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Research Article

PHYTOCHEMICAL PROFILING USING HIGH-RESOLUTION LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY AND EVALUATION OF THE *IN VITRO* ANTIOXIDANT AND ANTIDIABETIC POTENTIAL OF HYDROALCOHOLIC EXTRACT OF *IPOMOEA CAIRICA* LEAVES

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ABSTRACT

Objective: This paper examines the phytochemical composition and biological functions of hydroalcoholic extract of *Ipomoea cairica* (HEIC) leaf, which is a perennial herb traditionally used in the treatment of inflammation, diarrhea, and febrile rashes.

Methods: A range of bioactive compounds was detected with the help of high-resolution liquid chromatography-mass spectrometry, among which flavonoid and alkaloid compounds, phenolic acids, and glycosides are well-known as antioxidants and antidiabetics.

Results: HEIC *in vitro* antioxidant capacity was assessed using a series of assays, which showed that the solution has a dose-dependent radical-scavenging activity, but the activity of this compound is lower than the one of ascorbic acid. More so, the extract had moderate inhibitory effects on alpha-amylase and alpha-glucosidase enzymes with IC_{50} values of 130.67 µg/mL and 212 µg/mL, respectively, suggesting its possible clinical use in the control of postprandial glucose concentration.

Conclusion: Major phytochemicals, including quercetin 3-rhamanoside-7-glucoside, rutin, physalis K, and 1,4-Di-O-caffeoylquinic acid, were associated with the therapeutic value of the extract. These results support conventional uses of *I. cairica* in herbal medicine and emphasize its potential in the preparation of natural antioxidants and antidiabetic agents.

Keywords: *Ipomoea cairica*, 2,2-Diphenyl-1-picrylhydrazyl, High-resolution liquid chromatography-mass spectrometry, Anti diabetic.

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INTRODUCTION

Drugs isolated using herbs have gained a focal point of pharmaceutical studies due to their outstanding therapeutic efficiency, safety, and adherence by patients. With the perception of adverse effects by synthetic drugs possibly shifting, the world has begun to consider the concept of seeking solutions derived out of plant-based ones [1,2]. The fact that herbal medicines are composed of plant components and phytochemicals makes it beneficial in that it has better bioavailability, low toxicity, and low cost; thus, very desirable in treating chronic diseases [3].

Ranging from 20 to 50 cm, five-fingered morning glory is a perennial herb of the family Convolvulaceae, *Ipomoea cairica*. It is native to tropical Africa and Asia and currently has a pantropical distribution and can survive under various habitats [4]. The plant is ethnobotanically used in traditional medical systems to treat inflammation, syphilis, diarrhoea, febrile rashes, and so on [5]. Bioactive constituents are linked to its anti-inflammatory, neuroprotective, and hepatoprotective effects, and they comprise coumarins, lignans, and phenolic acids [6-9]. In addition, its larvicidal and allelopathic effects underscore ecological and pharmacological significance [10,11].

Diabetes mellitus is a chronic metabolic disorder that is closely connected to oxidative stress and has an excessive level of glucose in the blood. The damage of the cells is caused by overproduction of the reactive oxygen species (ROS), which makes neuropathy, nephropathy, and cardiovascular diseases [12-14], more complicated. Antioxidants play a critical role in neutralizing the ROS, suppressing the oxidative stress, and preventing its long-term complications [15,16]. The recent studies emphasize the significance of the natural antioxidants in the management of diabetes and other diseases [17-19].

The proposed study will fill the knowledge gap in the scientific field by profiling the phytochemicals in the hydroalcoholic extract of *Ipomoea cairica* (HEIC) leaves using the high-resolution liquid chromatography-mass spectrometry (HR-LCMS) technique. In addition, it will compare the *in vitro* antidiabetic and antioxidant activity of the extract with the aim of establishing its usefulness as a natural medicinal agent in the management of diabetes and prevention of oxidative stress.

METHODS

Collection and authentication of medicinal plants

During September and October 2023, the *I. cairica* plant was gathered from Shauwadi, Kolhapur, Maharashtra (GPS Co.: N 16° 52’ 22.7562” E 74° 3’ 53.1894). Collected plants were authenticated by the Botanical Survey of India, Central National Herbarium, Howrah-711103 vide voucher specimen no. NSHM/SS-01.

The leaves were removed, thoroughly cleaned with water, allowed to dry in the shade, compressed, and kept in an airtight container until needed again.

Extraction

Succeeding extraction was carried out following through the Soxhlet apparatus [20]. 250 g of powdered drug with 500 mL of solvent was used in a successive manner, such as petroleum ether, chloroform, and ethanol 70% for 24–26 h, and the heating mantle’s temperature was adjusted to 40–60°C. After extraction, the sample’s extract was filtered and concentrated by using Rotary evaporator. The percentage yield was 2.9%.

HR-LCMS study

HR-LCMS was carried out on the chemical composition of the HEIC. The instrument used: HR-LCMS-Quadrupole Time-of-Flight-Agilent Technologies, USA. Data Acquisition Software: Agilent MAss Hunter, Data Processing Software: Agilent MAss Hunter Qualitative Analysis B.06, Column details- ZORBAX Eclipse Plus -C18 150 × 2.1 MM, 5 microns (Agilent).

Solvent A is 0.1% formic acid in Milli-Q water and Solvent B is Acetonitrile.

***In vitro* antioxidant activity**

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The plant extract and DPPH solution were prepared in methanol. In the reaction mixture, add different concentrations of the extracts 1.5 mL with 1.5 mL of DPPH solution and incubated for 30 min at room temperature in dark conditions. After 30 min, the absorbance of each solution was taken against methanol (as blank) at 517 nm [21]. The same was done for standard ascorbic acid (AA) at the same concentration.

Hydrogen peroxide scavenging assay

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of extracts (10–100 µg/mL) were prepared in DDW and then added to a prepared hydrogen peroxide solution. The absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide [21]. The same was done for standard AA at the same concentration.

Reducing power assay

The extract (0.75 mL) of various concentrations was mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1%, w/v), followed by incubation at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 mL of trichloroacetic acid solution (10%) and the mixture was centrifuged at 800 g for 10 min. 1.5 mL of the obtained supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance of the reaction mixture was taken at 700 nm. Higher value absorbance of the reaction mixture indicated greater reducing power [22]. The same was done for standard AA at the same concentration.

Hydroxyl radical scavenging activity

The Fe^{3+} -ascorbate-EDTA-hydrogen peroxide (H_2O_2) system generated the hydroxyl radical. The assay was based on the quantification of the 2-deoxy-D-ribose degradation product, which produced a pink chromogen upon heating with thiobarbituric acid (TBA) at low pH. The reaction mixture consists of 0.8 ml of phosphate buffer saline (50 mmol/L, pH 7.4), extract/standard at different concentrations (100, 200, 300, 400 µg/ml), EDTA (1.04 mmol/L), $FeCl_3$ (1 mmol/L), and 2-deoxy-D-ribose (28 mmol/L). The mixture was heated in a water bath at 37°C for 1 h. AA and H_2O_2 (10 mmol/L) were added to this mixture to start the reaction. The reaction mixture was further incubated at 37°C for 1h. Cold TBA was added to the incubated mixture, followed by HCl (25%). The mixture was heated at 100°C for 15 min and then cooled. The absorbance of the solution was checked at 532 nm with a spectrophotometer [22]. The same was done for standard AA at the same concentration.

***In vitro* antidiabetic activity**

Alpha-amylase inhibition assay

Solutions were made in sufficient amounts of 10 µg/mL to 250 µg/mL in dimethyl sulfoxide (DMSO). The coloring reagent was the DNS solution (20 mL of 96 mM 3,5-dinitro salicylic acid, 12 g of sodium potassium tartrate in 8 mL of 2 M sodium hydroxide and 12 mL of deionized water). Three sets of experiments included test, blank, and control. A 1 mL-test solution was mixed with 1 mL-enzyme solution and incubated at 25°C in 30 min, followed by the addition of 1 mL-starch solution and 3 min incubation. After that, DNS (1 mL) was added and the tube was heated in a water bath at 85 C, 15 min. The mixture was allowed to cool and then diluted with 9 mL of distilled water and mixed afterward and the absorbance was recorded at 540 nm. In the blank, DNS was put in the presence of the starch. The control substituted the plant extract with the DMSO. Acarbose was taken as a positive control. The α-amylase inhibitory activity was calculated according to the equation given below:

$$\text{Inhibition \%} = (A_{Control} - A_{Sample})/A_{control} \times 100$$

Where $A_{control}$ is the absorbance of the control (without drug extracts); A_{Sample} is the absorbance in the presence of drug extracts [23]. The same was done for standard acarbose at same concentration.

Alpha-glucosidase inhibition assay

The α-glucosidase reaction mixture contained 2.9 mM p-nitrophenyl-α-glucopyranoside, varying concentrations (10–250 µg/mL) of drug extracts, and 1.0 U/mL α-glucosidase in sodium phosphate buffer, pH 6.9. Control tubes contained only DMSO, enzyme and substrate, while in positive controls sample extract was replaced with acarbose. Mixtures without enzyme, sample extract, and acarbose were served as blanks. The reaction mixtures were incubated at 25°C for 5 min and boiled for 2 min. The absorbance of the resulting p-nitrophenol was determined at 405 nm using a spectrophotometer and was considered directly proportional to the enzyme activity. The IC_{50} values were determined from percentage inhibition plots versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference drug for the alpha-glucosidase inhibition assay. All the tests were performed in triplicate [23]. The same was done for standard acarbose at the same concentration.

RESULTS

Statistical analysis

All results are expressed as the mean±standard error of the mean. The results were analysed for statistical significance by one-way analysis of variance, followed by Dunnett's test using Graph Pad InStat version 5 (Graph Pad Software, USA). Bonferroni's Multiple Comparison Test was then performed. *$p<0.05$ was the threshold for statistical significance in each test.

HR-LCMS report analysis

HR-LCMS was performed to separate and identify the phytoconstituents based on their retention time (RT), database difference (library), experimental m/z, MS/MS fragments, metabolite class, and proposed compounds. MS data were provided in negative and positive ionization modes. Based on the mass of the molecular ion peak and their fragments, considering the neutral mass loss and known fragmentation patterns, as well as comparison with the available literature phytochemical analysis of HEIC revealed the presence of several known and known compounds (Figs. 1 and 2). A list of identified compounds presents in abundance with their mass (m/z), RT and bioactivity, is presented in Table 1.

In Table 1, the abundance–RT scatter plot shows that only a few compounds dominate the extract, while most others are present at much lower levels (Fig. 3). The first major peak appears at around 1.5 min and corresponds to retronecine, which shows exceptionally high abundance ($>5\times10^6$). This suggests that highly polar, low-molecular-weight alkaloids elute early and strongly influence the chemical profile of the extract. Another cluster of relatively abundant compounds appears at mid-range RTs (8–9.5 min), mainly contributed by 1,4-di-O-caffeoylquinic acid, phytosphingosine, spinosin B, and arctiin. These represent mid-polarity phenolic acids, glycosides, and sphingolipids, forming the second major group of constituents. The

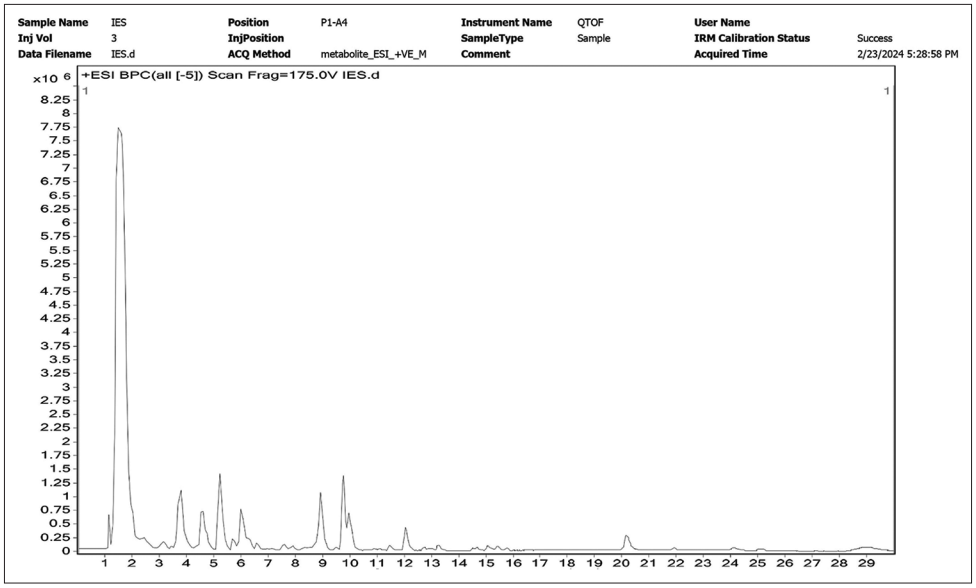

Fig. 1: High-resolution liquid chromatography-mass spectrometry chromatogram of HEIC (+ve). HEIC: Hydroalcoholic extract of *Ipomoea cairica*

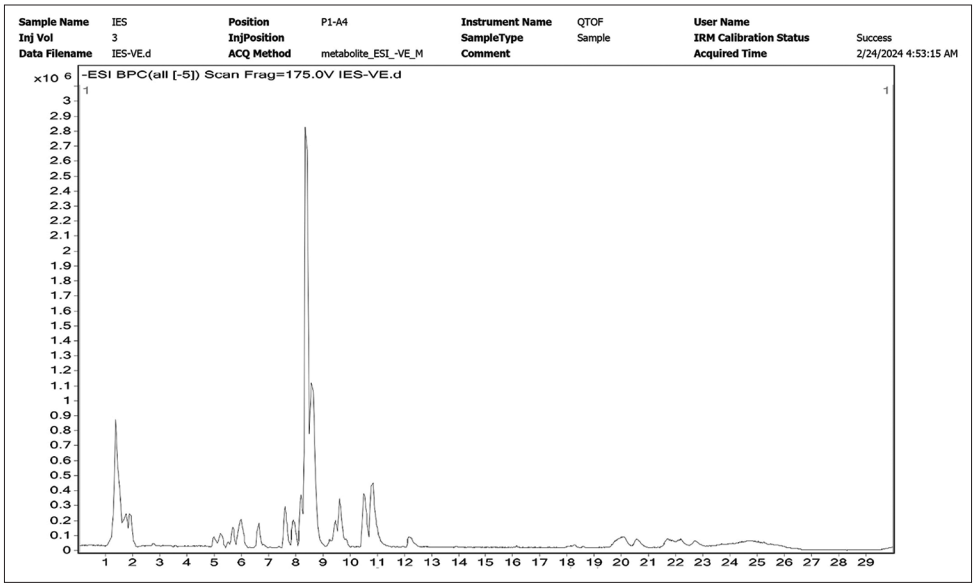

Fig. 2: High-resolution liquid chromatography-mass spectrometry chromatogram of HEIC (−ve). HEIC: Hydroalcoholic extract of *Ipomoea cairica*

remaining compounds are distributed across the RT range but occur at much lower abundances, reflecting the overall chemical diversity of the extract. Since no clear linear relationship was observed between RT and abundance, it is likely that the intrinsic chemical characteristics of each compound, rather than chromatographic behavior alone, determine their detected intensities. This pattern supports the idea that the extract's biological activity may result from interactions – both synergistic and antagonistic – among multiple classes of compounds.

***In vitro* antioxidant activity**

The antioxidant activity of the HEIC leaves was evaluated using four complementary *in vitro* assays: DPPH, hydrogen peroxide (H_2O_2), hydroxyl radical scavenging, and ferric-reducing power. AA was used as the standard reference antioxidant. In all assays, HEIC exhibited a clear dose-dependent increase in radical-scavenging activity; however, AA consistently showed higher inhibition across all tested concentrations.

For DPPH scavenging, HEIC produced 32.48% inhibition at 25 µg/mL, increasing to 91.00% at 100 µg/mL, whereas AA showed stronger activity ranging from 45.00% to 96.00% (Fig. 4). Correspondingly, the calculated IC_{50} values were 48.50±0.50 µg/mL for HEIC and 37.00±1.00 µg/mL for AA.

In the hydrogen peroxide assay, HEIC demonstrated inhibition values between 27.00% and 71.00%, compared with 32.00–79.00% for AA across the same concentration range (Fig. 5). The IC_{50} values further supported this difference, with 76.00±1.00 µg/mL for HEIC and 56.75±0.25 µg/mL for AA.

Similarly, in the hydroxyl radical scavenging assay (Fig. 7), HEIC showed moderate activity (21.00–68.00%), while AA produced slightly higher inhibition (27.00–72.00%). The IC_{50} values were 92.25±0.75 µg/mL for HEIC and 88.00±1.00 µg/mL for AA, again indicating lower potency compared to the standard.

Table 1: List of dominant compounds identified in HEIC by HR-LCMS-QTOF

S. No.	Compound Name	Molecular Formula	Class of Compounds/Biological Activity	Retention time (Min.)	Mass	m/z Ratio	Abundance
1.	Retronecine	$C_8H_{13}NO_2$	Alkaloid-genotoxic, neurotoxic, pneumotoxicity, and nucleotoxicity	1.500	155.0942	156.1014	5072421
2.	Anthranilic acid	$C_7H_7NO_2$	Aromatic amino acid derivative. Pre-cursor to tryptophan, used in plant defense.	1.742	137.0465	138.0538	952193
3.	Medicanine	$C_7H_{13}NO_3$	Alkaloid from natural sources	1.795	159.0882	160.0954	91212
4.	Lotaustralin	$C_{11}H_{19}NO_6$	Cyanogenic glycoside. Defensive compound in plants, releases cyanide (toxic); studied for ecological roles, not therapeutic.	2.002	261.1192	262.1265	287013
5.	Lentiginosine	$C_8H_{15}NO_2$	Polyhydroxylated alkaloid with glycosidase inhibitory activity	2.053	157.1091	158.1163	551344
6.	L-isoleucyl-L-proline	$C_{11}H_{20}N_2O_3$	Naturally occurring dipeptide. Antioxidant, antihypertensive, or anti-inflammatory	2.707	228.1454	229.1527	58157
7.	Fenapanil	$C_{16}H_{19}N_3$	Phenolic. Potential antioxidant or anti-inflammatory	2.813	253.153	276.1423	435029
8.	Coniine	$C_8H_{17}N$	Akaloid-nicotinic acetylcholine receptor antagonist. Found in poison hemlock	4.387	127.1352	128.1425	93088
9.	Larixinic Acid	$C_6H_6O_3$	Organic compound found in Larix species. Flavouring agent and fragrance	4.768	126.0308	127.0381	216098
10.	6-Methylquinoline	$C_{10}H_9N$	Antimalarial, antifungal, hypotensive, and antidepressant activity	5.197	143.0724	144.0797	78187
11.	Isocarbostyril	C9 H7 N O	Alkaloid/quinoline derivative. Antimicrobial or antimalarial activity	5.276	145.0517	146.059	108295
12.	Isopentenyladenine	$C_{10}H_{13}N_5$	Cytokinin (plant hormone). Plant growth regulator, used in agriculture to promote cell division and delay senescence.	5.283	203.1167	226.1055	46035
13.	Dihydroferulic acid 4-O-glucuronide	$C_{16}H_{20}O_{10}$	Phenolic acid derivative. Antioxidant, involved in plant defense, potential anti-inflammatory effects in human metabolism.	5.649	372.1051	417.1034	189253
14.	Cryptochlorogenic acid	$C_{16}H_{18}O_9$	Phenolic acid. Antioxidant, anti-inflammatory, and potential metabolic health benefits.	5.733	354.0945	353.0873	245884
15.	Leonusiride A	$C_{14}H_{20}O_9$	Glycoside. Potential sedative or cardiovascular effects (from Leonurus, used in traditional Chinese medicine).	6.16	332.1099	355.0991	91451
16.	p-Coumaroylagmatine	$C_{14}H_{20}N_4O_2$	Hydroxycinnamic acid amide.	6.374	276.1564	277.1637	66911
17.	Xanthotoxol glucoside	$C_{17}H_{16}O_9$	Coumarin glycoside. photosensitizer, antifungal, used in phototherapy.	6.764	364.0789	363.0721	27813
18.	Agnuside	$C_{22}H_{26}O_{11}$	Iridoid glycoside. Hormonal regulation, used in herbal medicine for menstrual disorders.	7.124	466.1495	465.1424	34788
19.	Quercetin 3-rhamnoside-7-glucoside	$C_{27}H_{30}O_{16}$	Flavonoid glycoside. Antioxidant, anti-inflammatory, potential anti-cancer and antiviral effects.	7.599	610.154	609.1469	375220
20.	Rutin	$C_{27}H_{30}O_{16}$	Flavonoid glycoside. Antioxidant, strengthens blood vessels, used in supplements for venous health.	7.645	610.1521	609.1451	112248
21.	1,4-Di-O-caffeoylquinic acid	$C_{25}H_{24}O_{12}$	Phenolic acid. Antioxidant, anti-inflammatory, and potential anti-diabetic effects.	8.118	516.1273	515.12	1722667
22.	Hispolone	$C_{12}H_{12}O_4$	Polyphenol. Antioxidant, antifungal, and potential anticancer activity.	8.34	220.0751	265.0733	
23.	Physalin K	$C_{28}H_{30}O_{12}$	Steroidal lactone. Anti-inflammatory, potential anti-cancer and antimicrobial effects.	8.35	558.1708	557.1673	
24.	Spinosin B	$C_{38}H_{40}O_{18}$	Flavone C-Glycoside. Sedative, anxiolytic, used in traditional Chinese medicine for sleep disorders.	8.373	784.2258	783.219	794276
25.	Uridine	$C_9H_{12}N_2O6$	Nucleoside. Supports cognitive function. Used in supplements.	8.434	244.0718	289.0704	84056
26.	Kaempferol 3-O-β-D-galactoside	$C_{21}H_{20}O_{11}$	Flavonoid glycoside. Antioxidant, anti-inflammatory, and potential anti-cancer effects.	8.474	448.0988	447.0916	
27.	Epitheaflagallin 3-O-gallate	$C_{27}H_{20}O_{13}$	Theaflavin derivative. Antioxidant, anti-inflammatory, and potential anti-viral properties.	8.628	552.0965	551.0939	
28.	(-)-Pinoresinol glucoside	$C_{26}H_{32}O_{11}$	Lignan glycoside. Antioxidant, anti-inflammatory, and potential cardiovascular benefits.	8.773	520.1917	519.1847	
29.	Scorzonoside	$C_{27}H_{34}O_{12}$	Iridoid glycoside. Potential anti-inflammatory or hepatoprotective effects.	8.805	550.2022	573.1917	250462

(Contd...)

Table 1: *(Continued)*

S. No.	Compound Name	Molecular Formula	Class of Compounds/Biological Activity	Retention time (Min.)	Mass	m/z Ratio	Abundance
30.	Hesperetin 7-O-glucuronide	$C_{23}H_{24}O_{11}$	Flavonoid (Flavanone). Antioxidant, anti-inflammatory, and potential cardiovascular benefits.	8.833	476.1309	499.1204	423197
31.	Pimentol	$C_{23}H_{26}O_{12}$	Phenolic/Terpenoid. Antioxidant, antimicrobial, used in flavoring and traditional medicine.	9.026	494.1406	517.1299	106045
32.	Flavine mononucleotide (FMN)	$C_{17}H_{21}N_4O_9P$	FMN is a naturally occurring coenzyme derived from riboflavin	9.12	456.1039	515.1177	32380
33.	Arctiin	$C_{27}H_{34}O_{11}$	Lignan glycoside. Antioxidant, anti-inflammatory, and potential cardiovascular benefits.	9.757	534.2069	557.1961	590769
34.	Cochlearine	$C_{15}H_{19}NO_3$	Glucosinolate/Alkaloid. Antimicrobial activity.	9.972	261.1363	284.1255	81437
35.	(S)-Edulinine	$C_{16}H_{21}NO_4$	Alkaloid. Potential antimicrobial effect.	9.981	291.1474	314.1367	241538
36.	Cinchonain Ib	$C_{24}H_{20}O_9$	Flavonoid. Antioxidant, potential anti-inflammatory and antimicrobial properties.	10.529	452.1126	451.1058	134555
37.	Carotamine	$C_{14}H_{13}N_3O_3$	Pigment/Carotenoid. Antioxidant, pre-cursor to vitamin A, used in food coloring, skin health, and eye health.	12.757	271.0949	272.102	25042
38.	Phytosphingosine	$C_{18}H_{39}NO_3$	Sphingolipid. Skin barrier function, antimicrobial, used in cosmetics and dermatology for anti-inflammatory effects.	13.006	317.2907	318.298	2049271
39.	Nigakilactone B	$C_{22}H_{32}O_6$	Lactone. Potential antimicrobial or cytotoxic activity.	15.846	392.2193	415.2087	499028

HEIC: Hydroalcoholic extract of *Ipomoea cairica*, HR-LCMS-QTOF: High-resolution liquid chromatography - quadrupole time-of-flight

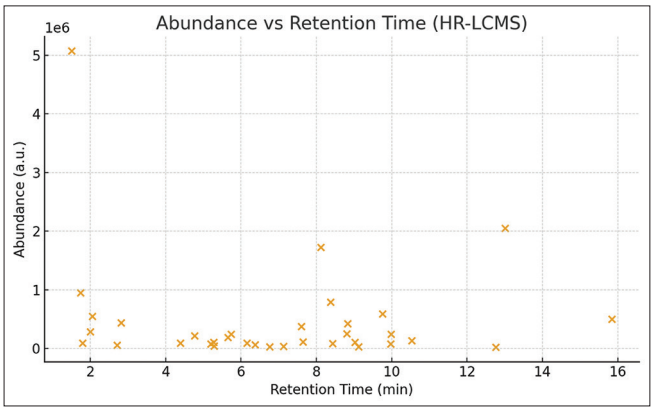

Fig. 3: Scatter plot showing the relationship between retention time (RT) and peak abundance obtained from High-resolution liquid chromatography-mass spectrometry analysis of the extract. A few highly abundant compounds appear at early and mid RT values, while most constituents show comparatively low signal intensities across the chromatographic range, reflecting the chemical diversity of the sample

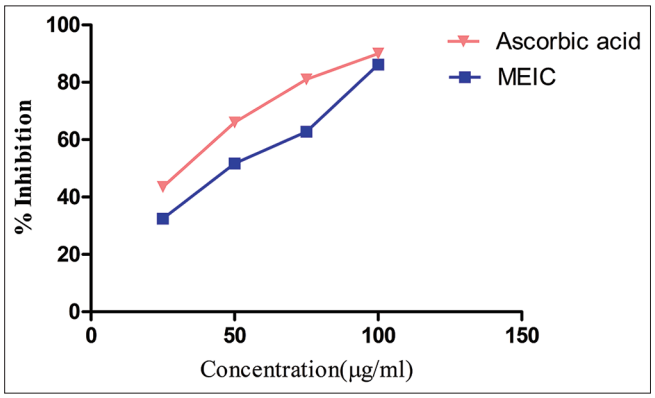

Fig. 4: The data represent the percentage inhibition of 2,2-diphenyl-1-picrylhydrazyl, by HEIC against ascorbic acid (standard). Each point represents the values obtained from experiments, performed in triplicate (mean±standard error of the mean). HEIC: Hydroalcoholic extract of *Ipomoea cairica*

In the ferric reducing antioxidant power assay (Fig. 6), HEIC demonstrated strong reducing capacity with inhibition values ranging from 39.00% to 90.00%. AA showed marginally better activity (45.00%–94.00%), reflected in the calculated IC_{50} values of 44.00±1.00 µg/mL for HEIC and 32.25±0.75 µg/mL for AA.

HEIC possesses moderate but notable antioxidant capacity (Fig. 8), supporting its potential role as a natural source of antioxidant compounds.

***In vitro* antidiabetic activity**

The HEIC leaves were evaluated for its *in vitro* antidiabetic potential using α-amylase and α-glucosidase inhibition assays. Acarbose, a well-known synthetic antidiabetic medication, was used as the standard reference in both assays.

Alpha-amylase inhibition assay

The HEIC exhibited a dose-dependent inhibition of α-amylase activity (Fig. 9), with inhibition percentages increasing from 15.95% at 10 µg/mL to 71.87% at 250 µg/mL. In comparison, acarbose demonstrated greater inhibitory activity, with percentages ranging from 23.00% to 87.00% across the same concentration range. The IC_{50} value of HEIC for α-amylase inhibition was determined to be 130.67 µg/mL, indicating its moderate efficacy as an inhibitor of this enzyme.

Alpha-glucosidase inhibition assay

HEIC also showed concentration-dependent inhibition of α-glucosidase activity (Fig. 10), with inhibition values rising from 11.52% at 10 µg/mL to 65.13% at 250 µg/mL. Acarbose displayed superior inhibition, with percentages ranging from 29.58% to 76.95% at the same concentration levels. The IC50 value of HEIC for α-glucosidase

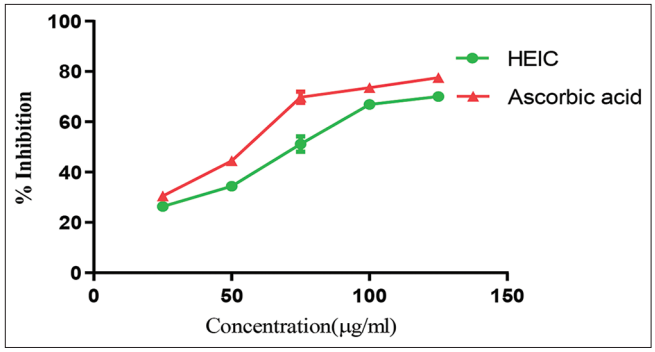

Fig. 5: The data represent the percentage inhibition of Hydrogen peroxide, by HEIC against ascorbic acid (standard). Each point represents the values obtained from experiments, performed in triplicate (mean±standard error of the mean). HEIC: Hydroalcoholic extract of *Ipomoea cairica*

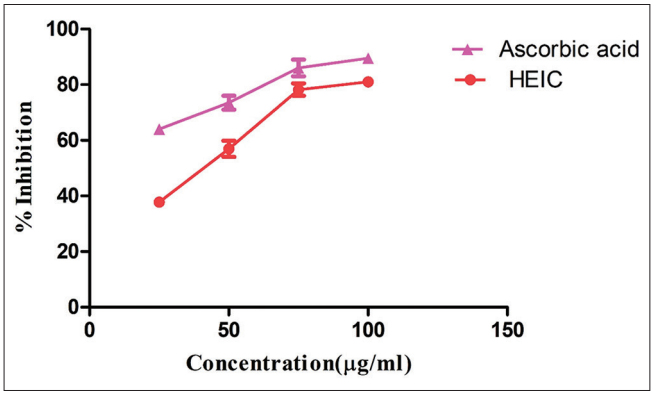

Fig. 6: The data represent the percentage inhibition of Ferric ion reduction by HEIC against ascorbic acid (standard). Each point represents the values obtained from experiments, performed in triplicate (mean±standard error of the mean). HEIC: Hydroalcoholic extract of *Ipomoea cairica*

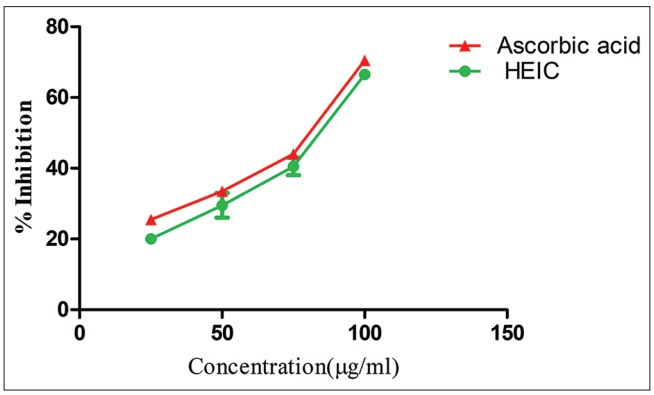

Fig. 7: The data represent the percentage inhibition of Hydroxyl radical reduction by HEIC against ascorbic acid (standard). Each point represents the values obtained from experiments, performed in triplicate (mean±standard error of the mean). HEIC: Hydroalcoholic extract of *Ipomoea cairica*

inhibition was calculated to be 212 µg/mL, reflecting a relatively lower potency compared to acarbose.

The results suggest that HEIC possesses significant inhibitory potential against key carbohydrate-digesting enzymes, α-amylase and α-glucosidase, which are critical targets in the management of postprandial hyperglycemia. This highlights the potential of HEIC as a natural antidiabetic agent.

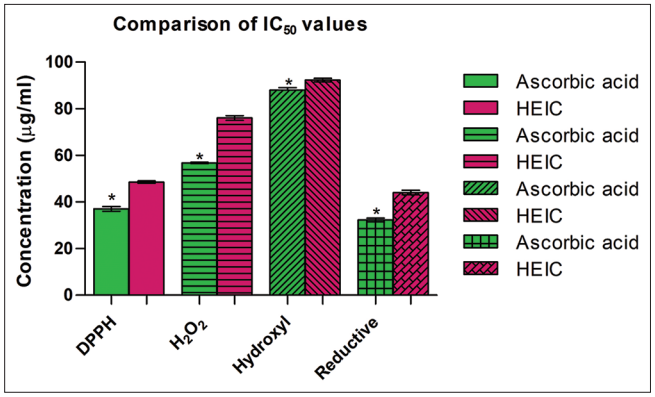

Fig. 8: The IC_{50} values of antioxidant activity of HEIC along with standard Ascorbic acid. All the data are represented as mean± standard error of the mean. One-way analysis of variance (was used to analyze the data for each group, and Bonferroni's Multiple Comparison Test was then performed. *p<0.05 was the threshold for statistical significance in each test. HEIC: Hydroalcoholic extract of *Ipomoea cairica*

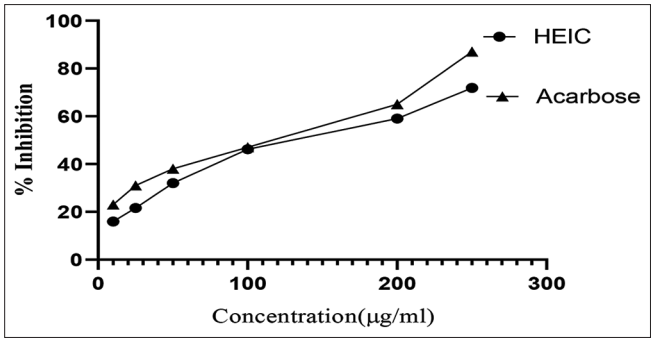

Fig. 9: Inhibition of alpha-amylase by HEIC: The data represent the percentage inhibition of *α*-amylase by HEIC against acarbose (standard). Each point represents the values obtained from experiments, performed in triplicate (mean±standard error of the mean)

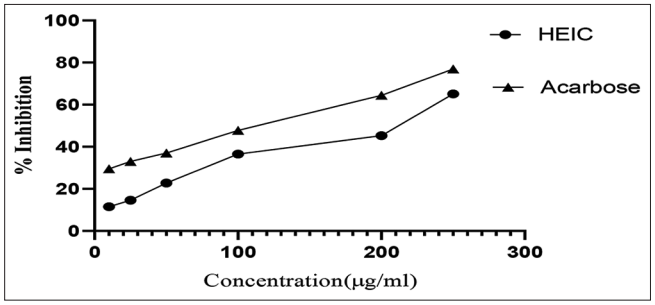

Fig. 10: Inhibition of alpha-glycosidase by HEIC: The data represent the percentage inhibition of α-glucosidase by HEIC against acarbose (standard). Each point represents the values obtained from experiments, performed in triplicate (mean± standard error of the mean)

DISCUSSION

The HR-LCMS analysis of *I. cairica* hydroalcoholic extract revealed a chemically diverse array of bioactive compounds with notable

pharmacological potential. Key nitrogen-containing metabolites, such as retronecine, anthranilic acid, and medicanine suggest active secondary metabolism linked to neuroactive, antimicrobial, and cytotoxic activities [6,7]. The presence of cyanogenic glycosides, such as lotaustralin points to roles in plant defense as well as enzyme inhibition and metabolic regulation [24,25].

Flavonoids, including quercetin 3-rhamanoside-7-glucoside and rutin, contribute established antioxidant, anti-inflammatory, and cardioprotective effects [14,26,27], while steroidal lactones, such as physalin K enhance the extract's therapeutic profile through anticancer, immunomodulatory, and antimicrobial properties [3]. Phenolic acids, such as 1,4-di-O-caffeoylquinic acid and cryptochlorogenic acid further add anti-inflammatory benefits [28].

Among the dominant compounds in the chromatographic profile were retronecine (5,072,421 a.u.), 1,4-di-O-caffeoylquinic acid, phytosphingosine, and spinosin B, highlighting alkaloids, phenolic acids, and sphingolipids as major chemical signatures. The abundance of phenolic acids and flavonoid glycosides, such as quercetin derivatives, rutin, hesperetin 7-O-glucuronide, and epitheaflagallin 3-O-gallate, suggests strong antioxidant potential that likely synergizes with moderate levels of polyphenols like hispolone and lignan glycosides (arctiin, pinoresinol glucoside) [3,11].

The co-existence of neuroactive or cytotoxic alkaloids (retronecine, coniine, edulinine) with bioactive phenolics implies complex interactions; retronecine's genotoxicity may counterbalance phenolics' antioxidant effects at sufficient concentrations. Conversely, synergistic actions are plausible – caffeoylquinic acids and flavonoid glycosides can potentiate antioxidant and anti-inflammatory responses via complementary radical scavenging. Lower abundance glycosides, such as spinosin B and agnuside may also modulate neuroprotection, stress responses, and metabolic equilibrium.

In addition, the discovery of phytosphingosine, with dermatological applications, and flavine mononucleotide, a vital metabolic coenzyme, underscores the extract's potential beyond medicine into cosmetics and nutraceuticals. Bioactive peptides like L-isoleucyl-L-proline further enrich the extract's antioxidant, antihypertensive, and metabolic regulatory capacity [3,11].

Overall, *I. cairica* hydroalcoholic extract's biological activity appears driven by a multifaceted chemical interplay where predominant alkaloids may pose safety considerations, while phenolic- and flavonoid-rich fractions provide antioxidant and anti-inflammatory efficacy. Such complexity necessitates targeted fractionation and bioactivity-guided research to clarify the balances of synergy and antagonism underlying observed therapeutic effects.

The antioxidant tests in this research established that HEIC is dose-effectively a radical scavenging agent, which targets DPPH, hydroxyl peroxide, hydroxyl radicals, and ferric ion reduction. Even though HEIC showed a bit lower efficacy in comparison to AA, its capacity to counteract oxidative stress is notable [29,30]. This ability to counteract ROS, oxidative injury, and lipid peroxidation is probably due to the presence of flavonoids and phenolic acids in HEIC [13,26,31]. Such results are in line with the prior studies on the benefits of plant-based extracts as antioxidants, as they help to decrease oxidative stress and promote cell health [1,32].

The HEIC showed inhibitory activity against both α-amylase (IC_{50} = 130.67 µg/mL) and α-glucosidase (IC_{50} = 212 µg/mL). When these values are compared with those reported for other antidiabetic plants, the activity of HEIC can be classified as moderate. For α-glucosidase, *Gymnema sylvestre* demonstrated much stronger inhibition, with an IC_{50} of 67 µg/mL [33], while *Rumex nepalensis* showed even greater potency at 9.45 µg/mL [34]. In contrast, *Cassia auriculata* exhibited far weaker activity, with an IC_{50} of about 7.19 mg/mL (≈7190 µg/mL) [35]. In this context, the IC_{50} of 212 µg/mL for HEIC places it midway between highly potent extracts and much weaker ones. A similar pattern is observed for α-amylase inhibition. *Gymnema sylvestre* again displayed strong activity with an IC_{50} of 82.34 µg/mL, while *Rumex nepalensis* showed an IC_{50} of 54.12 µg/mL, reflecting high potency. *Cassia auriculata*, however, recorded a much weaker effect with an IC_{50} of around 1.7 mg/mL (≈1700 µg/mL). Compared with these benchmark values, the α-amylase IC_{50} of 130.67 µg/mL for HEIC falls squarely in the moderate range, being less potent than the strongest extracts but substantially better than the weakest.

HEIC demonstrated a moderate inhibitory effect on both enzymes, likely driven by flavonoids, such as quercetin and kaempferol, which are known to inhibit carbohydrate-hydrolyzing enzymes effectively, which supports its potential in the treatment of postprandial hyperglycemia [36-38]. Isolation or further purification of these active constituents could enhance the inhibitory potency, potentially matching or surpassing the most effective plant extracts reported in existing studies.

CONCLUSION

The results of this research reveal the phytochemical content and biological potential of the HEIC leaves. The HR-LCMS analysis revealed the existence of a number of bioactive compounds that had known antioxidant, anti-inflammatory, and antidiabetic effects. The extract demonstrated significant free radical activity in various antioxidant tests, which supports its contribution to the reduction of the damage associated with oxidative stress. The inhibition of the enzymes α-amylase and α-glucosidase also demonstrates its potential in the management of postprandial hyperglycemia, which justifies the use of the substance in managing postprandial hyperglycemia as a natural antidiabetic.

Various phytoconstituents detected in HEIC, such as flavonoids, phenolic acid, alkaloids, and glycosides, make it a multifunctional therapeutic drug. The presence of certain toxic compounds in the extract has been reported, making fractionation and careful dose selection essential when considering its biological applications. The results are consistent with the common usage of *I. cairica* and give it scientific support in the use of the plant. Nonetheless, its efficacy, safety, and pharmacokinetic profile need to be evaluated with additional research and *in vivo* validation, as well as mechanistic research and clinical trials. Further research on the possibility of developing HEIC into nutraceutical or pharmaceutical preparations offers a promising area of research in the future and highlights the importance of the technique in contemporary herbal medicine and drug discovery.

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AUTHOR'S CONTRIBUTIONS

Arvind Kumar - Writing, experimentation, data collection, conceptualization, and visualization. Mrinmoy Basak - Evaluating the writing part, supervision.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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NA.

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