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REVIEW ARTICLE

Costus igneus N. E. Br. A molecular assessment of its pharmacological characteristics and bioactive compounds

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Abstract

Costus igneus, commonly referred to as the "Step ladder" or "Insulin plant," is a perennial herb native to the Western Ghats of India. Renowned for its hypoglycemic properties, the species is considered threatened, highlighting the need for urgent conservation efforts. *Costus igneus* is a rich source of valuable industrially bioactive compounds, including stigmasterol, lupeol, quercetin, diosgenin, and bis (2'-ethylhexyl)-1, 2-benzene-dicarboxylate. It is well-recognized for its ethonomedicinal applications in India, Africa, China. This plant exhibits a wide geographic distribution and is characterized by diverse descriptions, taxonomic classifications, and genetic variability. Extensive molecular level research has employed advanced genetic engineering techniques, focusing on identifying relevant markers, conducting protein analyses, and performing isozyme studies. Traditionally, *C. igneus* has been utilized for its stimulant, carminative, diuretic, digestive, hepatoprotective, and antidiabetic properties. This review highlights the urgent need for researchers and stakeholders to leverage advanced biotechnological methods, such as genetic diversity assessment, the implementation of conservation strategies, ecosystem monitoring, and species restoration programs for *C. igneus.* The comprehensive scope of the study encompasses various facets of *C. igneus*, including agro cultivation, medicinal chemistry, health applications, molecular and genetic approaches, and biotechnological advancements. In-depth research on this species has the potential to unveil its therapeutic potential, paving the way for the development of innovative plant-based treatments.

Keywords

antidiabetic; *Costus igneus*; insulin; markers; phytochemicals

Introduction

Medicinal plants are increasingly sought after for their therapeutic properties, making them as critical areas of focus in pharmacognosy, ethnobotany, and biotechnology. These disciplines bridge traditional knowledge with modern medical advancements. One such medicinal plant, *C. igneus* (N.E. Br.), commonly known as the "insulin plant," has gained significant attention for its potential antihypoglycemic activity (1) .

Native to Southeast Asia, particularly India, *C. igneus* belongs to the

Costaceae family, which was recently separated from the Zingiberaceae family (2). The monocot family Costaceae comprises approximately seven genera and around 143 identified species. In Ayurvedic medicine, *C. igneus* has traditionally utilized to manage diabetes and other health conditions, including inflammatory diseases and digestive disorders. The plant's medicinal value, combined with its unique morphological traits, has sparked scientific interest due to its potential role in treating diabetes and related ailments (3,4).

In Ayurvedic tradition, diabetes is traditionally managed by chewing the leaves of the plant daily over a one-month period to regulate blood glucose levels. The increasing prevalence of diabetes, largely attributed to lifestyle changes such as poor diet and reduced physical activity, has prompted further interest in alternative treatments like *C. igneus*. Recent research suggests that the plant contains a protein with oral hypoglycemic properties, potentially mimicking to the function of insulin.

One of the most remarkable features of *C. igneus* is its established use in Ayurvedic medicine for diabetes management (5). The leaves of the plant are believed to contain bioactive compounds that aid in regulating blood sugar levels, making it a promising natural remedy for diabetes.

Importance of the study

Glycogenolysis and gluconeogenesis play a critical role in elevating blood glucose levels, ensuring a continuous supply of glucose for the proper function and survival of organs that rely on a steady energy source. Insulin facilitates glucose entry into cells by binding to insulin receptors, enabling its utilization for energy production. Increased insulin secretion and subsequent glucose uptake by cells result in a reduction in blood glucose levels. The rising prevalence of diabetes is primarily attributed to a rapid epidemiological transition characterized by alterations in dietary habits and reduced physical activity (6).

Objectives

This study focuses on investigating an orally active hypoglycemic protein derived from *C. igneus* (N.E Br.), evaluating its effects using both in vitro and in vivo methods. (7). The present review aims to provide a detailed overview of the geographical distribution, taxonomic classification, molecular profiling, phytochemical constituents, and pharmacological properties of *C.* igneus.

Classified as a near-threatened species due to over-exploitation and other anthropogenic pressures, *C. igneus* necessitates urgent conservation measures. Consequently, this review seeks to alert researchers and stakeholders to adopt advanced biotechnological methods, including genetic diversity assessment, the implementation of conservation strategies, ecosystem monitoring, and species restoration programs for *C. igneus.*

This paper provides an in-depth analysis of various facets of *C.* igneus, encompassing its cultivation methods, medicinal chemistry, therapeutic applications, and modern molecular advancements in molecular, genetic, and biotechnological innovation.

Taxonomy and Botany

Botanical name	:	*Costus igneus* N.E.Br
Domain	:	Eukaryota
Kingdom	:	Plantae
Subkingdom	:	Viridaeplantae
Phylum	:	Tracheophyta
Subphylum	:	Euphyllophytina
Infraphylum	:	Radiatopses
Class	:	Liliopsida
Subclass	:	Commelinidae
Superorder	:	Zingiberanae
Order	:	Zingiberales
Family	:	Costaceae
Subfamily	:	Asteroideae
Tribe	:	Coreopsideae
Genus	:	*Costus*
Specific epithet	:	*igneus*

The chromosome number of *C. igneus* is 2n = 18 (8). Among its chromosomes, there is minimal variation in size, with most exhibiting a median centromere attachment. However, the AA and BB chromosomes pairs display a sub terminal centromere (9). Of the 9 chromosome pairs, 6 are rod-shaped, while the remaining three are crescent-shaped.

C. igneus, a member of the Costaceae family, is characterized by its upright growth habit. Its evergreen leaves are simple, alternate, and oblong, measuring 4 to 8 inches length and featuring a parallel venation system. These leaves are notably large, dark green, and soft, with light purple undersides. They are spirally arranged around the stems, forming attractive, arching clusters that emerge from underground rootstocks. Typically, the plant reaches a maximum height of 60 cm, with taller stems often bending to rest on the ground. The leaves are slender and uniformly smooth , exhibiting bilateral symmetry without distinct upper or lower halves. Structurally, leaves consist of four layers of broad, tangentially oblong mesophyll cells and two layers of thin epidermal cells (10). The epidermal cells are thin-walled and tangentially flattened. The bracts of *C. igneus* are light green.

The plants produce striking orange flowers on hot days, with flower measuring 2.5 to 12.5 cm in diameter and appearing on cone-like heads at the ends of branches. Low-growing plants, reaching heights of up to 50 cm, typically have purple-red stems 5-7 mm in diameter. The roots are fleshy, with some tips exhibiting slight thickening. Sheaths are purple-red, punctuated, smooth, and range from 5-10 mm in diameter.

The ligule is smooth and measures 1-2 mm in length. Petioles are sparsely minutely puberulous, measuring 1-3 mm in length. The plant bears 6 to 15 leaves that are narrowly elliptic to narrowly obovate, with short, acuminate tips terminating in filiform points 1-3 mm long. The leaves are cuneate at the base, measuring 11-18 cm in length and 3-5 and (or occasionally 6- 7.5) cm in width. They may be either glabrous or sparsely minutely puberulous on both sides.

The inflorescence consists of 3 to 8 flowers, with herbaceous bracts that are light green, fleshy, and measure up to 40 mm long and 15 mm wide. Over time, these bracts become less hairy. The appendages are leaf-like, green, narrowly triangular, measuring 2-11 cm in length and 1.5-4.5 cm in width. They are sparsely and minutely puberulous or smooth on both sides.

Bracteoles are membranous and tubular but often split deeply on the abaxial side. They measure 25-30 mm in length, are smooth, and features two keels on the adaxial side. The apex is two-lobed, with lobes that are narrowly triangular to deltoid and 2-8 mm long.

longitudinally from apex to base and contains glossy brown seeds. Bulbils are ellipsoid to ovoid, approximately 10 mm long, densely covered with brown appressed hairs, and borne in the axils of the upper leaves.

C. igneus is a rhizomatous shrub that propagate through stem cuttings and rhizomes (Fig.1). The rhizome is cylindrical, about 30 to 40 cm long, soft, fleshy, with a smooth pale-brown surface. Internally, a broad cortical zone exists in the radial plane. The cortical cells are thin walls, circular, wavy, and compactly structured. Small circular cortical circulatory bundles are erratically dispersed across the cortex. The bundles are collateral, consisting of a small cluster of phloem elements and a larger angular, somewhat thick walled xylem element, which is connected to the endodermiod layer.

C. igneus, also commonly known as Fiery Costus, Spiral Flag, Insulin plant, Step ladder, or spiral ginger, is a highly valued plant due to its unique characteristics and traditional medicinal applications.

Fig. 1. Different parts of *Costus igneus* (**A**) leaves, (**B**) rhizome, and (**C**) roots.

The pedicels are fleshy, green, and measure 1-5 mm in length and 3-5 mm in width, extending up to 25 mm during fruiting. The calyx is membranaceous to herbaceous, light green, 30-40 mm long, and densely minutely puberulous, with triangular lobes measuring 6-10 mm long and a callus 2-3 mm in length. The corolla is orange, approximately 60 mm long, either smooth or sparsely covered with tiny hairs, with a tube around 25 mm long and 3-5 mm in diameter. The lobes are narrowly ovate-elliptic, measuring 35 mm long and 9-12 mm wide.

The labellum is orange, suborbicular, and 50-70 mm in diameter, with irregularly dentate margins. The stamen is yellow, narrowly triangular, 15 mm long, 5-8 mm wide, reflexed, and golden yellow. Its apex is irregularly dentate, and the upper side is densely covered with white hairs. The anther, measuring 5-7 mm long, is attached at the base.

The basal part of the labellum and stamen are fused into a tube measuring 35-40 mm long. Pollen grains are 130-150 μm in size, with an exine approximately 8 μm thick and 11-14 pores, each measuring 14-20 μm in diameter. The stigma is cup-shaped. The ovary is densely covered with tiny hairs, measures 5-10 mm in length, and contains an ellipsoid capsule, 15 mm long, that dehisces

Distribution

Examining the morphology and cytogenetic background offers valuable insights into the role of chromosomal and genomic flexibility in the diversification of the species. The occurrence of functional 2n gametophytes in the diploids of *C. speciosus* strongly suggests that sexual polyploidzation, involving either the function of 2n male or female gamete (unilateral sexual polyploidization) or both (bilateral sexual polyploidization), may have played an important role in the origin of interspecific chromosomal races in this species (11).

Species of the genus *Chamaecostus* exhibit a clustered distribution pattern, characterized by elevated local densities yet infrequent presence across broader landscape. Presently, the genus comprises seven recognized species, all native to South America. Some of the important species of Costus across the world are listed in Table1. The geographic distribution of these species varies and includes the seasonally dry forests of Southwest Amazonia and the Cerrado forest ecosystems of Central Brazil, as seen with C*hamaecostus subsessilis*, as well as the central Atlantic Forest, which is home to C*hamaecostus cuspidatus*.

Table 1. List of some important species of "*Costus*" genera (https // powo.science.kew.org/taxon/urn lsid ipni.org names 329965-2)

S. No	Species	S. No	Species
1	*C. alleniopsis*	11	*C. lucanusianus*
2	*C. alticolus*	12	*C. malortieanus*
3	*C. convexus*	13	*C. montanus*
4	*C. comosus*	14	*C. pictus*
5	*C. dubius*	15	*C. specious*
6	*C. erythrophyllus*	16	*C. spectabilis*
7	*C. giganteus*	17	*C. spiralis*
8	*C. glaucus*	18	*C. stenophyllus*
9	*C. guanaiensis*	19	*C. tonkinensis*
10	*C. igneus*	20	*C. viridis*

The individuals clustered in this area exhibit the most extensive geographic spread among all *Chamaecostus* species. Together, these populations form a complex comprising eleven species documented in historical records (12). In monographs, it was underscored that these eleven identified species cannot be distinguished solely based on floral characteristics, as these traits remained consistent throughout their geographic range.

Morphology

Morphological and agronomic characteristics are useful for evaluation; however, their utility is limited by restricted variability and environmental factors that can influence phenotypic expression. Relying solely on these markers may lead to inaccurate conclusions about evolutionary history, as phylogeographic breaks can occur even in species with seemingly continuous distributions(13). Additionally, morphometric data may reflect phenotypic plasticity, potentially obscuring genetically driven variations that are crucial for accurate taxonomic classification.

Genetic variability

The assessment of genetic diversity within and between plants populations is carried out using various techniques, including cytological, morphological, and biochemical characterization. Morphological markers are based on visible characteristics such as flower colour, seed shape, and size, growth pattern, habitat, pigmentation, and it does not require expensive technology (14). Molecular tools can identify variations at the DNA level, offering valuable insights into genetic diversity. However, the morphological markers are often unreliable as they can be affected by climatic conditions and development stages (15).

Genetic diversity helps determine genetic distances within and between species, facilitating the identification of species and strains through distinct genetic profiles and the construction of phylogenetic trees. Genetic diversity is defined as the variation in genetic material within a population, providing crucial information about genotype commonness and distinctness, which is essential for the effective protection of genetic diversity (16, 17). High-Performance Thin-Layer Chromatography (HPTLC) helps verify the authenticity of herbal drugs, further underscoring the importance of understanding germplasm and evolutionary connections among medicinal plants for advancements in health promotion.

Currently, various techniques are employed to assess genetic polymorphism across breeding species and populations. The utility of molecular markers in elucidating genetic relationships among medicinal plants has been well-documented (18).

The adaptability and evolutionary potential of any species are directly influenced by the extent of genetic diversity within its populations. This diversityreflects the abundance of germplasm within a specific environment or locality (19). The proportion of polymorphic loci serves as a valuable indicator for evaluating genetic diversity level in *C. pictus*. Genetic diversity plays a crucial role in species' adaptability and evolutionary potential. High genetic diversity supports greater adaptation to environmental changes, while low diversity reduces resilience and increase the risk of extinction. Therefore, understanding genetic diversity and utilizing molecular markers are essential for the conservation, management, and sustainable use of plant genetic resources (20,21).

Active constituents

Phytochemical screening has revealed that the leaves of *C. igneus* are rich in protein, iron, and antioxidants such as ascorbic acid, tocopherol, carotene, terpenoids, steroids, and flavonoids. Furthermore, the methanol extract of *C. igneus* was found to contain significant quantities of various phytochemicals, including carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, and flavonoids. Ethyl Oleate, Oleic acid, bis (2'-Ethylhexyl)-1,2-benzene-dicarboxylate compounds constituted 59.04% of the leaf composition in *C. igneus*, (Table 2), alongside a diverse range of compounds such as tocopherol and the steroid ergosterol within the ether fraction.

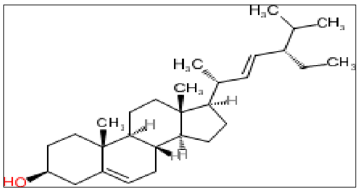
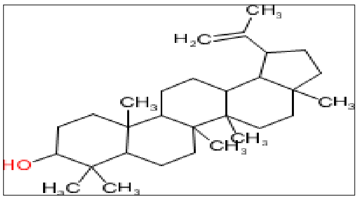
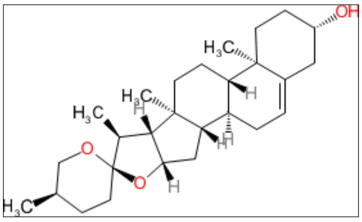
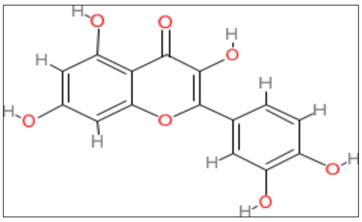
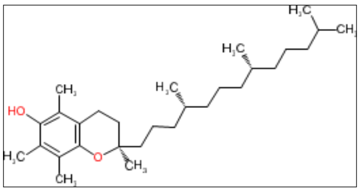
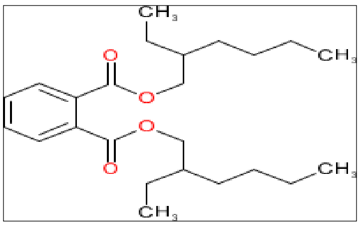
The stem of *C. igneus* was found to contain the terpenoid lupeol and the steroid stigmasterol, as well as N-hexadecanoic acid, 1, 6-Octadiene 5, 7 acid, and Di-n-octyl phthalate (Table 2). Moreover, bioactive compounds such as quercetin and diosgenin were isolated from the rhizome of *C. igneus* (22). Lupeol, a triterpene compound, was also isolated from *Crataeva nurvala* via HPTLC and exhibited antioxaluric and anticalciuric effects against hydroxyproline-induced hyperoxaluria in rats.

Two phytoconstituents, Lupenol and Stigmasterol, were extracted. Lupenol was isolated using an n-hexane :ethyl acetate (80:20 v/v) solvent system as the mobile phase, while stigmasterol was isolated using a toluene: acetone :acetic acid (8.9:0.9:0.2 v/v/v) solvent system as the mobile phase (23).

Pharmacological properties

Numerous plant species, along with their extracts and phytochemicals, have demonstrated significant effect on the mechanisms of insulin secretion. *Costus* plants are rich sources of bioactive compounds, including alkaloids, flavonoids, terpenoids, and phenolic compounds. These substances help prevent diabetes by scavenging free radicals to mitigate oxidative stress, increasing insulin sen-

Table 2. Role of each compounds pharmacological activites **(**49)

Parts	Compounds	Chemical Formula	Chemical Structure	Pharmacological.activites
Stem	Lupeol and Stigmasterol	$C_{30}H_{50}O$ & $C_{29}H_{48}O$	 Stigmasterol  Lupeol	Hyperoxaluria is a condition characterized by excessive oxalate levels in the urine, which can lead to kidney stones and, in severe cases, kidney damage.
Rhizome	Quercetin and Diosgenin.	$C_{15}H_{10}O_7$ & $C_{27}H_{42}O_3$	 Diosgenin  Quercetin	Antidiabetic refers to substances or medications that help lower blood sugar levels or manage diabetes by improving insulin function or reducing glucose production
Leaf	bis(2'-Ethylhexyl)-1,2-benzene -dicarboxylate and α-tocopherol	$C_{24}H_{38}O$ & $C_{29}H_{50}O_2$	 α-tocopherol  Bis(2-ethylhexyl) phthalate	Bis(2'-Ethylhexyl)-1,2 benzene-dicarboxylate It is also a subject of environmental and health studies due to its potential toxicity and endocrine-disrupting properties. α-Tocopherol This is the most active form of vitamin E, a fat-soluble antioxidant that protects cells from oxidative damage by neutralizing free radicals. It preventing lipid peroxidation in cell membranes

sitivity, promoting the secretion of insulin, and blocking gluconeogenesis. Many plant varieties are recognized for their antidiabetic properties, and various plant extracts have been noted for their beneficial effects in treating diabetes (Table 3). Notably, these plants and their extracts are considered to have lower toxicity and fewer adverse effects compared to synthetic medications.

Antidiabetic activity

The presence of quercetin in leaf extracts was confirmed through TLC and HPLC. Computational methods were employed to explore the antidiabetic potential of quercetin, which demonstrated superior binding energy compared

Table 3. Activity of *Costus igneus* herb in different sectors

Parts	Compounds	Activity	References
Rhizome	Quercetin and Diosgenin	Antidiabetic	(50)
	2, 2-diphenyl-1-picrylhydrazyl	Antioxidant	(33)
Leaves	α- tocopherol	Antihypoglycemic	(51)
	bis(2'-Ethylhexyl)-1,2-benzene-dicarboxylate	Antihyperglycemia	(52)
Stem	α- tocopherol	Anti-inflammatory	(53)
	Stigmasterol and Lupeol	Hyperoxaluria	(23)

to other flavonoids present in the plant, as well as the standard drug pioglitazone. This was attributed to both covalent and non-covalent interactions. Oral administration of ethanolic extract from *C. igneus* led to a notable increase in body weight and a reduction in blood glucose levels in diabetic rats (24). These effects are likely due to improved glycemic control mechanisms and enhanced insulin secretion from the remaining pancreatic β-cells in diabetic rats (25). Costus *pictus* rhizomes were reported to exhibit of the highest diosgenin content (2.54%), followed by *C. speciosus* (2.15%) and *C. igneus* (1.17%) rhizomes. Conversely, diosgenin levels were lower in leaf samples, with *C. pictus* leaves containing 0.83%, *C. speciosus* containing 0.58%, and *C.igneus* leaves containing 0.39%. Elevated levels of secondary metabolites, such as diosgenin, may contribute to the antidiabetic activity of *C. igneus*. Quantification of diosgenin in *C. igneus* rhizomes using HPTLC offers a precise and reliable method for assessing diosgenin content in extracts and formulations derived from *C. igneus* rhizomes. This methodology can also be extended to assess diosgenin content in various *Costaceae* species.

Despite the unidentified effector chemicals, leaf extracts of *C. igneus* have demonstrated significant inhibition of α-glucosidase and α-amylase enzymes, underscoring the potential of *Costus* plants in managing postprandial hyperglycemia (26). Compared to glibenclamide (0.5 mg/kg), methanolic leaf extracts of *C. igneus* (200 mg/kg) have shown a greater reduction in blood glucose levels over 30 days in diabetic rats. Furthermore, rhizome extracts of *C. igneus* have displayed antioxidant properties, enhanced glycolytic enzyme activity, and regulation of gluconeogenesis in diabetic rats. Methanol extracts of *C. igneus* rhizomes have also demonstrated potent antidiabetic and hypolipidemic effects in STZ-induced diabetic albino rats, showing comparable efficacy to the standard reference drug glibenclamide (27).

Antioxidant activity

Antioxidant components were found at substantial quantities in the *C. igneus* and *C. speciosus* samples (28). *C.igneus*, *C. speciosus* exhibited higher levels of ascorbic acid and glutathione, with *C.igneus* showing particularly elevated levels of β-carotene (667μg) and α-tocopherol (149 mg) (29). The methanolic extracts from the flower and stem of *C. pictus* possess in vitro antioxidant properties, effectively combating oxidative protein damage. These extracts represent a promising sources of natural antioxidants, demonstrating the protective effects against glucose-induced protein modifications and notably inhibiting the formation of advanced glycation end-products (AGEs).

C. speciosus reduces free radicals through interactions with hydrogen donors in antioxidant compounds. DPPH radicals react with suitable reducing agents, resulting in paired electrons and a colorless solution, where the extent of color loss is proportional to the number of electrons absorbed. It was observed that the benzene extract exhibited superior antioxidant activity compared to the standard antioxidant ascorbic acid in the DPPH assay(30). The leaves and rhizomes of *C. Pictus* exhibited notable antioxidant efficacy, measuring approximately 89.5% and 90.0% efficacy, respectively, when compared to the standard BHT (Butylated Hydroxy Toulene) at a concentration of 400 μg/ml.

Anti-inflammatory activity

The anti-inflammatory property of the extract was assessed using carrageenan-induced paw edema and cotton pellet-induced granuloma formation model (31). A notable anti-inflammatory effect was observed in rats with carrageenan-induced edema at a dosage of 800 mg/kg. Similarly, the extract demonstrated notable efficacy against cotton pellet granuloma formation in rats at dosages of 400 mg/kg and 800 mg/kg.

Anticancer activity

The study revealed significant cytotoxic effects of the extract on the MCF-7 cell line in comparison to the control group, which demonstrated 110.52±0.10 % cell viability (32). The investigation further unveiled that, when administered at a concentration of 2000 μg/mL, the extract exhibited potent anti-cancer properties, resulting in 97.46±0.74 % cytotoxicity at the maximum dosage tetsed. The IC50 value was calculated to be 950 μg/mL. The extract demonstrated dose-dependent cytotoxicity against the MCF-7 cell line. Additionally, at a concentration of 100 μg/mL, the leaf and rhizome extracts had significant antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl radicals, with 61% and 62% inhibition, respectively (33).

Furthermore, the extract exhibited a noteworthy ability to reduce ferric iron, as evidenced by absorbance values of 0.541 and 0.459 at 100 μg/mL for the leaf and rhizome extracts, respectively. *C. igneus*'s leaves, stems, and rhizomes showed strong hypoglycemic activity, confirming their long-standing use in the management of diabetes mellitus.

Antibacterial activity and antimicrobial activity

The compound isolated from the ethanolic extract of *Chemoecostus cuspidatus* demonstrated moderate antibacterial and antifungal effects against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (34). Various extracts from the rhizomes of *C. speciosus*, including petroleum ether, cyclohexane, benzene, ethyl acetate, chloroform, acetone, methanol, and water, were evaluated *in vitro* for their antioxidant potential. Several of these extracts demonstrated significant activities, including DPPH radical scavenging , total antioxidant capacity (TAC), Nitric oxide (NO), and hydroxyl radical scavenging, as well as ion chelation. These activities correlated with the phenolic content of *C. speciosus* (35). One study revealed that ethanolic leaf extract of *C. pictus* significantly elevated in the levels of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, vitamin A, vitamin C, vitamin E, and reduced glutathione levels. Consequently, it holds potential for mitigating oxidative stress and conditions caused by free radicals. The anti-oxidative potential of this botanical extract likely stems from its phenolic components. Notably, the benzene extract exhibited the highest phenolic content of 4.38% and showed significant antioxidant activity compared to other extracts.

Antimicrobial testing indicated that methanol extracts from insulin leaves are more effective than the viscous extract in inhibiting *Enterobacter. aerogens*, *Klebsiella oxycota*, and *Pseudomonas fragi*. Additionally, antimicrobial testing conducted on insulin roots revealed that the n-butanol fraction exhibited the strongest ability to inhibit bacterial and fungal growth.Recently, zinc oxide nanoparticles synthesised from *C. igneus* leaves (Ci-ZnO NPs) demonstrated superior anti-diabetic and antioxidant properties compared to the crude leaf extract. Additionally, they exhibited antibacterial and anti-biofilm activity against specific bacterial strains (36,37,38). The molecular weight of the isolated protein was determined to be 56118 da.

Studying genetics of Costus using Molecular tools

Distinct molecular markers exhibit varying genetic characteristics; such as dominance or co-dominance, the ability to amplify either anonymous or characterized loci, or the capacity to target expressed or non-expressed sequences. In this study, various explants, including leaves, nodes, internodes, and rhizomes, were employed to assess callus formation. The initiation of callus development occurred on the fourth day in MS medium and the third day in LS medium. This process was facilitated by a specific combination of hormones: BAP (0.4 mg/L), KIN (0.2 mg/L), NAA (0.1 mg/L), IAA (0.2 mg/L), and IBA (0.2 mg/L) (39).

The spectrophotometric approach stands out as a favored technique for quantifying bioactive compounds due to its simplicity, efficacy, and cost-effectiveness. This technique relies on color reactions involving specific compounds. However, the meticulous selection of compounds for reaction, optimization of color development conditions, and the selection of standards and wavelengths are intricate tasks that require precision, as the data obtained often pertain to total saponins rather than specific ones (40). Saponins, ubiquitous secondary metabolites in the plants, comprise a diverse array of compounds characterized by structures featuring a steroidal or triterpenoid aglycone along with one or more sugar chains attached at various positions (41). Due to their pharmacological properties, saponins are widely utilized in the food and pharmaceutical industries.

A study investigating the phylogenetic relationships, biogeography, and pollination history within the *Costus* subgenus *Costus* (Costaceae) used sequence data from the internal and external transcribed spacer (ITS and ETS) regions of the 18S–26S nuclear ribosomal DNA (42). DNA Sequencing and editing were performed on both strands. Significantly, samples of *C. dubius*, *C. malortieanus*, and *C. pulverulentus* from Barro Colorado Island in Panama, showcased various alleles of ITS, whereas *C. pictus* displayed multiple alleles for both ITS and ETS. Following this, PCR products were cloned, and six to ten favorable clones were selected from each PCR product for subsequent sequencing. The rDNA sequences of ITS and ETS emerged as valuable instruments for uncovering the phylogenetic connections within *Costus* species.

The integration of DNA markers into studies of crop genetic diversity has become increasingly common, revolutionizing biological research. Molecular markers have proven indispensable for evaluating genetic variation in germplasm collections (43). Over the past few decades, rapid advancements in molecular genetics have introduced numerous techniques for analyzing genetic variation (44). These advancements have led to the development of methods that are accurate, rapid, and cost-effective for assessing genetic diversity (45).The choice of marker technology depends on factors such as the intended application, expected level of polymorphism, availability of technical resources and expertise, time constraints, and financial considerations.

A study conducted on 14 closely related accessions of *Costus speciosus* revealed 73.33% polymorphism using RAPD markers, highlighting the genetic diversity within the species. Molecular markers are the primary tools for analyzing genetic variability and relationships among genotypes (46). In this study, marker technologies effectively separated *C. pictus* accessions into two distinct clusters. The combination of RAPD and ISSR banding patterns enhanced the reliability of the results, allowing for a more comprehensive screening of genomic segments. However, several studies have suggested that ISSR markers may outperform RAPD markers for phylogenetic analysis (47).

The development of molecular tools represents a significant advancement in molecular genetics, greatly facilitating plant breeding. Additionally, molecular docking and *in silico* screening have emerged as valuable computational techniques for identifying and evaluating potential antidiabetic compounds derived from plants. These methods accelerate the discovery process by enabling virtual screening of extensive plant chemical libraries and predicting their interactions with key biological targets involved in the pathogenesis of diabetes.

Future prospects

Studying the phenological behavior of wild plants is crucial for their successful cultivation. *C.igneus* presents challenges due to its diverse responses across various climatic conditions, making conventional breeding programs difficult to implement. Limited research exists on its genetic variability and the application of molecular tools. *C.igneus* thrives in moist, acidic soil conditions, making it well adapted as a shrub. These conditions offer an excellent opportunity for cultivating this crop within agroforestry systems, providing both economic and ecological benefits. However, achieving such ideal conditions on a large scale for cultivation remains impractical.

For centuries, traditional medical systems like Unani and Ayurveda have utilized plant-based treatments to manage diabetes. (48) However, further research is required to understand the precise mechanisms of action of certain bioactive compounds in *C. igneus*. This understanding is vital for the development of these compounds into effective medicines or chemical leads.

Moreover, the value of plant-derived chemicals extends beyond their therapeutic potential; they also play a crucial role in identifying and elucidating complex biochemical pathways and targets associated with various health disorders.

Thus, continued research on the phytochemicals of *C. igneus* could uncover novel therapeutic targets for addressing diabetes mellitus and other health conditions, paving the way for innovative treatments.

Conclusion

Scientific investigations are needed to validate the efficacy of *C. igneus* and elucidate its precise mechanisms of action. The extensive history underscores its diverse impacts and therapeutic potential. However, due to overexploitation *C. igneus* necessitates the development of alternate methods to protect the plant in its natural habitat. This highlights the opportunity for genetic research aimed at comprehending the genetic diversity, designing conservation tactics, and establishing breeding initiatives alongside molecular markers for this recently introduced plant.

The findings discussed herein can serve as a foundation for future studies dedicated to exploring the genetic variability of *C. igneus*. Research focused on discovering and producing therapeutically significant lead compounds through elite chemotype identification holds promise for enhancing the plant's industrial applications and nutraceutical properties. Furthermore, investigating the mechanisms of action of its bioactive compounds and standardizing herbal formulations are crucial steps toward maximizing its therapeutic potential.

Renowned for its ability to lower blood sugar levels, *C. igneus* demonstrates a multifaceted nature due to its wide range of beneficial phytochemicals. The therapeutic herbs that were once integral to ancient traditions are now emerging as essential components of modern medicine, bridging the gap between historical wisdom and contemporary scientific advancements.

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Authors' contributions

PR and DA participated in conceptualization of the review. PR and SB carried out the literature review. PR prepared the original draft. PR, DA, and SB reviewed and edited the manuscript. All authors read and approved the final manuscript

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