhile, an anti-nociceptive agent is a compound that can reduce pain without impairing consciousness or causing anesthesia that works in varied modes of action. Pain modulation is an intricate process involving many mediators and receptors at the peripheral and central levels. Pain management using currently available anti-nociceptive agents or analgesics could not ultimately thrive in alleviating pain [73]. Worse, the effectiveness of these analgesics, such as opiates,

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morphine, and nonsteroidal anti-inflammatory drugs (NSAIDs), has been negated by a variety of side effects and linked to dependence and tolerance upon usage for an extensive time [72, 73]. Therefore, natural plant-derived compounds as the source and safe substitutes for analgesic drugs are a popular research area among healthy federal bodies and researchers.

Abdul-Wahab et al. [26] reported a moderate anti-nociceptive activity for all different extracts of *P. bleo* using the formalin-induced test, acetic acid-induced abdominal writhing, and hot plate test. The preparation included the ethanolic Soxhlet extraction for 72 hours, treatment with activated charcoal overnight, and a liquid–liquid partition yielding hexane, dichloromethane, ethyl acetate, and butanol extracts. A study by [27] evaluated anti-nociceptive activity by hot plate test and sitosterol from hexane, vitexin from ethyl acetate, hexane, ethyl acetate, and butanol fractions from P. bleo. The results indicate that *P. bleo* fractions, sitosterol, and vitexin possessed a central anti-nociceptive effect mediated by opioid receptors and nitrergic pathways. Further study on the mechanism of extract as an anti-nociceptive agent is well needed to look for *P. bleo* as a source of safe substitute analgesic drugs.

Antimicrobial

The World Health Organization (WHO) has declared that antimicrobial resistance (AMR) is one of humankind's top 10 global public health crises. AMR happens when microorganisms such as bacteria, viruses, fungi, and parasites evolve, develop the ability to defeat the antimicrobial agents designed to kill them, and multiply in their presence [74]. In contrast, multidrug resistance (MDR) is when a single microbial strain is resistant to more than one antimicrobial drug that is structurally unrelated and has different molecular targets. The discovery and use of natural compounds that may boost the antibacterial activity of standard antibiotics represent a possible alternative to the ongoing fight against the increased events of MDR. Due to their therapeutic characteristics, various molecules derived from plants have been employed in treating human ailments. Several investigations over the preceding years have shown that combining naturally occurring plant compounds with antibacterial drugs may become a novel approach to treating diseases caused by multidrug-resistant bacteria. The following antibacterial findings on *P. bleo* are listed in Table 6.

Antimicrobial chemicals derived from medicinal plants may suppress the development of microbes via distinct mechanisms from those currently available antimicrobial products. They demonstrated promising results in preventing the development of drug-resistant infectious agents [3, 75]. Moreover, according to [3], some natural chemicals may pose intrinsic antibacterial and antibiotic resistance-modifying properties; if they have low efficacy, combining natural chemicals with synthetic antibiotics may exhibit a synergistic effect and inhibit the growth of bacteria.

Plant-derived phenolics, such as phenolic acids, flavonoids, and tannins, stand out as promising alternatives to antimicrobial compounds that are known to involve many sites of action at the cellular level. Studies show that the increased lipophilicity of phenolic compounds boosts antimicrobial activity by facilitating their contact with cell membranes [76, 77]. This may cause the irrevocable aggregation of cell contents and cytoplasmic damage, inhibiting intracellular enzymes [76]. While the presence of the hydroxyl group in phenolic compound forms hydrogen bonds with the microbial enzyme's active site, this delocalized electron system is thought to be responsible for the cytoplasmic membrane destabilization and the collapse of the proton motive force, thus inhibiting the catalytic activity [78]. Conversely, Flavonoid hinders DNA synthesis and energy metabolism, which could impact how protein and RNA are synthesized [76, 79]. Intracellular pH alteration and ATP-generating system interference in Gram-positive bacteria were reported [76, 80]. However, up to this date, the structure-activity relationships and mechanisms of action of natural compounds of *P. bleo* have primarily remained elusive and still need to be fully deciphered.

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Table 6. The antibacterial range was reported in various extraction methods and experimental dosages of the *P. bleo* leaves part.

Extraction	Bacteria	MIC value (µg/mL) or Diameter (mm)							Reference
		HEX	DMET	EA	C	MET	ETOH	AQ	
Minimum inhibitory concentration (MIC)									
Cold extraction method	*Staphylococcus aureus*	1800	N.I	N.I	225	225	N.I	N.I	[15]
	Streptococcus pyogenes	1800	N.I	N.I	225	225	N.I	N.I	
	Pseudomonas aeruginosa	1800	N.I	N.I	450	450	N.I	N.I	
	Escherichia coli	1800	N.I	N.I	225	450	N.I	N.I	
Disc diffusion assay (6 mm diameter)									
Soxhlet extraction (100 mg/mL)	Methicillin-resistant *S. aureus*	-	11	-	N.I	-	N.I	N.I	[16]
	P. aeruginosa	14	7	7	N.I	9	N.I	N.I	
	Salmonella choleraesuis	14	-	-	N.I	9	N.I	N.I	
	Bacillus subtilis	-	-	-	N.I	-	N.I	N.I	
Soxhlet extraction (100 mg/mL)	*S. epidermis*	N.I	N.I	N.I	17	NI	-	24	[28]
	S. pyogenes	N.I	N.I	N.I	-	NI	-	28	
	Enterococcus faecium	N.I	N.I	N.I	13	NI	20	-	
	Micrococcus luteus	N.I	N.I	N.I	14	NI	22	20	
	Proteus vulgaris	N.I	N.I	N.I	15	NI	24	22	
Maceration (500 mg/mL)	*E. coli*	-	N.I	-	N.I	-	N.I	N.I	[29]
	P. aeruginosa	9.5	N.I	8.5	N.I	9.8	N.I	N.I	
	S. aureus	-	N.I	-	N.I	-	N.I	N.I	
	B. subtilis	8.2	N.I	7.8	N.I	-	N.I	N.I	

HEX: Hexane; DMET: Dichloromethane; EA: Ethyl acetate; C: Chloroform; MET: Methanol; ETOH: Ethanol; AQ: Aqueous; (-): no activity; N.I: not indicated.

On the other hand, plants produce a variety of defensive AMPs, many of which are cysteine-rich peptides (CRPs), such as cyclotides, defensins, knottins, hevein-like peptides (HLPs), snakins, and thionins [81]. Antimicrobial peptides (AMPs) are short polycationic peptides synthesized by all living organisms as essential components of their innate immunity. In eukaryotes, AMPs are the first line of defense against microbial attacks, while in prokaryotes, AMPs are produced as a competitive strategy to restrict the activity of other microorganisms [82, 83]. AMPs were shown to engage in various biological activities, including antimicrobial and anti-mitogenic agents and antitumor, anti-inflammatory, and immune-modulatory properties [84]. In combating the multi-resistance strain pathogens, AMPs are promising as they can exert potent microbicidal activity in the micromolar range, rapid bacterial death action, and low resistance selection [82, 84, 85, 86]. Their antibacterial mechanism is multifunctional as it alters the cell membrane and attacks specific targets involved in developing various intracellular processes, such as transcription, translation, protein synthesis, and bacterial cell wall formation [82, 84, 85, 86].

A study by [30] reported that HLPs known as bleogen pB1 are present in P. bleo and contain cystine-knot disulfide, β-sheets, and a four loops motif [81]. Bleogen pB1 demonstrated antifungal activity against C. albicans and C. tropicalis, with low micromolar MIC of 5µM and 10µM, respectively, while showing no cytotoxicity toward mammalian cells [30]. Moreover, a recent investigation by [31] indicated that *P. bleo* leaves extract displayed MIC values in the 25% v/v and 100% v/v concentrations against Aspergillus niger and C. albicans, respectively. The leaf extract also synergizes against both microbes when combined with itraconazole.

A study by [32] evaluated the antifungal activity of four yeasts, C. albicans, C. parapsilosis, Issatchenkia orientalis, and Cryptococcus neoformans; two molds Aspergillus brasiliensis and Trichophyton mentagrophytes against P.bleo extract. The findings revealed that the *P. bleo* ethyl acetate extract displayed a broad fungicidal activity spectrum against all tested microorganisms, with a MIC range of 0.31 to 2.50. The extracts with the most robust fungicidal activity were chloroform, methanol, hexane, and ethanol with 0.16 mg/mL minimum fungicidal concentration. Another study also revealed antifungal activity on Penicillium chrysogenum, Mucor haemalis, Saccharomyces cerevisiae, and C. albicans with 20

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mm, 23 mm, 24 mm, and 20 mm zones of inhibition, respectively, when treated with 5 mg aqueous leaves extracts of *P. bleo* [28].

In conclusion, plants produce a variety of bioactive secondary metabolites that could be used to fuel the future discovery pipeline. Evidence indicates that secondary plant metabolites and their offshoots have a wide range of biological functions, including potential antimicrobial agents. As the most prevalent plant metabolites with unique antioxidant activity structures, the phenolic compounds have proven to exert a potent antimicrobial action among the secondary metabolites considered. The inclination of these secondary metabolites to act as resistance-modifying agents is a critical attribute in lowering the prevalence of microbial resistance.

Other potential activity - Larvicidal

The majority of mosquito control methods have relied on synthetic organic insecticides. In addition to environmental contamination, increased risk of toxicity in humans, and the driving up of pesticide prices, the widespread use of synthetic organic insecticides has led to the physiological development of resistance in vector species [87]. Therefore, this necessitates the production of organic compounds that work at various stages of an insect's life cycle. Natural agents control vectors differently since they are less dangerous and poisonous than synthetic insecticides [88]. According to some studies, numerous plant species have larvicidal and insecticidal capabilities [89, 90, 91].

According to [92], a preliminary study of aqueous *P. bleo* fruit endocarp extract has produced more promising findings than aqueous leaf extract regarding larvicidal efficacy against A. aegypti. Therefore, they evaluate the larvicidal activities of crude and fractionated *P. bleo* fruit endocarp against third-instar larvae of A. aegypti. In this study, the fruit was extracted with distilled water and ethanol. It was discovered that the activity of the extract 50% lethal concentration (LC50) values at post-24-hour exposure were high. However, the 48-hour extended exposure revealed superior activities. The ethanol extracts demonstrated greater activity than the aqueous extracts, as crude and fractionated ethanolic extracts had lower LC50 values than the aqueous extract. The maximum toxicity was found in one of the fractionated groups of the ethanolic extract, with significantly lower LC50 values of 707.94 ppm and 223.12 ppm for 24- and 48-hour detection periods, respectively. In conclusion, it can be inferred that *P. bleo* fruit contains larvicidal properties against A. aegypti. Therefore, to further discover novel, effective larvicidal agents, a correspondence study to extract a pure compound should be done, and studies on the larvicidal impact of this plant on the other mosquito vectors are required.

CONCLUSION

In conclusion, *P. bleo* demonstrated promising biological activities, particularly as antimicrobials, antiproliferatives, and antioxidants. It could also be a potential larvicidal agent. As contended in several studies, the extraction techniques have affected the extraction of the active phytochemicals, which governed the biological activity. Unfortunately, there is currently a dearth of knowledge regarding the phytochemicals and other biological functions of P. bleo. Further investigation on the pharmacological properties and phytoconstituents of *P. bleo* is warranted to exploit further its potential as a source of novel phytochemical-based therapeutic interventions or lead compounds that would benefit the researcher and economic sector of pharmaceutical and food. Future research should focus on comprehensive characterizations of the phytochemicals with a focus on factors that influence the extraction of secondary metabolites to expand knowledge regarding their biological activities to support their commercialization prospect.

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AUTHOR'S CONTRIBUTION

Nur Azwa Hanim Azizan: Data Curation, Writing-Original Draft Preparation, Nurul Aili Zakaria: Supervision, Data Curation, Writing and Editing; Farizan Aris: Co-supervised progress Writing-Reviewing and Editing, Mohd Taufiq Mat Jalil: Writing-Reviewing and Editing, Norfatimah Mohamed Yunus: Writing-Reviewing and Editing, Syarifah Ab Rashid: Writing-Reviewing and Editing.

CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted without any self-benefits or commercial or financial conflicts and declare the absence of conflicting interests with the funders.

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