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Comparative Phytochemical Screening by GC-MS and Assessment of Biological Activities of Leaves, Stem and Root of *Costus igneus*

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Costus igneus (insulin plant), is among the important medicinal plant known for its diverse medicinal properties; in particular, to manage blood glucose levels. The present study is a comparative analysis of phytochemical composition and pharmacological activities of leaves, stem and root of insulin plant through FTIR, GC-MS analysis, DPPH radical scavenging ability, protein denaturation inhibition and *in vitro* α-amylase inhibitory activity. The present study aimed to evaluate the antioxidant and anti-inflammatory potential of different plant parts. Among the extracts tested, the leaf extract exhibited the highest α-amylase inhibitory activity, showing efficacy comparable to that of the standard drug acarbose. The leaf extract exhibited the highest DPPH radical scavenging activity, reaching maximum inhibition at 100 µg/mL. In the protein denaturation assay, the stem extract showed anti-inflammatory activity comparable to salicylic acid and second only to the leaf extract. Phytochemical analysis revealed the presence of diverse bioactive compounds in all plant parts, with the root containing the highest concentration and greatest phytochemical diversity. While previous studies have largely focused on the leaves, the present findings demonstrate that the stem and root also possess considerable therapeutic potential, expanding their prospects for healthcare and related industrial applications.

Keywords: *Costus igneus*, GC-MS, FTIR, *In vitro* α-amylase inhibitory, DPPH-radical scavenging, Protein denaturation assay.

INTRODUCTION

Costus igneus, commonly known as the insulin plant, belongs to the Costaceae family and has long been used in traditional medicine because of its remarkable antidiabetic properties [1]. Native to tropical regions of South and Central America, the plant is now extensively cultivated in several countries, particularly India, where it has gained considerable therapeutic and commercial importance [2]. It is popularly referred to as the "insulin plant" due to its reputed ability to regulate blood glucose levels and is widely consumed by diabetic patients [3]. *C. igneus* is a perennial herb that grows to a height of 1-2 m, bearing thick spirally arranged leaves of approximately 30 cm in length and distinctive yellow flowers [2]. The plant possesses fleshy, fibrous roots that adapt well to a wide range of soil conditions. In Ayurvedic and Siddha systems of medicine, fresh or powdered leaves are traditionally consumed, typically one leaf per day, for glycaemic control [4]. Besides its antidiabetic application, the plant has also been used for the management of fever, skin rashes, asthma, bronchitis, intestinal worm infestations, oedema and respiratory infections [5].

Phytochemical investigations have revealed that different parts of *C. igneus* contain a diverse range of bioactive constituents responsible for its pharmacological activities. The leaves are rich in flavonoids, which exhibit significant α-amylase inhibitory activity and contribute to the plant's hypoglycaemic potential [6]. They also possess strong DPPH radical-scavenging activity and enhance the activities of antioxidant enzymes including superoxide dismutase and catalase, thereby protecting cells against oxidative stress [7]. The stem has been reported to exhibit notable anti-urolithiatic activity by inhibiting crystal nucleation and growth, reducing the risk of renal stone formation [8]. The presence of terpenoids and steroids also

imparts broad-spectrum antimicrobial activity against several pathogenic microorganisms [9]. The rhizomes (tubers) have attracted attention since they exhibit stronger DPPH radical-scavenging and protein denaturation inhibitory activities than the leaves and stems [8]. Their pronounced antibacterial and antifungal activities, together with the abundance of flavonoids and terpenoids, indicate that the rhizomes represent a valuable source of bioactive compounds with potential applications in the management of diabetes, inflammatory disorders, and microbial infections.

Recent investigations have further highlighted the diverse pharmacological potential of *C. igneus*, particularly its in vitro α-amylase inhibitory activity, supporting its traditional use in diabetes management [7]. Studies have also reported significant DPPH radical-scavenging activity, protein denaturation inhibitory activity, antibacterial and antifungal properties, indicating its broad therapeutic potential [10]. Phytochemical characterisation using Fourier-transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS) has identified several bioactive constituents, including flavonoids, terpenoids and alkaloids, which are believed to contribute to these biological activities.

Previous reports have shown that the leaves exhibit strong DPPH radical-scavenging activity and α-amylase inhibition, whereas the rhizomes possess superior antioxidant and protein denaturation inhibitory activities [11,12]. These findings support the medicinal significance of *C. igneus* in both traditional and modern healthcare systems. Riaz *et al.* [13] identified bioactive phytochemicals from the leaves and demonstrated their potential in managing disorders associated with oxidative stress and inflammation. Despite these advances, most biological and phytochemical investigations have been confined to the leaves, leaving the therapeutic potential of the stems and roots largely unexplored. In view of this research gap, the present study was undertaken to characterise the phytochemical composition of different parts of *C. igneus* and evaluate their biological activities through protein denaturation, DPPH radical-scavenging and *in vitro* α-amylase inhibition assays.

EXPERIMENTAL

Preparation of plant extracts: Plant materials used in the present study were collected from the local Jadi Buti Farm, Dehradun, India and authenticated by a qualified taxonomist. Following authentication, *C. igneus* plants were cultivated and maintained in the Botanical Garden of the School of Applied and Life Sciences (SALS), Uttaranchal University, Dehradun. The leaves, stems and roots were harvested, thoroughly washed with distilled water to remove dust and other impurities, and finally shade-dried. The dried plant materials were powdered, and 50 g of each sample was extracted with 500 mL of methanol (1:10, w/v) using a Soxhlet apparatus at 40-45 ºC for 5 h (12 extraction cycles). The extracts were filtered and concentrated under reduced pressure to obtain the crude methanolic extracts. The percentage yield of a plant extract was calculated using the following formula [14] and the details are given in Table-1.

$$\text{Yield (\%)} = \frac{\text{Weight of extract obtained}}{\text{Weight of plant material used}} \times 100$$

TABLE-1
COMPARATIVE EXTRACTION YIELD AND EXTRACT WEIGHT OBTAINED FROM THE LEAF, STEM AND ROOT OF *C. igneus*

Plant part	Extract weight (g)	Yield (%)
Leaf	4.15	8.3
Stem	2.50	5.0
Root	3.40	6.8

All extractions were performed in triplicates and the mean extraction yield was recorded.

FTIR analysis: FTIR spectroscopy was performed to identify the functional groups present in the plant extracts using an FTIR spectrophotometer equipped with a DTGS detector. Spectra were recorded over the 4000-400 cm^{-1}.

GC-MS analysis: Methanolic extracts were analysed using a PerkinElmer AutoSystem GC-MS. Helium was used as the carrier gas at a constant flow rate of 1.51 mL cm^{-1} and 2 μL of each extract was injected for analysis. Mass spectra were processed using TurboMass software and phytocompounds were identified by comparing their retention times and mass spectra with the NIST98 and NIST spectral databases.

DPPH-radical scavenging: The antioxidant activity of the plant extracts was evaluated using the DPPH radical scavenging assay following the method of Abbas *et al*. [15]. Stock solutions (10 mg/mL) of the plant extracts and standards (ascorbic acid and rutin) were prepared in methanol and serially diluted to the desired concentrations. Each sample was mixed with 2 mL of freshly prepared 80 μg/mL DPPH solution and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm, with the corresponding extract or standard serving as the blank. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (1)$$

Protein denaturation assay activity: The anti-inflammatory activity of the plant extracts was evaluated using the bovine serum albumin (BSA) protein denaturation assay according to the method of Williams *et al.* [16]. The reaction mixture consisted of methanolic plant extract and 1% aqueous bovine serum albumin, with the pH adjusted to 7. The mixture was incubated at 37 ºC for 20 min, followed by heating at 57 ºC for 20 min. After cooling, the absorbance was measured at 660 nm. The percentage inhibition of protein denaturation was calculated using the following equation.:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (2)$$

***In vitro* α-amylase inhibitory activity:** The α-amylase inhibitory activity of the plant extracts was determined using the 3,5-dinitrosalicylic acid (DNSA) method [17]. A 0.5% (w/v) starch solution was prepared in 20 mM sodium phosphate buffer containing 6.7 mM NaCl (pH 6.9), while α-amylase solution (2 U/mL) was prepared in ice-cold distilled water. Plant extracts and acarbose (standard) were tested at concentrations of 100-400 μg/mL. The reaction mixture containing extract, starch solution and α-amylase was incubated for 3 min,

followed by the addition of DNSA reagent and heating at 85 ºC for 15 min. After cooling, the absorbance was recorded at 540 nm to determine maltose formation. Samples without enzyme served as the control and the percentage inhibition of α-amylase activity was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}}}{\text{Abs}_{\text{control}}} \times 100 \quad (3)$$

Statistical analysis: All the experiments were tripled (n = 3) and the results are presented as the mean ± SD. The linear regression analysis of concentration-response data was used to derive values of IC_{50}.

RESULTS AND DISCUSSION

FTIR analysis: The FTIR spectra of the leaves, stem and root extracts of *C. igneus* revealed the presence of diverse phytoconstituents associated with their biological activities. The leaf extract exhibited a broad O–H stretching band at 3428.7 cm^{-1} (Fig. 1) indicating the presence of polyphenols and glycosidic compounds associated with the antioxidant activity. A strong absorption band at 1638.5 cm^{-1} corresponded to aromatic or conjugated systems and possible proteinaceous residues, while the band at 1385.6 cm^{-1} was assigned to aliphatic $–CH_3$ bending vibrations characteristic of terpenoids and steroids [18]. The stem extract showed a broad O–H stretching band at 3489 cm^{-1} confirmed the presence of hydrogen-bonded hydroxyl groups from polyphenols, glycolsides and carbohydrates (Fig. 2). The prominent band at 1639.6 cm^{-1} is due to the characteristic conjugated aromatic systems, whereas the strong C–O stretching vibration at 1050 cm^{-1} reflected the high carbohydrate content typical of plant stem tissues. The absorption bands at 1385 and 826.7 cm^{-1} were attributed to $–CH_3$ bending of terpenoids and aromatic C–H out-of-plane bending, respectively [18]. The root extract displayed a broad O–H/N–H stretching band at 3494 cm^{-1}, together with sharper bands at 3616 and 3751 cm^{-1} confirmed the presence of hydroxyl- and amine-containing compounds (Fig. 3). The strong band at 1639 cm^{-1} was assigned to the aromatic C=C and amide C=O vibrations, suggesting the presence of flavonoids, while the absorption at 1764 cm^{-1} indicated carbonyl groups associated with lactone functionalities. The band at 1385 cm^{-1} corresponded to $–CH_3$ bending vibrations of terpenoids and phenolic compounds, whereas the weak absorptions at 2090 and 2428 cm^{-1} were attributed to spectral artefacts, such as alkyne or atmospheric CO_2 absorption [19].

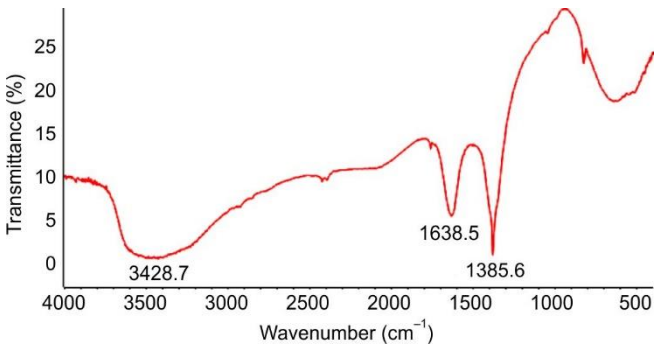

Fig. 1. FTIR spectrum of *C. igneus* leave extract

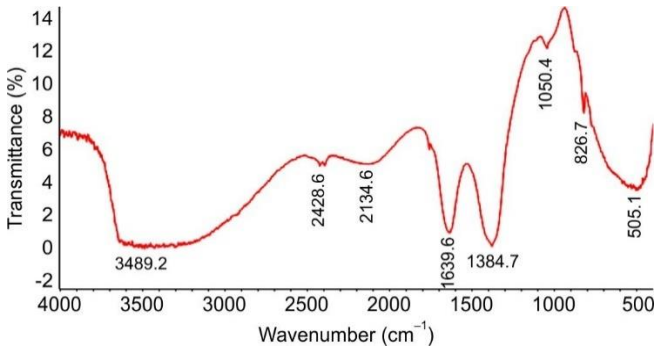

Fig. 2. FTIR spectrum of *C. igneus* stem extract

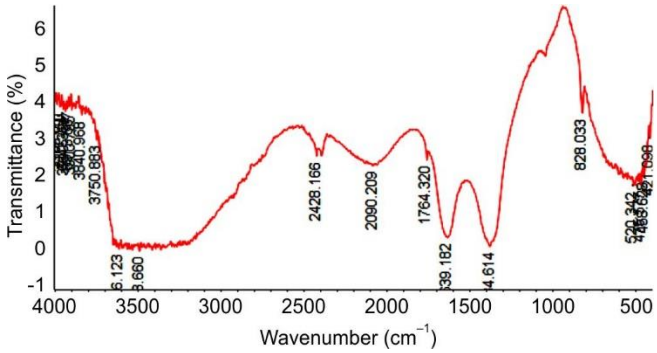

Fig. 3. FTIR spectrum of *C. igneus* root extract

GC-MS analysis: GC-MS analysis of the leaves, stem and root extracts of *C. igneus* identified several phytoconstituents responsible for the medicinal properties of the plant. The leaf extract contained 11 compounds (Fig. 4, Table-2) with reported antioxidant, antidiabetic, anti-inflammatory and antimicrobial activities [12]. Phenolic constituents such as phloroglucinol and benzopyran derivatives possess excellent free radical-scavenging ability and effectively suppress lipid peroxidation, accounting for the antioxidant potential of the extract [21]. Steroidal and triterpenoid molecules, namely cholestene analogues, thiositosterol disulphide and urs-12-ene, have been associated with α-glucosidase inhibition, improved insulin sensitivity and regulation of glucose metabolism. These compounds also reduce inflammatory responses through inhibition of the cyclooxygenase (COX) and lipoxygenase (LOX) pathways [22]. Fatty acid derivatives such as decanoic acid, palmitic acid and 14-heptadecenal exhibit antibacterial activity by disrupting microbial cell membranes [23]. The coexistence of phenolics, sterols, triterpenoids and fatty acids provides a chemical basis for the traditional use of *C. igneus* leaves in diabetes and inflammatory disorders.

The stem extract yielded 16 phytoconstituents belonging to phenolics, terpenoids, fatty acids, sterols, indole deriva-

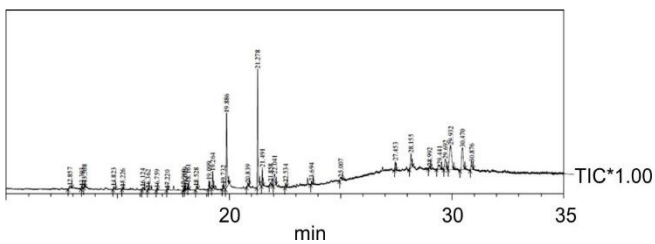

Fig. 4. GC-MS chromatogram of *C. igneus* (leave extract)

TABLE-2
MAJOR PHYTOCOMPOUNDS IDENTIFIED IN METHANOLIC EXTRACT PREPARED FROM LEAVES OF *C. igneus*

S. No.	Retention time (min)	Area (%)	Name
1	15.226	0.27	Phloroglucinol,*o,o'-bis*(trimethylsilyl)-
2	18.528	0.63	Decanoic acid
3	19.099	1.16	14-Heptadecenal
4	19.264	2.70	Palmitic acid,tmsderivative
5	21.858	0.66	Ethyl 3-hydroxy-4-methylpentanoate
6	22.534	0.51	Phosphoricacid,triphenylester
7	28.155	4.79	2*H*-1-Benzopyran-6-ol,3,4-dihydro-2,5,7,8-tet
8	29.441	2.50	Cholest-22-ene-21-ol,3,5-dehydro-6-methoxy, pivalate
9	29.692	4.41	5-Cholene,3,24-dihydroxy-
10	30.470	9.99	Thiositosteroldisulfide
11	30.876	2.77	Urs-12-ene

tives and glycosidic compounds (Fig. 5, Table-3). Benzopyran derivatives and 1-naphthalenol possess strong antioxidant properties due to their ability to neutralize free radicals and inhibit lipid oxidation [24]. Tocopherol-related compounds together with 3,4-dihydroxy-5-methyl-dihydrofuran-2-one contribute to cellular antioxidant defence through hydrogen-donating reactions [25]. Terpenoids such as neophytadiene, squalene and *cis*-3,14-clerodadien-13-ol are well known for anti-inflammatory and antimicrobial effects. Fatty acid derivatives, namely hexadecanoic acid methyl ester, tridecanoic acid and 11,14,17-eicosatrienoic acid methyl ester, regulate inflammatory processes, improve insulin sensitivity and inhibit microbial growth [26]. Steroidal compounds such as cholest-5-en-3-ol, together with indole and glycosidic derivatives, strengthen the pharmacological profile by influencing lipid metabolism, glucose homeostasis and antioxidant defence [27]. The diversity of these metabolites explains the broad therapeutic potential of the stem extract.

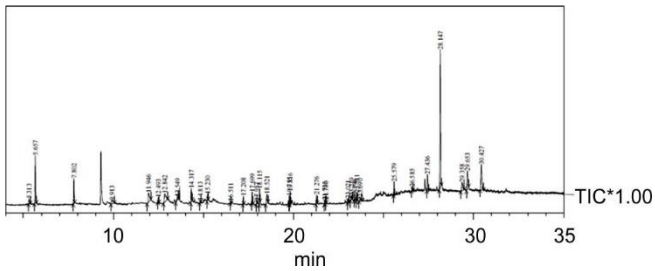

Fig. 5. GC-MS chromatogram of *C. igneus* (stem extract)

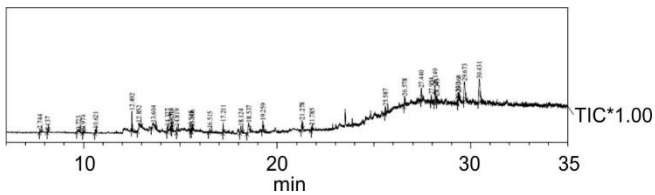

Fig. 6. GC-MS chromatogram of *C. igneus* (root extract)

The root extract contained 13 bioactive constituents (Fig. 6, Table-4) dominated by terpenoids, fatty acid derivatives, phenolic compounds, tocopherols, amino acids and steroidal analogues. Terpenoids such as linalool-type compounds, *trans*-cyclobutylsesquilavandulol, farnesene-type compounds and *Z*-3-pinen-2-ol possess pronounced antioxidant, antimicrobial and anti-inflammatory properties through scavenging of ROS, suppression of inflammatory mediators and disruption of microbial membranes [28]. Farnesol has received considerable attention due to its potent antimicrobial and anti-inflammatory activities. Fatty acid derivatives such as tridecanoic acid methyl ester and hexadecanoic acid trimethylsilyl ester influence

TABLE-3
MAJOR PHYTOCOMPOUNDS IDENTIFIED IN METHANOLIC EXTRACT PREPARED FROM STEM OF *C. igneus*

S. No.	Retention time (min)	Area (%)	Name
1	9.913	1.83	4-Methyl benzaldehyde
2	12.842	4.65	α-D-Glucopyranoside, β-D-fructofuranosyl
3	13.549	0.97	3,4-Dihydroxy-5-methyl-dihydrofuran-2-one
4	15.230	0.95	1-Naphthalenol,1,2,3,4,4a,7,8,8a-octahydro-1
5	17.208	1.12	Neophytadiene
6	17.699	1.96	1-Tetradecanol
7	18.115	1.75	Hexadecanoic acid, methyl ester
8	18.521	3.01	Tridecanoic acid
9	19.816	1.58	11,14,17-Eicosatrienoic acid, methyl ester
10	23.021	0.65	1H-Indole-3-ethanamine
11	23.511	1.27	1,2-Benzenedicarboxylic acid
12	25.579	2.02	Squalene
13	28.147	29.49	2,5,7,8-Tetramethyl-3,4-dihydro-2*H*-1-benzopyran-6-ol
14	29.358	3.54	*cis*-3,14-Clerodadien-13-ol
15	29.653	5.62	[(3β)-6-Nitrocholest-5-en-3-yl]acetate
16	30.427	9.22	16-[2-(Formylthio)ethyl]cholestane-3,22,26-triol

TABLE-4
MAJOR PHYTOCOMPOUNDS IDENTIFIED IN METHANOLIC EXTRACT PREPARED FROM ROOT OF *C. igneus*

S. No.	Retention time (min)	Area (%)	Name
1	7.744	0.83	3,7-Dimethylocta-1,6-dien-3-ol
2	9.974	0.58	*trans*-Cyclobutyl sesquilavandulol
3	12.492	4.58	(3*Z*,6*E*)-3,7,11-Trimethyldodeca-1,3,6,10-tetraene
4	13.604	5.24	l-Serine
5	15.606	1.01	Z-3-Pinen-2-ol
6	17.211	2.25	2-Decen-1-ol
7	18.124	1.95	methyl tridecanoic acid ester
8	19.259	3.15	Hexadecanoic acid, trimethylsilylester
9	25.587	2.45	2z,6e-Farnesol
10	26.578	3.32	3,4-Dihydroxymandelic acid, 4TMS derivative
11	27.440	4.88	β-tocopherol
12	28.149	6.41	3,4-Dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-6-ol
13	30.431	18.87	6-Methoxy-3,5-dehydrocholest-22-en-21-yl pivalate

membrane permeability and regulate inflammatory signalling pathways [29]. 3,4-Dihydroxymandelic acid contributes to antioxidant activity through its hydroxyl-rich structure, whereas β-tocopherol and benzopyran derivatives protect cellular membranes by preventing lipid peroxidation [30,31]. L-Serine supports antioxidant defence and glucose metabolism, while cholest-22-ene-21-ol has been linked to improved insulin sensitivity and regulation of lipid metabolism [32].

Thus, FTIR and GC-MS analyses showed that the phytochemical composition varied across the leaves, stem and root of *C. igneus*. The leaf extract possesses compounds predominantly associated with antioxidant and antidiabetic activities, whereas the stem contains the widest variety of pharmacologically active metabolites. The root is particularly rich in terpenoids, tocopherols and phenolic antioxidants that contribute to antioxidant, antimicrobial, anti-inflammatory and glucose regulating activities. This phytochemical diversity supports the medicinal value of the entire plant and demonstrates that the stem and root, in addition to the leaves, represent valuable sources of therapeutic compounds.

In vitro pharmacological activities

DPPH-radical scavenging activity: The leaf, stem and root extracts exhibited concentration-dependent DPPH radical scavenging activity over the 25-100 µg/mL range (Fig. 7). At 25 µg/mL, radical inhibition reached 44.64 ± 0.73%, 39.77 ± 0.60% and 46.28 ± 2.74% for the leaf, stem and root extracts, respectively. At 100 µg/mL, the inhibition increased to 84.96 ± 1.46%, 71.82 ± 1.98% and 73.43 ± 3.74%, respectively. Ascorbic acid, used as the reference standard, produced higher scavenging activity throughout the concentration range, increasing from 88.25 ± 1.94% at 25 µg/mL to 95.41 ± 2.97% at 100 µg/mL.

Among the plant extracts, the leaf exhibited the lowest IC_{50} value and the strongest antioxidant activity, followed by the root and stem extracts. This activity can be attributed to the phenolic and flavonoid constituents identified by FTIR and GC-MS analyses, which readily donate hydrogen atoms or electrons to quench DPPH radicals [33].

Protein denaturation assay: Protein denaturation is a widely accepted *in vitro* model for evaluating anti-inflammatory activity as denatured proteins can trigger inflammatory

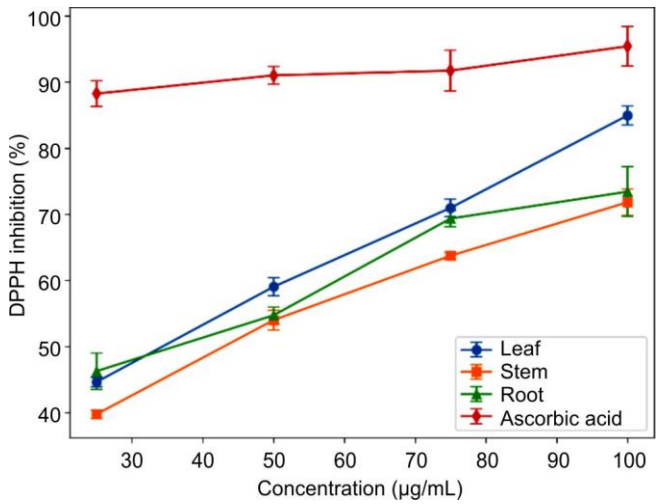

Fig. 7. DPPH radical scavenging activity of plant part extracts compared with ascorbic acid at different concentration (25-100 µg/mL). Values are expressed as mean ± SD (n = 3)

responses. The leaf, stem and root extracts of *C. igneus* inhibited protein denaturation in a concentration-dependent manner (Fig. 8). The leaf extract exhibited the strongest activity with IC_{50} of 30.1 µg/mL, followed by the stem (33.3 µg/mL) and root (41.3 µg/mL) extracts. Salicylic acid, used as the reference standard, exhibit a lower IC_{50} of 14.4 µg/mL, reflecting its superior anti-inflammatory potency. At concentrations of 75-100 µg/mL, the leaf extract exhibited protein denaturation inhibition comparable to that of salicylic acid (Fig. 8). This activity may be associated with the abundance of phenolic compounds, which are known to stabilize proteins and suppress inflammatory mediators such as C-reactive protein and tumour necrosis factor [34,35].

***In vitro* α-amylase inhibitory activity:** The α-amylase inhibitory activity of the *C. igneus* extracts was evaluated using the DNSA colorimetric assay, where a reduction in absorbance at 540 nm reflects inhibition of enzyme activity through decreased maltose formation. All extracts exhibited concentration-dependent inhibition of α-amylase (Fig. 9). The leaf extract showed the strongest inhibitory activity with IC_{50} of 85.0 µg/mL, followed by the root extract (90.7 µg/mL), whereas the stem extract displayed comparatively lower acti-

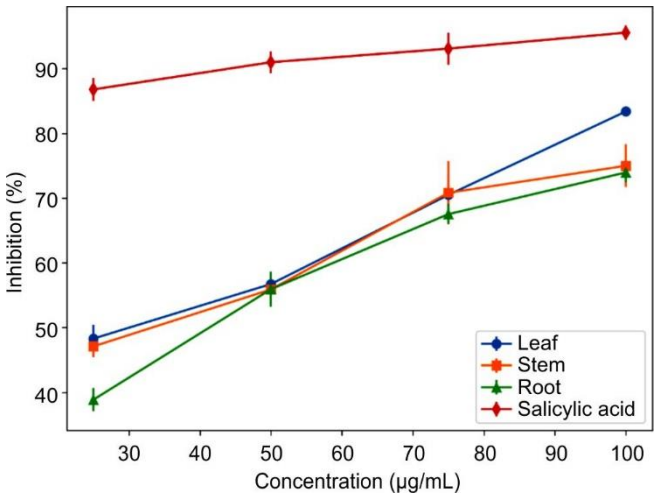

Fig. 8. Protein denaturation assay of plant part extracts compared with ascorbic acid at different concentration (25-100 µg/mL). Values are expressed as mean ± SD (n = 3)

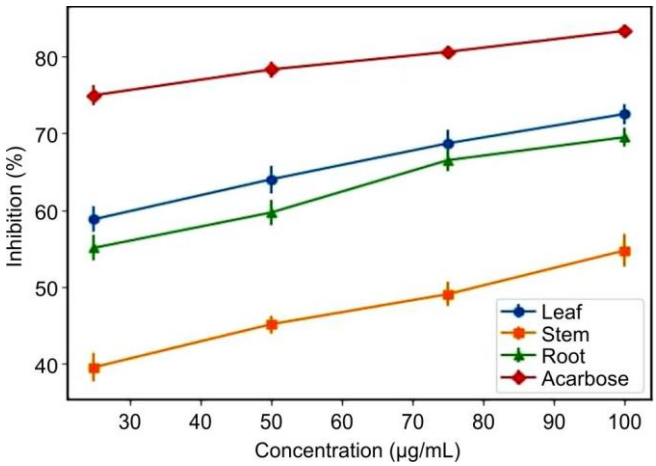

Fig. 9. *In vitro* α-amylase inhibitory activity of plant part extracts compared with ascorbic acid at different concentration (25-100 µg/mL). Values are expressed as mean ± SD (n = 3)

vity (IC_{50} = 317.5 µg/mL). Acarbose, used as the reference standard, exhibit IC_{50} of 66.7 µg/mL. The superior activity of the leaf extract may be attributed to its higher abundance of phenolic and flavonoid constituents identified by FTIR and GC-MS analyses, which have been widely associated with α-amylase inhibition. The results support the traditional use of *C. igneus* in diabetes management and indicate its potential as a natural source of α-amylase inhibitors for healthcare and nutraceutical applications.

Conclusion

The present study provides a comparative evaluation of the phytochemical composition and biological activities of the leaf, stem and root extracts of *C. igneus*. FTIR analysis confirmed the presence of hydroxyl, carbonyl, amine and other functional groups associated with phenolics, flavonoids and related bioactive constituents. GC-MS analysis identified a diverse range of secondary metabolites that account for the antioxidant, anti-inflammatory and α-amylase inhibitory activities observed in the extracts. Among the plant parts examined, the leaf extract exhibited the strongest antioxidant and α-amylase inhibitory activities, whereas the stem extract showed anti-inflammatory activity comparable to the leaf. Although previous investigations have largely focused on the leaves and rhizomes, the present work establishes that the stem also possesses considerable pharmacological potential. These findings broaden the medicinal significance of *C. igneus* and support the use of its stem, alongside the traditionally used leaves and roots, as a promising source of bioactive compounds for pharmaceutical, nutraceutical and healthcare applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

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