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Phytoconstituent screening and *in vitro* hypoglycemic and antioxidant properties of terpenoid fraction of *Kaempferia pulchra* extracts in Indian traditional medicine

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ABSTRACT

Introduction: The current healthcare system is insufficient to deal with the global impact of rising diabetes, necessitating the development of better alternatives, such as plant-derived natural compounds, to improve glucose tolerance. Therefore, the present study evaluated the phytoconstituents of the aqueous leaf and rhizome extracts of an unnoticed plant species, *Kaempferia pulchra*. In addition, the *in vitro* anti-hyperglycemic efficacy and antioxidant activities of the derived terpenoid fraction from the plant extracts were also assessed.

Methods: The aqueous extracts of *K. pulchra* leaves and rhizomes were screened for phytoconstituents using gas chromatography-mass spectrometry. Colorimetric techniques were used to determine the terpenoid fraction's total phenolic and flavonoid content. Terpenoids' *in vitro* antioxidant properties were examined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (RSA) and ferric reducing power assay, proceeded by α-amylase and α-glucosidase inhibition tests, and anti-hyperglycemic potential was determined utilizing the terpenoid's fraction.

Results: The preliminary phytochemical analysis revealed that the terpenoids obtained from the leaf had the highest total phenol and flavonoid content. Both leaf and rhizome extracts had modest antioxidant capacities compared to ascorbic acid. Similarly, the rhizome extract had significantly higher α-amylase inhibitory activity than the standard acarbose ($P<0.05$). Overall, the rhizome extract of *K. pulchra* outperformed the leaf extract in terms of antioxidant and antidiabetic potential.

Conclusion: *Kaempferia pulchra* is a natural source of terpenoids with several therapeutic qualities, especially for managing diabetes. However, further research is needed to validate some of the claims ascribed to this plant.

Implication for health policy/practice/research/medical education:
The phytoconstituents of aqueous leaf and rhizome extracts of *Kaempferia pulchra* were discovered to have many therapeutic properties, particularly for diabetes management.
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Introduction

Hyperglycemia, insulin resistance, and abnormalities in the metabolism of a variety of carbohydrates, proteins, and lipids are all features of the severe chronic endocrine pancreatic condition known as diabetes mellitus (DM), which has been associated with significant morbidity and mortality (1). According to the World Health Organization (WHO), 422 million people worldwide

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have diabetes, with the majority living in low- to middle-income nations (2). India has the second-most diabetic population in the world, with an estimate of more than 70 million adult diabetics, with that number expected to climb to 135 million by 2045 (3). Most of these increased diabetes patients (>90%) have type 2 diabetes caused either by pancreatic dysfunction or relative increased insulin resistance, leading to impaired glucose tolerance (4).

Furthermore, scientific evidence suggests that postprandial hyperglycaemia during diabetes strongly stimulates endothelial dysfunction, inflammatory reactions, and oxidative stress, contributing to the development and progression of pathological complications such as nephropathy, retinopathy, neuropathies, cardiovascular diseases, and atherosclerosis (5). Currently, insulin therapy and synthetic oral drugs such as insulin secretagogues, α-glucosidase inhibitors, and biguanides are available for treating all types of diabetes. To make matters worse, these drugs are accompanied by a slew of unfavourable side effects, growing costs and resistance, prompting researchers to look for alternate sources of antidiabetic drugs, such as herbal medicines.

Plants are a valuable source of a wide range of bioactive chemicals, frequently recommended as the medicine of choice due to their low cost and lack of negative side effects. Indeed, 80% of the population relies on herbal drugs and supplements for their primary healthcare needs (6-8). In recent decades, approximately half of all pharmaceutical medications have been generated directly or indirectly from the active parts of medicinal plants, putting them at the forefront of drug research initiatives (9,10).

According to ethnobotanical information, approximately 800 plants have therapeutic benefits for alleviating diabetes and its associated complications (11,12). Numerous studies have documented various plant-derived active principles, including glycosides, galactomannan, polysaccharides, terpenoids, steroids, carbohydrates, glycopeptides, amino acids, and inorganic ions that modulate metabolic cascades, affecting blood glucose levels in the body (9). Some reports also suggest that these bioactive substances influence the pancreatic secretion of insulin, apart from their insulin-mimetic properties. There are additional claims in the literature about their antioxidant potential (13). For example, many epidemiological and meta-analytical studies have highlighted the pleiotropic health advantages of dietary polyphenols due to their antioxidative, anti-inflammatory, enzyme inhibitory, and cell signalling modulator mode of action (14).

Kaempferia is a genus of the *Zingiberaceae* family comprising about 60 species of small perennial rhizomatic herbs indigenous to Malaysia, Thailand, Myanmar, Indonesia, and Singapore, with some having vast ethnomedicinal importance (15). One medicinally important species is *Kaempferia pulchra* Ridl, which is widely known in tropical Southeast Asian countries; however, it lacks the same *status quo* in the Indian peninsula (16). *K. pulchra* is widely used in folk medicine to treat coughs, stomach problems, fevers, and urinary tract infections. Furthermore, it is said to have heat-quenching, blood-stimulating, carminative, and diuretic qualities (17).

Furthermore, the terpenoids derived from *K. pulchra* rhizomes are reported with anti-mutagenic, anticancer, antimicrobial, and anti-inflammatory effects (18). Nevertheless, the scarcity of studies exploring *K. pulchra's* antidiabetic and antioxidant potential piqued our curiosity. This study investigated the antioxidant and anti-hyperglycemic properties of terpenoids obtained from *K. pulchra* leaves and rhizome extracts *in vitro*. Gas chromatography–mass spectrometry (GC-MS) analysis was used in this investigation to detect and describe the many phytoconstituents identified in both extracts.

Materials and Methods

Materials and reagents needed

Plant materials, Muslin clothes, sterile distilled water, separating funnel, Soxhlet apparatus, hexane, dichloromethane, ethyl acetate, thin layer chromatography (TLC) plate, rotary evaporator, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, aluminium chloride, ethanol, terpenoid rich fraction (TRF) solution, quercetin, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, acetate buffer, 2,4,6-tris(2-pyridyl)-s-triazine, hydrochloric acid, iron (III), chloride hexahydrate, sodium phosphate buffer, α-amylase solution, 3,5-dinitrosalicylic acid, 50mM phosphate buffer, sodium carbonate, and *p*-nitrophenol all chemicals and reagents obtained were analytical grade.

Collection and authentication

The fresh *K. pulchra* plant was collected from various locations in the Thiruvananthapuram district of Kerala, India (8.17°N 76.41°E) from April to September because, in winter, the plants die off. The plant specimens were authenticated at Nagaland University, Department of Forest Science Lumami, Zunheboto, Nagaland. The specimens were kept in the University's Centre for Biotechnology greenhouse (NUFS/2021/1004).

Preparation of extract

Fresh *K. pulchra* leaves and rhizomes were cleaned with distilled water, cut into pieces, and air-dried for 15-20 days at 25°C in the shade. The powdered dry plant material was then milled (Retsch's Mortar grinder - RM 200, Haan, Germany). Each dried powder weighing 500 g was Soxhlet extracted for 24 hours using water solvent. This process was repeated three times to isolate the plant material exhaustively. Finally, the attained extracts from each plant material were filtered using a muslin cloth. The

resultant filtrate was evaporated to dry mass at 40°C and stored at 4°C until further use.

Phytochemical analysis

Preliminary qualitative phytochemical analysis was performed using the standard methods.

Fractionation of *Kaempferia pulchra* extract

The crude extracts of leaf and rhizome from *K. pulchra* were dissolved in sterile distilled water in two different separating funnels. They were sequentially extracted using equal hexane, dichloromethane, and ethyl acetate solvents. The different organic fractions were separated and collected based on their similarities in thin-layer chromatography (CAMAG, Muttenz, Switzerland). The prospective portion was collected on a rotary evaporator at 40°C in a low-pressure environment, and then dried at room temperature for further examination.

Total phenolic content

With only minor adjustments, the Folin-Ciocalteu procedure was employed to determine the total phenolic content (TPC) of an ethyl acetate extract of *K. pulchra* leaves and rhizomes (19). Diluted leaf and rhizome extracts (250 mL) were combined with 2.5 mLof 7% sodium carbonate after an aliquot of 250 µL of Folin-Ciocalteu reagent was added. A final volume of 6 ml was created using distilled water. A spectrophotometer was used to test the mixture's absorbance at 760 nm after incubating for 90 minutes. The calibration curve was produced using gallic acid (reference standard) at the concentrations of 100, 200, 300, 400, and 500 µg/mL in order to determine the phenol content in the extracts. The TPC for triplicate determinants was expressed as mg of gallic acid equivalents (GAE)/g of extract dry weight.

$$C = C_1 \times V/m$$

Where *V* is the extract volume in mL, *m* is the weight of the plant extract in g, and *C1* is the concentration of gallic acid determined from the calibration curve in mg/mL. *C* stands for TPC in mg/g.

Determination of total flavonoid content (TFC)

The TFC of the ethyl acetate fraction was calculated using the colorimetric technique of aluminium chloride (20). Briefly, 1 mL of 2% AlCl3-ethanol solution was mixed with 1 mL of TRF solution, and the combination was then let to sit at room temperature for 60 minutes in the dark. A Ultraviolet-visible (UV-Vis) spectrophotometer was used to measure the absorbance at 420 nm wavelength (Waters Corp., Milford, MA). As a standard, quercetin in concentrations of 100, 200, 300, 400, and 500 µg/mL was utilised. The TFC for the triplicate determinants was determined from the quercetin calibration curve and expressed as mg quercetin equivalent/g of the dry weight of the extract.

$$TFC = (R \times D.F \times V \times 100) / W$$

Where *R* is the result from the standard curve, *D.F* is the dilution factor, *V* is the volume of stock solution, 100 is the weight of the experiment's plants (100 g dry plant), and *W* is the weight of the plants utilised.

Chemical identification and characterization by GC-MS analysis

Component identification and characterization of ethyl acetate terpenoid fraction were performed by GC-MS analysis. This experiment employed a 6890 series instrument (Agilent Technologies, Palo Alto, CA, USA) interfaced with a mass selective detector and a fused silica capillary column HP-5 (30 m × 0.25 mm internal diameter and 0.25 m film thickness). At a flow rate of 1.0 mL/min, helium was employed as the carrier gas. The sample injection volume was 1 L, the split ratio was 1:100, the injector temperature was kept at 250°C, the ion source temperature was held at 230°C, and the interface/detector temperature was kept at 280°C.

The oven temperature was designed to range from 50°C to 270°C at a rate of 4-15°C/min, with isothermal periods of 1 minute at 50°C and 15 minutes at 270°C. PerkinElmer Turbomass Gold detector was utilised in this investigation, and the software adopted to handle mass spectra and chromatograms was a TurboMass version 5.2 employed for data acquisition from GC-MS (70eV), which was accomplished on the same gas chromatography column coupled with the MSD 5973, at scan speeds of 1 scan/s for mass ranges 50-600 amu. The National Institute of Standard and Technology (NIST) library's mass spectra and retention index were used to identify compounds.

Antioxidant activity

DPPH assay

Free radical scavenging activity (RSA) of *K. pulchra* terpenoid fraction was determined through DPPH based on a previously reported method. Briefly, a fresh stock of methanolic DPPH solution was prepared by dissolving 1.5 mg of DPPH into 100 mL in methanol. Then, a 1.5 mL methanolic DPPH was mixed with an equivalent volume of methanolic plant extract at the concentrations of 100, 200, 300, 400, and 500 µg.

The reaction mixture was successively incubated for 30 minutes at room temperature in the dark. At 517 nm, the absorbance of the reaction mixture was measured spectrophotometrically against the absolute methanol (absorbance of DPPH without antioxidant) as blank. Ascorbic acid was set as a positive control. All determinants were performed three times. The proportion (%) of RSA was estimated using equation as the percentage inhibition of the DPPH radical by the samples:

$RSA\ (\%) = [(A_0\text{-}A_{test})/A_0] \times 100$

The A_0 denotes the absorbance of the control (solution without extract), and A_{test} is the absorbance of samples (extract and ascorbic acid). A calibration curve graph was plotted with % RSA against different extract concentrations (21-23).

Ferric reducing antioxidant power (FRAP) assay

The TRF samples of various concentrations (100, 200, 300, 400, and 500 µg/mL) were subjected to FRAP-reducing power using the method described by Musa and colleagues (19). In short, 300mM acetate buffer (pH 3.6) and 10mM of 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) were mixed in 40mM hydrochloric acid (HCl), followed by the addition of 20mM iron(III) chloride hexahydrate ($FeCl_3\text{-}6H_2O$) to prepare a fresh FRAP reagent. Then, a volume of 1.0 mL of FRAP reagent was allowed to react with 50 µL of extract and subsequently incubated at room temperature for 30 minutes in the dark. The absorbance value at 595 nm was measured as reducing power, and the relative concentration providing 50% of absorbance was computed by plotting the absorbance against the corresponding sample extract concentration (24).

Enzyme inhibition assay

α-Amylase inhibition assay

The enzyme inhibition activity of α-amylase was performed according to the earlier described standard method. To begin, an aliquot of 250 µL of 0.02M sodium phosphate buffer containing α-amylase solution (1 mg/mL) and 500 µL of terpenoid fraction of varying concentrations (100, 200, 300, 400, and 500 µg/mL) were incubated at 37°C for 10 min. Then, 250 µL of 0.5% starch solution prepared in 0.02M sodium phosphate buffer (pH = 6.8) was added to the reaction mixture, which was allowed to incubate further at room temperature for another 10 minutes. Finally, 1 mL of 3,5-dinitrosalicylic acid (DNSA) colour reagent was added to terminate the reaction. The absorbance was read at 540 nm using UV-spectrophotometer. Acarbose was used as the reference α-amylase inhibitor (100, 200, 300, 400, and 500 µg/mL)) and was estimated using the formula given below:

$\%\ Inhibition = [Abs_{blank} - Abs_{extract}/ (Abs_{Blank})] \times 100$

Where Abs_{blank} is the absorbance read without the extract and $Abs_{extract}$ is the absorbance read in the presence of the extract.

α-Glucosidase inhibition assay

This assay's protocol was altered from Kumar et al (25). The tube containing 1 mM p-nitrophenyl-D-glucopyranoside (p-NPG) produced in 50 mM phosphate buffer received various quantities of 200 µL of the plant extract (100, 200, 300, 400, and 500 µg/mL) (pH 6.8). 1.25 u/mL of -glucosidase (20 µL) dissolved in phosphate buffer was added to the reaction mixture after it had been pre-incubated at 37°C for 10 minutes. The reaction mixture was then briefly incubated at room temperature for 20 minutes. Subsequently, the reaction was terminated by adding 20 µL of 1M sodium carbonate (Na_2CO_3), and the final volume was made up to 1 mL with phosphate buffer. At 405 nm, the *α*-glucosidase activity was determined spectrophotometrically by evaluating the *p*-nitrophenol quantity released from p-NPG. The reference α-glucosidase inhibitor was acarbose (100, 200, 300, 400, and 500 µg/mL). By graphing the percentage inhibition versus the extract concentration, the extract concentration necessary to block 50% of α-glucosidase activity was estimated. The sample containing α-glucosidase served as a positive control in the absence of plant extract.

Statistical analysis

On several days, triplicates of each experiment were run with newly made reagents. Standard deviation (SD) of the mean was used to represent the results after analysis. The Student's *t* test was used to statistically compare the test chemical and the positive control compound. It was deemed significant at $P < 0.05$.

Results

Qualitative phytochemical screening of leaf and rhizome extracts

There was a 56.02% yield in the leaf extract and a 68.91% yield in the rhizome extract of each plant material. The phytochemistry of the leaf and rhizome extract of *K. pulchra* is shown in Table 1. An initial screening of phytochemicals revealed alkaloids, flavonoids, triterpenoids, amino acids, and glycosides in the leaf extract, whereas the rhizome extract constituted alkaloids, steroids, triterpenoids, saponins, and amino acids.

Isolation of terpenoid rich fraction (TRF)

The aqueous extracts of *K. pulchra* leaves and rhizomes were partitioned with hexane, dichloromethane, and ethyl

Table 1. Preliminary phytochemical screening of leaf and rhizome extracts of *Kaempferia pulchra*

Test for secondary metabolites	Leaf	Rhizome
Dragendorff's test, Mayer's test (Alkaloids)	+	+
Salkowski test (Steroids)	-	+
Alkaline reagent test, Shinoda test (Flavonoids)	+	-
Liebermann–Burchard test, Salkowski test (Triterpenoids)	+	+
Barfoed's test, Benedict's test (Carbohydrates)	-	-
$FeCl_2$ test (Tannins)	-	-
Foam test (Saponins)	-	+
Millon's test, Ninhydrin test (Amino acids	+	+
Keller-Kiliani test, Borntrager's test (Glycosides)	+	-

acetate solvents. A TLC analysis confirmed the presence of various terpenoids in all organic solvents (data not shown), but the yield obtained through hexane was 10-16% w/w, and dichloromethane was 8-12% w/w. However, the ethyl acetate elutes comprised most terpenoids, which were subsequently studied for antioxidant and antidiabetic activities. On repetitive fractionation, the percentage yield of terpenoid achieved was 35–40% w/w.

Identification of chemical constituents by GC-MS analysis

The different chemical constituents identified in leaf and rhizome extracts are given in Table 2. Total of seven compounds, namely phytol, hexadecanoic acid methyl ester, hexahydrofarnesyl acetone, dibutyl phthalate, 9,12-octadecenoic acid, methyl ester, methyl linolenate, and β-farnesene, accounting for 92.1%, were identified in the leaf extract. The rhizome extract showed the presence of eight compounds, including terpenoid, membrane, L-bornyl acetate, aromadendrene, ledol, dehydrobietan, and borneol, with a quantity of 82.86%. Figure 1 illustrates the base peak chromatogram profiles of different phytoconstituents in various *K. pulchra* extracts.

Quantitative analysis of TRF

Gallic acid equivalent (GAE)/g and Quercetin equivalent/g were used to express the ethyl acetate fraction's total phenolic and flavonoid contents in *K. pulchra* extracts. In leaf extract, TPC was 31.75±0.44 mg GAE/g, whereas in rhizome extract, it was 27±0.36 mg GAE/g. The results of TFC in the fraction were 39.46±0.1 (leaf) and 27.30 ± 0.43 mg (rhizome) Quercetin equivalent/g, respectively.

Determination of antioxidant properties of TRF

DPPH assay

The DPPH test was used to evaluate the terpenoid fraction's non-enzymatic antioxidant capabilities. The DPPH radical, a chromogenic redox reagent, served as an endpoint indicator for the experiment. As can be seen in Figure 2, there was a concentration-dependent increase or decrease in DPPH RSA across the samples (fractions). When concentration increased, the OD value decreased, indicating that the extracts actively scavenged the free radicals in the solution. In the meantime, the pink color solution was automatically turned to pale brown when

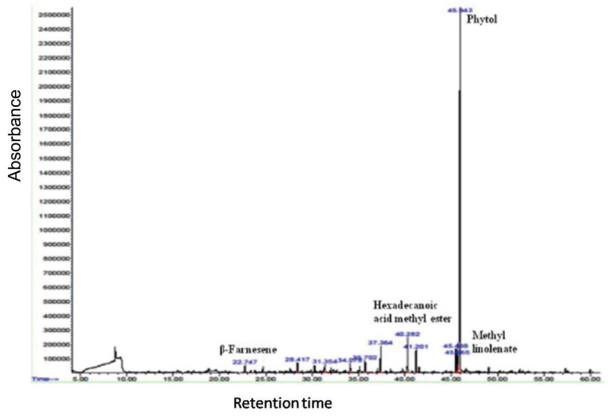

Figure 1. The base peak chromatogram profile of different phytoconstituents present in *Kaempferia pulchra* extract.

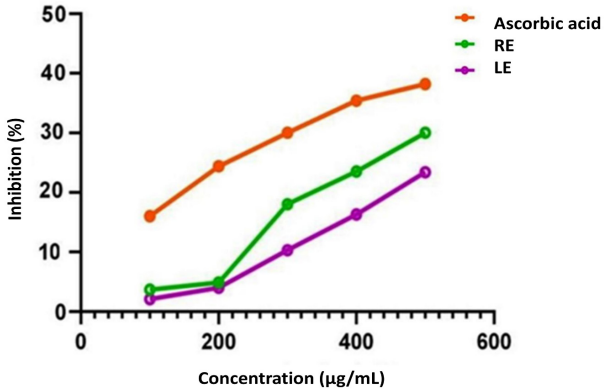

Figure 2. DPPH (2,2-diphenyl-1-picrylhydrazyl) activity of the leaf and rhizome extract obtained from *Kaempferia pulchra*. Ascorbic acid was used as a standard. LE: leaf extract; RE: rhizome extract.

the concentration increased. The graph shows the plotted value of the percentage of scavenging effect in which higher activity at 500 µg concentration was shown by ascorbic acid followed by Rhizome extract and then leaf extract, indicating they had modest antioxidant activity. In other words, the extract could neutralize the DPPH free radicals by donating hydrogen.

FRAP assay

FRAP assay measures the reduction of ferric-

Table 2. Chemical composition of terpenoid rich fraction from leaf extract of *Kaempferia pulchra*

Retention time (min)	Compound name	Area %
20.758	(6E)-2,6-dimethyl-10-methylidenedodeca-2,6-diene	1.05
38.372	6,10,14-Trimethylpentadecan-2-one	3.80
41.280	Hexadecanoic acid, 6-methyl, methyl ester	4.98
42.202	Dibutyl phthalate	3.39
45.889	9,12-Octadecenoic acid, methyl ester	3.78
45.763	Methyl linolenate	3.40
46.442	3,7,11,15-tetramethylhexadec-2-en-1-ol	73.4

tripyridyltriazine (Fe^{3+}-TPTZ) to ferrous tripyridyltriazine (Fe^{2+}-TPTZ) by the antioxidants under evaluation at low pH. Figure 3 depicts the reductive effect of the terpenoid fraction derived from both leaf and rhizome extracts of *K. pulchra*, which increased dose-dependently. Furthermore, the terpenoid in the isolated compounds displayed modest lowering capacity when compared to the conventional ascorbic acid, similar to the DPPH RSA.

In vitro hypoglycemic activity

α-Amylase inhibitory activity

Terpenoids are a group of natural compounds with reported antidiabetic effects. In the present study, the inhibitory activity of α-amylase by the terpenoid fraction revealed that enzyme inhibition activity of both leaf and rhizome extracts was significantly higher when compared to the values of acarbose standard. The rhizome extract showed greater activity than the leaf extract at 500 µg concentration. The ratio of inhibition increased with increasing extract-terpenoid concentration (Figure 4).

α-Glucosidase inhibition activity

Figure 5 provides the α-glucosidase inhibition activity of the leaf and rhizome extracts of *K. pulchra*. The overall enzyme inhibition activity for both plant extracts (leaf and rhizome) was observed to be significant at 500 µg concentration compared to the acarbose positive control.

Discussion

Diabetes is a complicated worldwide public health threat that necessitates novel, cost-effective, and safer treatment strategies in order to reduce the rising economic burden on poor and middle-income nations. As a result, diabetes specialists believe that plants that synthesise a variety of phytochemicals have future therapeutic promise in the treatment of diabetes or are at least beneficial as a supplement to establish therapies with fewer side effects (20). However, understanding the mechanism of action of certain physiologically active chemicals is required. Thus, phytochemical screening and phytoconstituent extraction are key steps in the pharmacological processing of plant-derived substances. The aqueous *K. pulchra* leaf and rhizome extracts were screened for different phytochemicals in the current study, which revealed the presence of phenols, alkaloids, terpenoids, amino acids, and flavonoids in the crude aqueous extracts, which are likely to exert their antidiabetic effects via various regulatory mechanisms. Further, the liquid column chromatogram provided terpenoids as the primary component of *K. pulchra* extracts, with peak regions also being recognized on the GC-MS spectrum, as summarised in Figures 1 and 2. Notably, the rhizome extract had abundant terpenoids, with a concentration of 74.96 ± 0.86%.

In a recent review on *Kaempferia* phytochemistry, diterpenoids were reported as one of the dominant

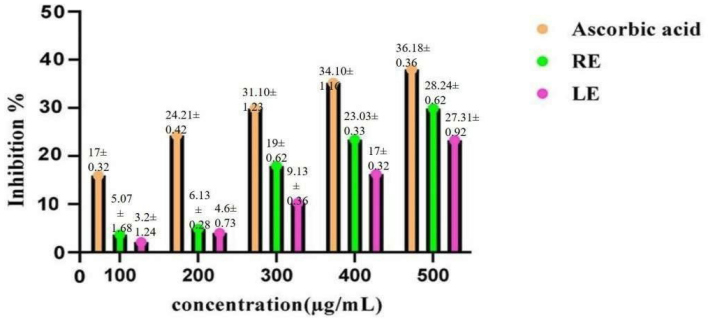

Figure 3. FRAP activity of the leaf and rhizome extracts obtained from *Kaempferia pulchra*. Ascorbic acid was used as a standard. LE: leaf extract; RE: rhizome extract.

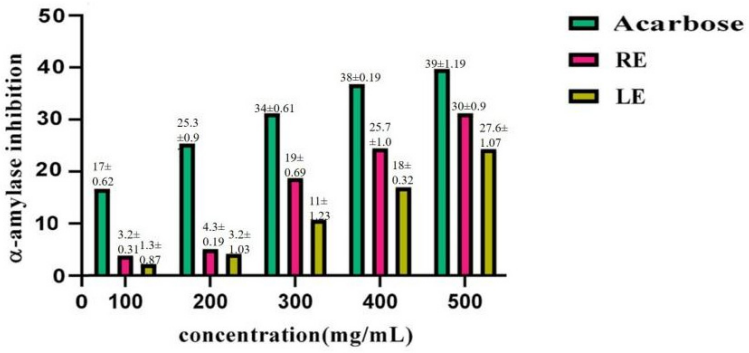

Figure 4. α-Amylase inhibition activity of the leaf and rhizome extracts obtained from *Kaempferia pulchra*. Acarbose was used as a standard. LE: leaf extract; RE: rhizome extract.

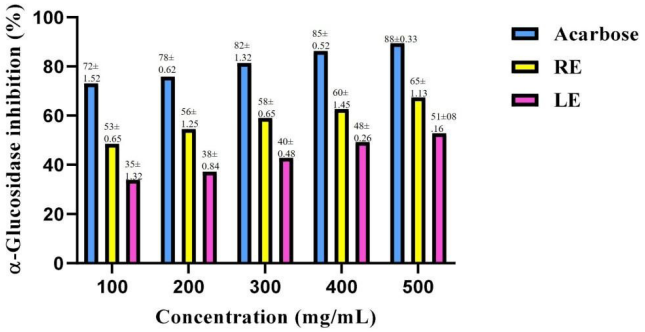

Figure 5. α-Glucosidase inhibition activity of the leaf and rhizome extracts obtained from *Kaempferia pulchra*. Ascorbic acid was used as a standard. LE: leaf extract; RE: rhizome extract.

compounds among the chemical repertory of the genus exhibiting diverse pharmacological properties (21). Previous chemical composition investigations on *K. pulchra* rhizome extract have revealed that at least 21 isopimarane diterpenoids, along with numerous additional flavonoids, have anti-inflammatory and anti-proliferative activities (22). Likewise, Chawengrum and colleagues (23-25) discovered a novel clerodane-type diterpenoid and eight other compounds in the rhizome extract of *K. pulchra*, of which some exhibited cytotoxic effects against human leukaemia cells (HL-60).

Diabetes mellitus, according to growing data, produces highly reactive free radicals that induce persistent oxidative stress in the tissues, which is exacerbated by an inadequate endogenous antioxidant defence response. Our research discovered that terpenoids obtained from plant extracts had a moderate antioxidant impact when compared to the cited ascorbic acid. However, rhizome extract surpassed leaf extract in terms of antioxidant capacity, exhibiting a concentration-dependent impact, presumably due to the interaction of natural terpenoids with other antioxidants such as flavonoids and phenolic chemicals. Furthermore, these findings are similar to the reports from other *Kaempferia* species. For example, in an earlier study, *K. angustifolia* rhizomes displayed significant RSA against DPPH in the chloroform and methanol extracts, expressed as 615.92 mg Trolox equivalent (TE)/g.

Plant-based α-amylase and α-glucosidase inhibitors have recently been proven in investigations to offer a promising therapeutic method for treating postprandial hyperglycemia. As shown in Figure 5, compared to the acarbose standard, the ethyl acetate-terpenoid fraction of *K. pulchra* rhizome showed a dose-dependent reduction in α-amylase activity, indicating the likely presence of semi-polar compounds acting synergistically or independently on the glycolytic and gluconeogenic pathways. As a result, it is obvious that *K. pulchra* terpenoids have enormous therapeutic promise in reducing postprandial hyperglycemia. More study is needed, however, to determine if these natural compounds directly alter numerous unique aspects influencing better glucose metabolism and insulin resistance.

Conclusion

The present study has established the phytochemical compositions of *K. pulchra* plant extracts known to have pharmacological properties. The study showed that the percentage of yield was significantly higher in the rhizome part of the plant than the leaf. The ethyl acetate fraction from the rhizome extract was rich in terpenoids (35-40%) and displayed strong antidiabetic potential through delayed carbohydrate digestion. Moreover, it displayed moderate antioxidant activity compared to the standard ascorbic acid. Nevertheless, we still believe that the terpenoids derived from *K. pulchra* have strong hypoglycaemic potentials, which require a careful scientific assessment to establish their mechanism of actions before being offered as effective anti-hyperglycaemic agents in preventive diabetology.

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Authors' contribution

AD supervised the study, KS, G, AA, P and M reviewed and contributed to data collection and preparation of manuscript. KS and AD prepared the first draft. All authors read the final version and confirmed it for publication.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

Ethical approval for the study was granted by the Ethics Committee of Nagaland University, Nagaland, India.

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