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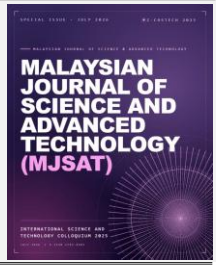

From Rhizome and Leaf to Bioactivity: Extraction Approaches and Bioactive Compounds of *Kaempferia pulchra*

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ABSTRACT

Kaempferia pulchra is an herbaceous plant of the Zingiberaceae family that has received increasing attention due to its secondary metabolite content and potential bioactivity. This narrative review aims to critically evaluate the extraction strategies used and their relationship with the types of bioactive compounds and biological activities reported. A qualitative literature review was conducted using major scientific databases to identify peer-reviewed studies on extraction techniques, solvent systems, and bioactivity. Dominant secondary metabolites were identified, including diterpenoids, flavonoids, phenolic compounds, and terpenoids. There is an increasing trend towards the use of modern extraction methods, which demonstrate higher efficiency and shorter extraction times than conventional methods. However, a critical evaluation of the available literature reveals notable gaps in the standardization of research methodologies, particularly regarding the types of solvents and extraction techniques employed. Moreover, studies that directly compare different extraction approaches used on *K. pulchra* remain limited. In conclusion, the existing studies discovered the potential of *K. pulchra* as a source of bioactive compounds with the variation of methods. Future studies are needed to isolate the key compounds for direct application in natural pharmaceutical products.

1. INTRODUCTION

The herbaceous perennial plant, *Kaempferia pulchra,* is a member of the family Zingiberaceae. The local name for this plant is cekur hitam. This plant is also known as "peacock ginger" due to its unique arrangement of leaves with small purplish flowers. This species is a rhizomatous geophyte that predominantly thrives in the humid tropical habitat. It is indigenous to Southern Thailand and Northern Peninsular Malaysia [1].

K. pulchra has a long history in local medicinal practices to treat various diseases. The rhizome and leaves of this herb are often used in traditional formulations for the management of constipation [2], as a carminative agent to treat digestive disorders [3], [4], and for the treatment of infectious coughs, diabetes [5]. In addition, *Kaempferia species* are also used externally as poultices to treat certain skin diseases or taken internally in the form of decoctions or infusions for the treatment of stomach disorders and other diseases involving the digestive and respiratory systems, which are partly attributed to the presence of bioactive compounds such as kaempferol [5].The variety of unique and structurally intricate bioactive compounds found in medicinal plants enables the creation and introduction of modern and novel therapeutic agents. Historically, these compounds have served as the foundation for drugs since they are naturally occurring [6] and carry far less toxicity as compared to their synthetic equivalents [7]. In recent years, there has been an upsurge in interest in medicinal plants and bioactive phytochemicals due to the increased global demand for natural products that are effective as well as safe [7]. Vastly classified bioactive phytochemicals include flavonoids, terpenoids, phenolic acids, and alkaloids, and these constituents have proven to possess a variety of desirable biological attributes, such as anticancer, antimicrobial, and anti-inflammatory activities, as well as acting as antioxidants.

While extensive phytochemical and pharmacological profile investigations have been conducted on *Kaempferia* species, for instances *K. galanga* (kencur), *K. rotunda* (kunir putih), and *K. pandurata* (temu kunci) and *K. pulchra* (cekur hitam) have received comparatively limited scientific attention, despite their established ethnomedicinal relevance [8]. Although several studies [2], [4], [9], [10] have reported the

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presence of diterpenoids, flavonoids, phenolics, saponins, alkaloid compounds, and terpenoids in *K. pulchra*, the available literature is still scattered and descriptive. In particular, there is a lack of integrated analyses examining how different extraction methods and solvent systems affect the phytochemical profile and biological activities of *K. pulchra* extracts. Furthermore, existing discussions often rely on general phytochemical patterns within the Zingiberaceae family or make assumptions based on findings from other *Kaempferia* species, which may obscure species-specific characteristics and limit the translational value of these findings for *K. pulchra*.

Therefore, the present review aims to systematically examine various extraction methods utilized for isolating bioactive compounds from *K. pulchra*, evaluating the influence of extraction solvents and techniques on phytochemical constituents. Moreover, it discusses the biological activities of these isolated compounds, emphasizing their potential therapeutic applications and providing insights for future research.

2. METHODOLOGY

A comprehensive literature search was conducted between 2004 and 2024 as a search timeline to capture relevant peer-reviewed studies published within the last twenty years. Literature search was performed using multiple electronic databases including ScienceDirect, Scopus, PubMed, Web of Science, Google Scholar, Google Search Engine, and Google Patents. The literature search was conducted using a Boolean search strategy that combined species-specific terms, methodology, phytochemistry, and bioactivity. The search included the terms "*Kaempferia pulchra*" or "*K. pulchra*" along with "*Zingiberaceae*" and combined using the AND operator with extraction-related terms such as "*extraction method*", "*maceration*", "*Soxhlet*", and "*ultrasound-assisted extraction* (UAE)". These terms were then linked with phytochemical-related keywords, including "*bioactive compounds*", "*flavonoids*", "*terpenoids*", "*phenolics*", and "*alkaloids*", as well as biological activity-related terms such as "*antibacterial*", "*antioxidant*", "*anti-inflammatory*", "*anticancer*", "*antidiabetic*", and "*UV protection*". As this study was a narrative review, the search strategy was iteratively applied and refined during the screening process to prioritize relevance to extraction strategies and bioactivity results specific to *Kaempferia pulchra*.

Only peer-reviewed articles published in English clearly mentioning plant authentication, voucher specimen deposition, extraction techniques, and biological activities were included. A total of 46 relevant references from journal articles and books were ultimately included in this review. Relevant data, including extraction methods, solvents used, isolated compound classes, and reported biological activities, were compiled and qualitatively synthesised. The collected information was critically analysed to summarise existing evidence, identify methodological trends, and highlight research gaps related to extraction strategies and bioactivity outcomes in *Kaempferia pulchra*.

3. RESULTS AND DISCUSSION

3.1 Extraction techniques for Kaempferia pulchra

The first step in natural drug discovery from plant sources is to identify each biological activity of plant compounds. Therefore, to obtain plant compounds, plant extraction is crucial to isolating plant metabolites from plant cells using either conventional extraction methods or modern extraction methods [11]. The chosen method must be cost-effective, easy to use, and also environmentally friendly. This method requires the use of organic solvents, and the extraction will be successful with high yields when the selection of the solvent type is appropriate [12]. Table 1 shows in detail the extraction methods used for *K. pulchra* with specific solvents, as well as the biological activities obtained from *K. pulchra* extracts.

Table 1. Extraction methods and the *K. pulchra* extracts' biological activities

Extraction technique	Solvent	Duration	Phytochemical compounds	Biological activities
Maceration	Ethanol	48 h	Alkaloid, flavonoid, saponin, phenolic	Antimicrobial
	Methanol	-	Clerodane-type diterpenoids, Propadane A, Propadane B, Propadane C, Kolavelool, 2β hydroxykolavelool	Antimicrobial
Soxhlet	Water	72 h	phytol, hexadecanoic acid methyl ester, hexahydrofarnesyl acetone, dibutyl phthalate, 9,12-octadecenoic acid, methyl ester, methyl linolenate, β-farnesene, terpenoid, membrane, L-bornyl acetate, aromadendrene, ledol, dehydrobietan, and borneol	Antioxidant and anti-diabetic
	Hexane, Chloroform, Methanol	-	2α-Acetoxysandaracopimaradien-14α-ol and Sandaracopimaradien-12,22-diol	Anti-inflammatory
UAE	Chloroform	90 min	Diterpenoids (kaempulchraols A-H)	Anti-cancer and Anti-inflammatory
	Ethanol	30 min	Flavonoids and Phenylpropanoids (Flavokawain B, Ethyl p methoxycinnamate, Methoxyflavones)	Antioxidant ad UV-protecting agent

Several conventional extraction methods have been employed to isolate bioactive compounds from *K. pulchra*. The most commonly utilized techniques include maceration, Soxhlet extraction, and ultrasound-assisted extraction (UAE). However, no studies have been conducted with direct and standardized comparisons under the same solvent system or extraction conditions, such as duration and temperature. Therefore, the differences in biological activity reported cannot be attributed solely to the extraction technique itself, but need to consider the polarity of the solvent, the duration of extraction, as well as variations of biological activities that have been attributed to each of the plant compounds.

Maceration can also be identified as a soaking method [13]. The extraction solvent is typically ethanol, methanol, water, and acetone, a widely used solvent in plant extraction due to its simple extraction method involving soaking powdered plant materials, such as leaves, rhizomes or flowers, into a container with organic solvents at room temperature for several days [10], [14]. This method has the advantage of simplicity and does not require complex equipment at a low temperature setting [15], making it suitable for thermosensitive compounds and reduced energy requirement [16]. However, it is time-consuming and requires a large amount of solvent compared to modern extraction methods [17].

The reviewed studies show that the choice of solvent in the same extraction technique can produce significantly different secondary metabolite profiles in *K. pulchra,* thus highlighting an important source of method diversity. For example, the 48-hour ethanol maceration method yielded mostly polar and semi-polar compounds such as alkaloids, flavonoids, saponins, and phenolic compounds that were associated with antimicrobial [10]. In contrast, methanol maceration, despite using a similar extraction approach, resulted in the recovery of clerodane-type diterpenoid compounds such as Propadane A, Propadane B, Propadane C, kolavelool, and 2β-hydroxykolavelool that also showed antimicrobial effects [2]. This difference can be explained by the higher polarity and broader solubility of methanol compared to ethanol, which allows methanol to dissolve not only polar compounds but also moderately lipophilic diterpenoids. Ethanol, which is slightly less polar, tends to extract compounds such as phenolics, flavonoids, saponins, and alkaloids. Therefore, the antimicrobial activity reported in maceration studies may be due to different classes of compounds, depending on the choice of solvent [18].

These differences indicate that variations in the polarity and solubilization capacity of the solvent, rather than the extraction duration alone, play a significant role in shaping the phytochemical composition and bioactivity observed in *K. pulchra* extracts. Therefore, the antimicrobial activities reported in various studies may be due to different classes of compounds depending on the choice of solvent, making it difficult to directly compare efficacy between studies. These findings highlight the need for more standardized or comparative solvent studies to accurately identify the biological activities associated with specific classes of metabolites in *K. pulchra*.

The process of Soxhlet extraction entails continuous hot extraction. Like many others, Soxhlet is one of the first that comes to mind because it is considered to be particularly efficient for target compounds of large metabolite compounds, as it employs a continuous heating and solvent recycling method. Some of the solvent extractions can include water, hexane, chloroform, acetone, propanol, methanol, ethanol and ethyl acetate [19], [20]. As to the apparatus setup, it consists of a bottom flask, an extraction chamber, a siphon tube, and a condenser on top. Solvents are placed in the bottom flask, while the powdered plant materials are put in the thimble or porous bag. The moment heat is applied, extraction starts. Heat induces vaporization of the organic solvents. The organic solvents then go to the condenser and then fall to the extraction chamber, where the organic solvents interact with the plant metabolites and dissolve them [21]. For example, by using water as a solvent for 72 hours, Soxhlet extraction isolated phytol, hexadecanoic acid methyl ester, dibutyl phthalate, hexahydrofarnesyl acetone, methyl linolenate, β-farnesene, terpenoids, aromadendrene, ledol, dehydrobietan, and borneol, exhibiting antioxidant and antidiabetic activities [3]. Furthermore, the use of sequential Soxhlet extraction with hexane, chloroform, and methanol isolated anti-inflammatory diterpenoids 2α-acetoxysandaracopimaradien-14α-ol and sandaracopimaradien-12,22-diol [9], [22]. From the methodological standpoint, Soxhlet and maceration are particularly simple to perform, cost-effective, and effective in extracting a wide range of plant compounds. However, they have a few disadvantages, such as the lengthy extraction time at higher temperatures may lead to degradation of bioactive compounds, a negative effect on the environment due to overuse of organic solvents, and a complex setup during the extraction process [23]. To date, no studies have quantified the degradation potential of *K. pulchra* thermolabile plant compounds under prolonged extraction conditions for the Soxhlet and maceration processes.

Modern extraction techniques like ultrasound-assisted extraction (UAE) can overcome these limitations by reducing extraction time and increasing extract yields of specific bioactive compounds such as phenolics, flavonoids, and oils. UAE is an advanced extraction method. Its working principle is based on the disruption of plant cells by using sound waves to create tiny bubbles in the liquid known as the acoustic cavitation process. These bubbles grow and then collapse suddenly, producing high temperatures and pressure in small areas. This helps improve the extraction process of secondary metabolites in several ways. It breaks the plant material into smaller pieces, causing surface erosion of materials, and breaks the cell walls. Subsequently, this mechanism increases the mass transfer within the plant cell and the solvent due to increasing solvent penetration and cell permeability [24], [25]. Such impacts include higher extraction yields at lower temperatures, lower energy and solvent consumption, and faster processing times relative to conventional means. UAE is capable of low thermal extraction and can retain the functionality of secondary metabolites extracted from the plant [26]. UAE with chloroform has been used for 90 minutes to obtain the anticancer and anti-inflammatory derivatives of diterpenoids, kaempulchraols A–H [27], [28]. Reported that the UAE of ethanol for 30 minutes extracted Flavokawain B, along with ethyl p-methoxycinnamate, and methoxyflavones, which exhibited good antioxidant activity and potential for use as UV-protecting agents [29]. Taken together, these findings indicate that differences solvents polarity and duration during UAE extraction. The difference in extraction time between chloroform (90 min) and ethanol (30 min) in the UAE method of *K. pulchra* reflects the mass transfer kinetics and compound–solvent interactions that depend on the type of solvent, rather than a method incompatibility. Chloroform is a non-polar solvent with low polarity that primarily solubilizes lipophilic diterpenoids, which are typically embedded in hydrophobic cell matrices such as resinous parts or membrane-associated compartments [30]. Therefore, longer sonication times are required to facilitate cavitation-induced disruption of plant tissues and enhance the diffusion of these compounds into the solvent phase [31]. In contrast, ethanol is a polar solvent with strong hydrogen bonding capabilities, allowing rapid solubilization of polar and semi-polar compounds, including flavonoids and phenylpropanoids. These compounds are generally more accessible in plant cells and diffuse more rapidly into ethanol, allowing for shorter extraction times without compromising yields [32]. Additionally, prolonged ultrasonic exposure in ethanol may increase the risk of oxidation or degradation of plant compounds, especially flavonoids and phenolics, making shorter extraction times more suitable to maintain bioactivity. Typically, 60 °C minutes at 40 °C is the optimal condition to obtain plant compounds without the risk of degradation, as it is in accordance with previous studies by [27], [29]. However, the extraction period can be extended if lower temperatures are used [29], [33], [34]. In contrast, diterpenoids have a more stable structure compared to certain flavonoids and

phenolics that are less susceptible to ultrasonic-induced degradation, thus allowing for longer extraction times [33], [35].

In conclusion, evaluation of the extraction technique *K. pulchra* as a whole, a consistent pattern can be seen as polarity of solvent, times of extraction plays a major role in obtaining types of plant compounds, while the extraction technique influences efficiency as well as potential degradation risk.

3.2 Biological activities of Kaempferia pulchra extracts

3.2.1 Antibacterial

K. pulchra, as an unfamiliar member of the Zingiberaceae family, has recently gained attention for its diverse bioactive properties and is traditionally used across Southeast Asia to treat ailments such as coughs, fever, and gastrointestinal disorders [36].

A number of researchers have studied the antibacterial properties in *K. pulchra*, and the alternative method to increase antibacterial activity [2], [3], [10], [37], [38], [39]. According to [37] the presence of lipophilic diterpenoids, particularly clerodane-type diterpenoids (propadanes A, B, C) and labdane-type diterpenoids (anticopalic acid and anticopalol diterpenoids), was identified as a valuable secondary metabolite phytochemical in *K. pulchra.* Clerodane and labdane were identified and separated, which were constituents within the *K. pulchra* rhizomes that were aimed at serving as antibacterial were identified, a common member of the class of naturally occurring organic compounds, a bicyclic diterpenoid that is abundantly located in plants, more so within essential oils and resins. Labdane displayed potent antibacterial activity against *Bacillus cereus,* a gram-positive bacterium [37]. This finding is consistent with the fact that lipophilic diterpenoid compounds in *Kaempferia* spp. are effective in targeting Gram-positive bacteria because Gram-positive bacteria lack a lipopolysaccharide outer membrane. The absence of this outer membrane allows labdane compounds to interact directly with the cell membrane and cytoplasm, leading to cell lysis [40], [41]. Collectively, these findings indicate that lipophilic diterpenoid-rich fractions represent a major antibacterial component of *K. pulchra*, particularly against Gram-positive bacteria, suggesting a compound class–specific contribution rather than a uniform effect across all phytochemical groups.

Antibacterial activity can be determined based on the standard guidelines of the Clinical and Laboratory Standards Institute (CLSI), specifically by the Kirby-Bauer disc diffusion method [42]. This approach has been used by several researchers to evaluate the antibacterial potential of *K. pulchra* rhizome and leaf extracts, where the obtained samples were analyzed to identify the phytochemical profile before being tested using the method to determine the effectiveness against bacterial growth. The results of phytochemical screening on *K. pulchra* confirmed the presence of alkaloids, flavonoids, saponins, anthraquinone glycoside, terpenoids, and steroids in the water, methanol, ethyl acetate, and n-hexane macerated extracts. These extracts showed inhibitory action in the Kirby–Bauer disc diffusion method against seven pathogenic bacteria that cause respiratory tract infections. The most active of these was the ethyl acetate extract, with inhibition zones of 17.3 ± 0.57 mm against *Lactobacillus acidophilus*, 16.6 ± 0.28 mm against *Streptococcus pneumonia* and *Streptococcus pyogenes*, and 15.3 ± 0.28 mm against *Pseudomonas aeruginosa*. Phytochemical profiling showed that terpenoids and steroids were most abundant in the ethyl acetate fraction, while anthraquinone glycosides, alkaloids, and flavonoids were minor constituents. The low concentration of flavonoids and phenolics in the fraction suggests that these compounds were not the major antibacterial constituents [2], [10]. This pattern highlights a clear solvent-dependent trend, whereby extracts enriched in moderately lipophilic diterpenoids exhibit stronger antibacterial effects than extracts containing low levels of phenolic and flavonoid constituents. However, this difference does not necessarily indicate that phenolics and flavonoids are intrinsically less active but may be influenced by the relative levels of these compounds in the fractions tested. Antibacterial activity is highly dose dependent. Therefore, low phenolic and flavonoid content may limit biological effects, even though their mechanism of action may involve important intracellular targets [43]. Thus, the observed solvent-dependent trends may reflect differences in the concentration and bioavailability of bioactive compounds in each fraction, rather than an absolute superiority of one group of phytochemicals over another. Other studies have noted similar findings where ethyl acetate extracts showed significant antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, yielding Kirby–Bauer disc diffusion method inhibition zones of 5.32 ± 0.12 mm and 5.21 ± 0.01 mm, respectively [3]. When critically compared, these two findings show a similar pattern, with ethyl acetate extract exhibiting significant antimicrobial activity. However, the magnitude of the inhibition zone differed significantly between studies.

In the first study, ethyl acetate extract showed relatively large inhibition zones, such as 17.3 ± 0.57 mm against Lactobacillus acidophilus, 16.6 ± 0.28 mm against Streptococcus pneumoniae and Streptococcus pyogenes, and 15.3 ± 0.28 mm against Pseudomonas aeruginosa. These values indicate strong antibacterial activity, especially against Gram-positive bacteria. In contrast, a study by Solanki et al. (2023) reported much smaller inhibition zones for ethyl acetate extract, namely 5.32 ± 0.12 mm against Staphylococcus aureus and 5.21 ± 0.01 mm against Escherichia coli. Although both studies showed a tendency for activity by ethyl acetate extract, the almost threefold difference in the diameter of the inhibition zone indicates that the strength of activity was not uniform across studies. This difference is likely influenced by several methodological factors, including the concentration of recovered plant compounds, the types of plant compounds obtained, and the type of bacterial strain. In addition, variations in the content of diterpenoids and other semi-lipophilic compounds in the ethyl acetate fraction may influence the observed antibacterial potency. Therefore, although both studies support the role of the ethyl acetate extract as the active fraction, direct comparisons of the magnitude of the inhibition zones should be interpreted with caution, as these values are highly dependent on the experimental conditions and the chemical composition of the extract tested.

The antibacterial activity of the isolated compounds of the acetone extracts, done using the Kirby–Bauer disc diffusion method, showed that the diterpenoid (crotepoxide) and the benzyl benzoate (ester) had less activity than their corresponding crude acetone extract and n-hexane fraction. Benzyl benzoate had moderate activity against *Bacillus cereus* (5.9–9.9 mm inhibition zone at 50–500 µg/mL) and inhibited *E. coli* (5.2–7.0 mm), *S. aureus* (6.1–9.1 mm), and *Enterobacter aerogenes* (8.9 mm at 500 µg/mL) as well. In contrast, crotepoxide only showed moderate activity against *E. aerogenes* (6.1–8.6 mm at 100–500 µg/mL) and *B. cereus* (7.0 mm at 500 µg/mL) [38]. In conclusion, all the researchers mentioned agree that diterpenoid compounds are the main source of antibacterial activity in *K. pulchra*.

Another study by [39] stated that to enhance antimicrobial activity, a delipidation step is required to remove inactive lipid components that may interfere with the efficacy of bioactive compounds. After this process, the fractions that are more

concentrated in active compounds have the potential to show higher inhibitory effects on bacteria. The purified ethanol extract by the de-lipidation process exhibited strong *S. aureus* antimicrobial activity with a MIC_{50} of 0.5 µg/mL and showed anti-biofilm activity with IC_{50} values of 0.125 mg/mL for biofilm formation inhibition and 0.5 mg/mL for biofilm degradation against *S. aureus* and *P. aeruginosa*. The activity is related to bioactive compounds such as alkaloids, flavonoids, and terpenoids. Basically, antimicrobial activity is determined by the ability of bioactive compounds to disrupt the structure and function of bacterial cell membranes, inhibit protein synthesis, or affect the stability of essential components such as nucleic acids. In this context, the presence of compounds such as alkaloids, flavonoids, and terpenoids is believed to play a major role through synergistic mechanisms that result in membrane leakage, lipid oxidation, and interference with bacterial enzymatic systems. The observed anti-biofilm activity also indicates that the active components in the extract are able to inhibit the initial adhesion of bacteria and reduce the integrity of the extracellular polysaccharide matrix that is important in biofilm formation.

Overall, these findings reinforce the view that the reduced activity of isolated compounds relative to crude extracts indicates that antibacterial efficacy in *K. pulchra* is not solely dependent on single molecules but is likely enhanced by synergistic interactions among multiple secondary metabolites. The relatively low bioactivity of purified compounds as compared to their crude extract counterparts suggests that multiple secondary metabolites are likely to operate in synergy in the crude mixtures. Such interactions may act in the same way as the constitutive elements, but potentiating the antibacterial activity, a phenomenon that has been extensively studied in phytomedicine.

3.2.2 Antioxidant

There are 3 researchers that discover antioxidant properties in *K. pulchra*. Table 2 shows in detail the extraction methods used for *K. pulchra* with specific solvents, as well as the antioxidant test obtained from *K. pulchra* extracts.

Table 2. Comparison of Extraction Techniques, Solvents, and Antioxidant Activity of *K. pulchra*

Plant Part / Solvent	Extraction Method	DPPH	FRAP	ABTS	Interpretation / Main Findings	Ref
Rhizome and leaf / Water	Aqueous extract	✓	✓		Rhizome shows the strongest radical scavenging; phenolic and flavonoid content correlate with antioxidant activity.	[3]
Rhizome / 95% Ethanol	Sonication	✓	✓	✓	Moderate antioxidant activity; weak against DPPH, moderate for ABTS and FRAP; comparison with K. elegans, K. galanga, K. parviflora.	[29]
Rhizome / EtOH, EtOAc, n-Hexane	Reflux	✓	✓	✓	Moderate antioxidant activity; polar solvents are more effective; prolonged heating may degrade thermolabile flavonoid glycosides.	[10]

To evaluate the antioxidant potential of *K. pulchra* rhizome and leaf extract, two tests were conducted: 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and ferric reducing antioxidant power (FRAP) assay [3]. The aqueous rhizome extract demonstrated the strongest free radical scavenging activity (RSA) against DPPH radicals, exhibiting an IC_{50} = 420 µg/mL compared to an IC_{50} = 520 µg/mL leaf extracts. The rhizome extracts of *K. pulchra* at a concentration of 500 µg/mL have been reported to exhibit the highest reducing capacity in the FRAP assay, measured at 28.24 ± 0.62% inhibition, followed by the leaf extract at 27.31 ± 0.92% inhibition. The aqueous extract also recorded the highest total phenolic content (TPC) and total flavonoid content (TFC) for leaf extract at 31.75 ± 0.44 mg GAE/g and 39.46±0.1 mg QUE/g, respectively, followed by rhizome extract TPC and TFC value of 27 ± 0.36 mg GAE/g and 27.30 ± 0.43 mg QUE/g, respectively. Phenolic and flavonoid constituents contribute significantly to antioxidant activity [44] by donating hydrogen atoms or electrons to neutralise free radicals, as well as by chelating transition metals involved in oxidative processes.

Another study was done by [29] regarding the antioxidant activity of *K. pulchra* rhizome parts. The extraction was done with the sonication method using 95% ethanol as the solvent extractor. The antioxidant test was conducted with 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay, DPPH, and FRAP. Results found that *K. pulchra* has moderate antioxidant properties with values of 54.87 ± 2.45 µM TE g^{-1} DW (ABTS), 19.60± 0.17 µM TE g^{-1} DW (DPPH), and 43.00 ± 0.43 µM TE g^{-1} DW (FRAP) by quantitatively analysing the antioxidant capacity of plant extract, which was compared to standard Trolox and expressed as micromoles of Trolox equivalents (TE) per gram dry extract weight (µM TE g^{-1} DW). In this study, *K. pulchra* shows moderately antioxidant activity as compared to *K. elegans*, *K. galanga* and *K. parviflora* that range between 53.39 – 369.21 µM TE g^{-1} DW for ABTS, 26.16-91.73 µM TE g^{-1} DW DPPH, and FRAP range values of 42.30-155.41 µM TE g^{-1} DW. *K. pulchra* falls under this range for ABTS and FRAP but has weak activities against DPPH radicals. This necessitates TPC analysis before embarking on the antioxidant test.

In another investigation [10], the ethanolic extract of *K. pulchra* rhizome, prepared via reflux extraction, exhibited moderate DPPH radical scavenging activity (IC_{50} = 185.92 µg/mL). Phytochemical screening confirmed the presence of flavonoids, saponins, triterpenoids, and volatile oils, which are recognized for their electron-donating and radical-stabilizing capacities. However, the relatively higher IC_{50} suggests that prolonged heating during reflux may degrade thermolabile compounds, such as certain flavonoid glycosides, thereby reducing antioxidant potential. This underlines the necessity of quantifying total flavonoid content (TFC) prior to antioxidant assays to account for solvent and extraction-induced variations.

The ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) assay further revealed substantial antioxidant capacity in the ethyl acetate extract, with a TEAC (Trolox Equivalent Antioxidant Capacity) value of 298.15 mg TEAC/g extract, compared to 275.43 mg TEAC/g for the ethanol extract and 64.27 mg TEAC/g for the hexane extract. Using the FRAP (Ferric Reducing Antioxidant Power) method, the ethanolic extract achieved the highest reduced capacity, measured at 121.38 ± 4.12 µM Trolox/g dry weight. These findings suggest that *K. pulchra* contains a diverse array of phenolic and flavonoid compounds whose antioxidant efficacy varies according to extraction solvent polarity and processing conditions.

Overall, all three researchers agree that *K. pulchra* has antioxidant activity that is closely related to its phenolic and flavonoid content. However, the reported levels of activity vary, influenced by the type of solvent, extraction technique, and antioxidant assay method. These differences emphasize that, despite the recognized antioxidant potential, methodological standardization is needed to allow for more consistent comparisons between studies.

3.2.3 Anti-inflammatory

Anti-inflammatory activity was detected in *K. pulchra* and it was attributed to two diterpenes, namely 2 α-acetoxysandraracopimaradien-1 α-ol and sandaracopimaradien-1 α, 2 α-diol, which could reduce rat ear edema induced by phorbol ester [9]. A study evaluated the anti-inflammatory activity of *K. pulchra* rhizome through sonication with chloroform as the solvent extractor [27]. The anti-inflammation test was done on isopimarane diterpenoids (isopimara-8(9),15-diene diterpenoids, and isopimara-8-(14),15-diene diterpenoids), which are able to suppress inflammatory mediators like nitric oxide. For instance, kaempulchraols P and Q have effectively inhibited NF-κB-mediated transactivation, IL-6 production, and COX-2 expression and suppressed nitric oxide production in LPS-stimulated RAW264.7 macrophages, indicating that it's able to treat detrimental conditions with excessive nitric oxide production, like tissue damage and immune disorders.

However, the evidence is still limited to a few in vivo and in vitro studies, with little quantitative data on efficacy doses or comparisons with commercial anti-inflammatory standards. Therefore, although *K. pulchra* shows promising potential, additional studies are needed to confirm the mechanism, bioactive compound profile, and clinical efficacy. In the context of other *Kaempferia* spp. Several species also show anti-inflammatory activity, but specialization to *K. pulchra* is important to ensure the relevance of the data and the accuracy of the conclusions.

3.2.4 UV-protecting agents

A study had tested the ultraviolet (UV) protective properties of *K. pulchra* ethanolic extract using a UV-Vis spectrophotometer that records absorbance at UVA range (320-400 nm) and UVB range (290-320 nm), showing insufficient UVA, UVB protection based on the Boots star rating system [29]. However, it shows significant values for the sun protection factor (SPF) indicator with values of 1.40 μg mL^{-1} at a 100 μg mL^{-1} concentration of crude extract. In contrast, *K. rotunda*, *K. angustiflora* and *K. marginata* showed significantly lower SPF value with values of 1.10 μg mL^{-1}, 0.78 μg $mL^{-1,}$ and 0.50 μg $mL^{-1,}$ respectively. This indicates potential for *K. pulchra* extract to be used for UV protection.

However, the evidence is still limited to a single study, with data focusing on SPF alone without in vivo testing or cosmetic formulations. Therefore, while the potential for UV protection appears promising, additional studies are needed to assess efficacy on real skin, extract stability, and mechanisms of UV absorption.

3.2.5 Anti-cancer

Cancer continues to represent a global health challenge, with natural products gaining increasing attention as sources of novel chemotherapeutic agents due to their pleiotropic mechanisms and generally lower toxicity [45]. Although direct anticancer studies on *K. pulchra* remain limited, several pieces of evidence from related *Kaempferia* species or exact species provide valuable insights into its potential bioactivity. For instance, two compounds known as kolavelool and 2β-hydroxykolavelool have been found in the rhizome, which exhibit the ability to kill HL-60 leukaemia cancer cells [2]. Kaempferol F and kaempulchraol L are from the diterpenoid group and were isolated from the rhizome, and they possess the highest antiproliferative activity against human pancreatic cancer cells, specifically PANC-1 and PSN-1 [28].

In addition, *K. pulchra* extracts may also limit cell cycle progression and thus metastasis by G0/G1 and G2/M phase arrest. The terpenoids and diarylheptanoids of this genus are suggested to target angiogenesis, cell survival, and intercellular signaling through modulation of the PI3K/Akt pathway. Support for this comes from *K. parviflora* (black ginger) studies, where polymethoxyflavones are reported to induce mitochondria-mediated apoptosis, increase mitochondrial oxidative bursts, membrane potential depolarisation, and markedly less migration and invasion of melanoma by inhibition of MMP-2 and MMP-9 [46].

Alongside these direct methods, the antioxidant activity of *K. pulchra* phytochemicals may contribute to anticancer activity through the mitigation of oxidative DNA, one of the foundational causes of cancer, which is often considered a form of oxidative injury. The anticancer activity of flavonoids, alkaloids, and terpenoids in combination may also be appreciated from the enhanced cell-destructive ability directed at cancerous cells, which spares the host tissues. At the same time, the current evidence rests largely on in vitro assays and detailed in vivo studies; pharmacokinetics and toxicity studies of *K. pulchra* are necessary to prove the therapeutic potential of the plant.

Plants of the Zingiberaceae family are undoubtedly a source of secondary metabolites that can target cancer cell survival, proliferation, and metastasis. Direct anticancer studies on *Kaempferia pulchra* are sparse, but the phytochemicals of the plant, along with the related Kaempferia species, strongly suggest its use as an anticancer agent.

UAE of *K. pulchra* rhizomes has produced kaempulchraols A-H, a group of diterpenoids that have been reported to exhibit cytotoxicity towards leukemia (HL-60) and breast (MCF-7) cancer cell lines [28]. These compounds induce apoptotic cell death through caspase-dependent mechanisms as well as through the disruption of the mitochondrial membrane potential. Likewise, labdane and clerodane type diterpenoids from *K. pulchra* have shown antiproliferative activity against several cancer cell lines [2].

Flavonoids and phenolic compounds isolated from *K. pulchra* are also proposed to exert anticancer effects by modulating cell cycle progression. These compounds downregulate CDK and alter the cell cycle to G0/G1 or G2/M phases, therefore slowing down unchecked proliferation. Also, from this genus, terpenoids and diarylheptanoids have been noted to target the PI3K/Akt signalling pathway, which is important for the regulation of tumour growth, angiogenesis, and metastasis [46].

Oxidative DNA damage and inflammation, coupled with delayed tumor progression, may collaboratively benefit from the *K. pulchra* vascular plant's anticancer properties, which are indirectly associated with it. However, these studies are still in an elementary stage. From in vitro models, much more is needed, including in vivo studies and pharmacokinetics, to actually prove empirically that *K. pulchra* is a plant with anticancer effects, as shown in Table 3.

Table 3. Anti-cancer activity and mechanism of action towards cells target in *K. pulchra*

Compound	Class	Cancer Cell Target	Anticancer Mechanism	Ref
Kolavelool	Diterpenoid	HL-60 (leukemia)	Direct cytotoxic activity against leukemia cells	[2]

2β-hydroxykolavelool	Diterpenoid	HL-60 (leukemia)	Direct cytotoxic activity against leukemia cells	[2]
Kaempulchraol F	Diterpenoid	PANC-1, PSN-1 (pancreatic cancer)	High antiproliferative activity	[28]
Kaempulchraol L	Diterpenoid	PANC-1, PSN-1 (pancreatic cancer)	High antiproliferative activity	[28]
Kaempulchraols A–H	Diterpenoid	HL-60 (leukemia), MCF-7 (breast cancer)	Induce apoptosis via caspase-dependent mechanisms and mitochondrial membrane disruption	[28]
Labdane-type diterpenoids	Diterpenoid	Various cancer cell lines	Antiproliferative activity	[2]
Clerodane-type diterpenoids	Diterpenoid	Various cancer cell lines	Antiproliferative activity	[2]
Polymethoxy flavones	Flavonoid	Melanoma cells	Mitochondria-mediated apoptosis, increased ROS, mitochondrial depolarization, inhibition of MMP-2 and MMP-9 reducing migration and invasion	[46]
Flavonoids	Flavonoid	Various cancer cells	Cell cycle arrest at G0/G1 and G2/M phases via CDK down-regulation	[46]
Terpenoids	Terpenoid	Tumor cells (general)	Modulation of the PI3K/Akt pathway inhibits proliferation, angiogenesis, and metastasis	[46]
Diarylheptanoids	Diarylheptanoid	Tumor cells (general)	Regulation of PI3K/Akt signaling and cell survival pathways	[46]

Overall, the reported compounds showed consistent anticancer activity at the in vitro level, especially the diterpenoid group, such as kolavelool, 2β-hydroxykolavelool, and kaempulchraol derivatives, which showed cytotoxic and antiproliferative effects on leukemia, pancreatic, and breast cells. At the same time, flavonoids and related compounds were found to regulate the cell cycle through G0/G1 and G2/M phase arrest and modulate the PI3K/Akt signaling pathway involved in tumor survival, angiogenesis, and metastasis. From a mechanistic point of view, these findings are complementary because induction of mitochondria-dependent apoptosis, caspase activation, increased reactive oxygen species, decreased CDK expression, and inhibition of MMP-2 and MMP-9 are components of an interconnected biological network in the regulation of cell proliferation and death. However, the strength of the conclusions is still limited to cell culture models, and the absence of pharmacokinetic, bioavailability, and systemic toxicity data limits inferences on the actual therapeutic efficacy. Furthermore, the dual role of oxidative stress, either as an inducer of apoptosis or as a target of antioxidant activity, demonstrates a context-dependent nature that requires a more detailed dose-response elucidation. Thus, the available evidence supports the potential of anticancer biology through multi-target mechanisms, but in vivo validation and clinical translational evaluation are still required before definitive therapeutic conclusions can be drawn.

3.2.6 Anti-hyperglycemic

The rhizomes of *Kaempferia pulchra* have shown remarkable anti-hyperglycaemic activity for diabetes management, through inhibition of α-amylase [3]. The α-amylase inhibitory rhizome extracts at 100, 200, 300, 400, and 500 µg/mL concentrations demonstrated bioactivity in a dose-dependent manner, with the greatest effect at 500 µg/mL. Also, at the same concentration, the *K. pulchra* leaf extracts had less α-amylase inhibitory activity than the rhizome extract. Such activity could be due to the presence of some terpenoid bioactive compounds. Hence, their role in postprandial hyperglycaemia needs to be explored further. The presence of *K. pulchra* constituents warrants further studies aimed at deriving the terpenoid constituents and their precise action on *K. pulchra* in hyperglycaemia, which will help in better therapeutic application.

Although these initial findings are promising, evidence is still limited to in vitro evaluation, and the mechanism of action of specific terpenoid compounds in *K. pulchra* has not been fully explored. Therefore, further studies are necessary to determine the main terpenoid components and their mechanisms in regulating glucose, thereby maximizing the therapeutic potential of *K. pulchra* in diabetes management.

4. CONCLUSION

K. pulchra is one of the least explored species in the Zingiberaceae family with high pharmacological potential. Based on phytochemical studies, this plant contains various classes of metabolites such as flavonoids, diterpenoids, phenolic acids, diarylheptanoids, and alkaloids, which contribute to the biological activities of the plant.

Biological studies have shown that extracts and isolated components from *K. pulchra* have antibacterial, antioxidant, anti-inflammatory, anticancer, and anti-hyperglycemic activities. Phenolic and flavonoid contents are believed to be responsible for antioxidant activities, while diterpenoids and flavonoids play important roles in anti-inflammatory and anticancer activities. Antibacterial activity is more effective when secondary metabolites act synergistically, and the anti-hyperglycemic effect highlights the potential of terpenoids in regulating metabolism.

Although many of these findings indicate the pharmacological potential of *K. pulchra*, most of the evidence is still limited to in vitro studies without in vivo or clinical confirmation. Therefore, standardization of extraction protocols, bioassay-guided isolation, and mechanistic studies are needed to more comprehensively evaluate the therapeutic potential of this plant. Further studies on pharmacokinetics, toxicity, and formulation are also important for translation into therapeutic research.

Overall, *K. pulchra* remains underutilized, but its unique characteristics and potential make it an important source for the discovery and development of new drugs. Future studies are recommended using modern extraction techniques with green

solvents to support a green technology approach in the production of plant-based therapeutics.

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