

Phytochemical Profiling, Physicochemical Evaluation, and In Vitro Biological Activities of *Blepharis integrifolia* Extracts

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Abstract

Blepharis integrifolia (Acanthaceae) is a traditionally valued medicinal herb utilized for a wide spectrum of ailments, yet its systematic phytochemical and pharmacological validation remain limited. This study aimed to comprehensively evaluate the qualitative and quantitative phytochemical profiles, physicochemical parameters, and in vitro biological activities of *B. integrifolia* extracts. Shade-dried plant material was subjected to parallel cold maceration using 70% ethanol (BIE) and chloroform (BIC). Baseline physicochemical analysis established a moisture content of 2.41% and a total ash value of 8.31%. Preliminary qualitative screening confirmed the presence of phenols, flavonoids, proteins, and carbohydrates in both extracts. Notably, solvent polarity influenced the extraction profile: terpenoids were exclusive to the hydroethanolic extract, while alkaloids were specifically extracted by chloroform. Quantitative spectrophotometric analysis revealed that the chloroform extract yielded a significantly higher total phenolic content ($342.24 \pm 16.13 \mu\text{g GAE/mg}$), whereas the 70% ethanol extract exhibited a superior total flavonoid concentration ($197.51 \pm 8.41 \mu\text{g QE/mg}$). The extracts were subsequently evaluated for their in vitro biological efficacies. Both extracts demonstrated potent, broad-spectrum antibacterial activity against *S. aureus* and *E. coli* (zones of inhibition up to 15.66 mm at 3 mg). Furthermore, the ethanol extract exhibited superior antioxidant capacity (DPPH $\text{IC}_{50} = 88.84 \mu\text{g/mL}$), whereas the chloroform extract demonstrated stronger antidiabetic potential (α -glucosidase $\text{IC}_{50} = 60.16 \mu\text{g/mL}$). These findings validate the rich secondary metabolite profile of *B. integrifolia*, substantiating its ethnomedicinal applications and providing a concrete scientific rationale for its potential therapeutic use in managing oxidative stress, microbial infections, and metabolic conditions.

Introduction

Nature has long served as a primary reservoir for the discovery of novel therapeutic agents, with medicinal plants forming the backbone of traditional healthcare systems globally. The genus *Blepharis* (family Acanthaceae), comprising over 120 species widely distributed across arid and semi-arid regions of Asia and Africa, holds a prominent place in indigenous medicine (Dirar *et al.*, 2021). *Blepharis integrifolia* (syn. *Blepharis repens*, *Blepharis molluginifolia*), a prostrate and creeping herb, is particularly valued in traditional practices. Historically, various parts of this plant have been utilized to treat a wide spectrum of ailments, including

inflammatory conditions, bone fractures, wound healing, and gastrointestinal disorders (Coudhary *et al.*, 2023; Dirar *et al.*, 2021). Despite its widespread ethnomedicinal use, the systematic scientific validation of its biochemical constituents and therapeutic mechanisms remains an emerging focus in botanical research. The therapeutic efficacy of medicinal plants like *B. integrifolia* is fundamentally linked to their rich profile of secondary metabolites. Preliminary qualitative phytochemical screenings have indicated the presence of several vital chemical classes within the *Blepharis* genus, notably flavonoids, phenolic acid derivatives,

alkaloids, and phytosterols (Dirar *et al.*, 2021). However, establishing the pharmaceutical viability of this specific species necessitates comprehensive qualitative and quantitative profiling. Qualitative analysis provides the foundational fingerprint of the plant's biochemical makeup, while quantitative analysis, specifically the determination of total phenolic and total flavonoid contents, are imperative. These concentrations serve as critical biomarkers for standardizing the plant material and ensuring reproducibility in biological applications (Coudhary *et al.*, 2023).

The pharmacological potential of *B. integrifolia* is hypothesized to be a direct consequence of this complex phytochemical landscape. Recent pharmacological paradigms emphasize the role of plant-derived polyphenols and flavonoids as potent antioxidants capable of mitigating oxidative stress, a primary catalyst in the pathogenesis of chronic inflammation (Coudhary *et al.*, 2023). Furthermore, while traditional

applications strongly suggest robust antimicrobial and wound-healing properties, detailed empirical data exploring the specific *in vitro* biological activities of *B. integrifolia* plant extracts remain relatively limited (Dirar *et al.*, 2021).

To bridge the gap between traditional ethnomedicinal knowledge and modern phytopharmacology, a rigorous, targeted evaluation of this species is required. Therefore, the present study was designed to comprehensively evaluate the phytochemical profile of *B. integrifolia*. Specifically, this research aims to (1) perform qualitative and quantitative analyses of the plant extracts to identify major bioactive constituents, and (2) evaluate the *in vitro* biological activities of these extracts, focusing on their antioxidant, antidiabetic, and antimicrobial potential, thereby establishing a concrete scientific rationale for their therapeutic use.



Fig 1a: *Blepharis integrifolia* Plant,



Fig 1b: Figure: Filtration of the solvent extract

Materials and methods

Collection of the plant material: The fresh plant specimens of *Blepharis integrifolia* used in this study (**Figure 1a**) were collected in December 2024 from Lakkanahalli, Chitradurga district, Karnataka, India.

Preparation of plant extract: The shade-dried whole plant material was pulverized into a coarse powder. For the extraction process, the plant powder was divided to perform parallel cold macerations: a 20 g portion was macerated using 200 mL of 70% ethanol, and a separate 20 g portion was macerated using 200 mL of chloroform. Both mixtures were maintained at room temperature for 48h with occasional shaking to facilitate optimal extraction of the phytoconstituents. The mixtures were then independently filtered through Whatman No. 1 filter paper (**Figure 1b**). A small portion of each resulting liquid filtrate was reserved for preliminary qualitative phytochemical screening. The remaining filtrates were concentrated under reduced pressure to evaporate their respective solvents, yielding the dried crude hydroethanolic (BIE) and chloroform (BIC) extracts. The dried extracts were stored at 4 °C in airtight containers until further use for quantitative estimations and the evaluation of in vitro biological activities, specifically the antibacterial, antioxidant, and antidiabetic properties of *Blepharis integrifolia*.

Qualitative phytochemical analysis:

The extracts were subjected to preliminary qualitative phytochemical screening using standard protocols to detect the presence of various diverse bioactive compounds (Harborne *et al.*, 1998; Kokate *et al.*, 2007; Sofowora *et al.*, 1993).

Test for Phenols (Lead Acetate Test): One milliliter (1 mL) of the sample was

treated with a few drops of 10% lead acetate solution. The formation of a bulky white precipitate indicated the presence of phenolic compounds.

Test for Alkaloids:

Wagner's Test: Wagner's reagent was added to 1 mL of the sample. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

Dragendorff's Test: Dragendorff's reagent was carefully added to the sample. The formation of a prominent orange-red precipitate confirmed the presence of alkaloids.

Test for Flavonoids:

Alkaline Reagent Test: The sample was treated with a few drops of 20% sodium hydroxide (NaOH) solution. The appearance of an intense yellow color, which became colorless upon the addition of dilute hydrochloric acid (HCl), indicated the presence of flavonoids.

Lead Acetate Test: A few drops of lead acetate solution were added to the sample. The formation of a yellow precipitate indicated the presence of flavonoids.

Test for Tannins (Ferric Chloride Test): One milliliter (1 mL) of the sample was treated with a few drops of 5% neutral ferric chloride (FeCl₃) solution. The appearance of a dark blue or blue-green color indicated the presence of tannins.

Test for Terpenoids (Salkowski Test): Five milliliters (5 mL) of the sample were mixed with 2 mL of chloroform, followed by the careful addition of 3 mL of concentrated sulfuric acid (H₂SO₄) along the sides of the test tube. The development of a reddish-brown coloration at the interface indicated the presence of terpenoids.

Test for Saponins (Foam Test): A small quantity of the extract (approx. 1 mL) was diluted with 5 mL of distilled water in a graduated cylinder and vigorously shaken. The formation of a persistent, stable froth indicated the

presence of saponins.

Test for Glycosides (Keller-Killiani Test): The sample was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride (FeCl_3) solution, followed by the careful addition of 1 mL of concentrated sulfuric acid (H_2SO_4) along the walls of the tube. The formation of a brown ring at the junction of the two liquids indicated the presence of cardiac glycosides.

Test for Steroids (Liebermann-Burchard Test): The sample was dissolved in 2 mL of chloroform and treated with 2 mL of acetic anhydride, followed by the addition of a few drops of concentrated sulfuric acid (H_2SO_4). A color change from blue to green indicated the presence of steroids.

Test for Proteins (Biuret Test): Two milliliters (2 mL) of the sample were treated with 2 mL of 40% sodium hydroxide (NaOH) solution and a few drops of 1% copper sulphate (CuSO_4) solution. The appearance of a violet or purple color indicated the presence of proteins.

Test for Carbohydrates (Benedict's Test): Two milliliters (2 mL) of the sample were treated with 5 drops of Benedict's reagent and heated in a boiling water bath for 5 minutes. The appearance of a light green, yellow, or reddish-brown precipitate indicated the presence of reducing sugars.

Quantitative phytochemical estimation:

Total Phenolic estimation:

The total phenolic content of the extracts was determined following the method described by Singleton et al. (1999) using the Folin-Ciocalteu (FC) reagent, with gallic acid used as the standard. Briefly, to an aliquot of the appropriately diluted extract (equivalent to 100 μg of the sample), 0.5 mL of FC reagent was added. The mixture was incubated at room temperature for 10 min, followed by the addition of 2 mL of 7% Na_2CO_3 solution. The reaction

mixture was then boiled for 1 min, and the absorbance was recorded at 750 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The results were expressed as micrograms of gallic acid equivalents per milligram of extract ($\mu\text{g GAE/mg}$).

Total flavonoid estimation:

The total flavonoid content of the extracts was determined following the method described by Delcour and de Varebeke (1985). Briefly, to an aliquot of the sample (equivalent to 200 μg), 5 mL of chromogen reagent (0.1% cinnamaldehyde solution in a chilled mixture of 75 mL methanol and 25 mL concentrated HCl) was added. After a 10 min incubation period, the absorbance was recorded at 640 nm using the UV-Vis spectrophotometer. The total flavonoid content was expressed as micrograms of quercetin equivalents per milligram of extract ($\mu\text{g QE/mg}$).

Physicochemical Evaluation of Plant Material

The physicochemical parameters of the plant powder, specifically moisture content and total ash value, were determined following standard guidelines to assess the quality and purity of the sample (World Health Organization, 1998).

Determination of Moisture Content

The moisture content was evaluated using the standard loss-on-drying method. A precisely weighed amount of the initial plant biomass was placed in a pre-weighed crucible and dried in a hot air oven at 100 $^{\circ}\text{C}$ for 10 h. Following the drying period, the sample was allowed to cool, and the final constant weight was recorded. The moisture content was calculated based on the weight loss and expressed as a percentage of the initial sample weight using the following formula:

Moisture Content (%) = $\frac{[(\text{Initial weight} -$

Final weight) / Initial weight] $\times$ 100

Determination of Total Ash Content

The total ash content was determined to evaluate the amount of residual inorganic matter. Briefly, 5 g of the accurately weighed plant powder was placed in a previously tared crucible. The sample was then incinerated in a muffle furnace at 550 °C for 10 min. After incineration, the crucible was cooled, and the final ash weight was recorded. The total ash value was calculated based on the weight of the residual ash and expressed as a percentage of the total initial sample weight using the following formula:

Total Ash (%) = (Weight of ash / Weight of sample taken) $\times$ 100

Antibacterial Activity (Agar Well Diffusion Method)

The antimicrobial activity of the samples was evaluated using the agar well diffusion method, following the protocol described by Ranjitha et al. (2023) with minor modifications. The samples were tested against two bacterial strains: Gram-negative *Escherichia coli* (MTCC-7410) and Gram-positive *Staphylococcus aureus* (MTCC-7443). The bacterial inocula were adjusted to a concentration of approximately 5×10^5 CFU/mL using sterile saline solution. A stock solution was prepared by dissolving the samples in dimethyl sulfoxide (DMSO) at a concentration of 20 mg/mL, from which varying concentrations were subsequently loaded into the respective wells on Mueller-Hinton agar (MHA) plates. Following incubation at 37 °C, the antibacterial activity was quantified by measuring the diameter of the resulting zones of inhibition (mm).

In vitro Antioxidant Activity

DPPH Free Radical Scavenging Assay

The free-radical scavenging activity of the samples was evaluated using the

stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, following the methodology described by Blois (1958). Briefly, varying concentrations of the sample extracts were mixed with 2 mL of a DPPH methanolic solution (100 μ M), and the final volume was adjusted to 3 mL with methanol. The reaction mixture was thoroughly mixed and incubated in the dark for 45 min at room temperature. Following the incubation period, the absorbance of the mixture was recorded at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) against a control (reaction mixture without the test sample or standard). The free radical scavenging capacity of the samples was calculated and expressed as the half-maximal inhibitory concentration (IC₅₀) value, utilizing ascorbic acid (Vitamin C) as the standard reference antioxidant.

Ferrous-Ion Chelating Assay

The ferrous-ion chelating ability of the samples was determined according to the method described by Dinis et al. (1994). Briefly, varying concentrations of appropriately diluted samples were separately mixed with 0.05 mL of 2 mM FeCl₂. The reaction was initiated by the addition of 0.1 mL of 5 mM ferrozine, followed by thorough mixing and incubation for 10 min at room temperature. The absorbance of the resulting ferrozine-Fe²⁺ complex was measured at 562 nm using a UV-Vis spectrophotometer against a control blank (reaction mixture without the test sample or standard). The percentage inhibition of the ferrozine-Fe²⁺ complex formation was calculated, and the results were expressed as the half-maximal inhibitory concentration (IC₅₀) value, utilizing ethylenediaminetetraacetic acid (EDTA) as the standard reference chelator.

In vitro Antidiabetic Activity

Inhibitory Activity Against α -Amylase

The α -amylase inhibitory activity of the samples was evaluated following the method described by Gharge et al. (2024) with minor modifications. Porcine pancreatic α -amylase (1 U/mL; SRL, Bangalore, India) was prepared in 0.1 M phosphate-buffered saline (PBS, pH 6.9). Varying concentrations of the sample extracts (0–100 μ g/mL) were pre-incubated with 100 μ L of the enzyme solution for 10 min at 37 °C. The reaction was initiated by the addition of a substrate solution (0.1% starch) to the incubation medium. Following an additional 10 min of incubation, the reaction was terminated by adding 250 μ L of dinitrosalicylic acid (DNS) reagent (comprising 1% 3,5-dinitrosalicylic acid, 0.2% phenol, 0.05% Na_2SO_3 , and 1% NaOH in an aqueous solution) and subsequently placing the reaction mixture in a boiling water bath for 10 min. Thereafter, 250 μ L of a 40% potassium sodium tartrate solution was added. After cooling the mixture to room temperature in a cold-water bath, the absorbance was recorded at 540 nm using a UV-Vis spectrophotometer. The percentage of α -amylase inhibition was calculated, and the results were expressed as the half-maximal inhibitory concentration (IC_{50}) value, utilizing acarbose as the positive standard reference.

Inhibitory Activity Against α -Glucosidase

The α -glucosidase inhibitory activity of the samples was evaluated following the method described by Patil et al. (2024). Briefly, 50 μ L of yeast α -glucosidase (1 U/mL; SRL, Bangalore, India) was dissolved in phosphate buffer (50 mM, pH 6.9) and pre-

incubated with various concentrations of the sample extracts (0–100 μ g/mL) independently for 10 min at 37 °C. The reaction was initiated by the addition of 50 μ L of 5 mM p-nitrophenyl- α -D-glucopyranoside in phosphate buffer. The enzyme reaction was carried out at 37 °C for 30 min. The reaction was terminated by the addition of Na_2CO_3 (1 M), and the absorbance was recorded at 405 nm. The percentage of α -glucosidase inhibition was calculated, and the results were expressed as the half-maximal inhibitory concentration (IC_{50}) value, utilizing acarbose as the positive reference standard.

Results and discussion

Preliminary Qualitative Phytochemical Analysis

The preliminary qualitative phytochemical screening of *Blepharis integrifolia* extracts revealed the presence of various primary and secondary metabolites, demonstrating distinct profiles based on the solvent used for extraction (**Table 1**). Both the 70% ethanol extract (BIE) and the chloroform extract (BIC) tested positive for several vital bioactive constituents, including phenols, flavonoids, proteins, and carbohydrates. However, the varying polarities of the solvents resulted in specific differences between the two extracts. Terpenoids were exclusively detected in the ethanol extract (BIE), whereas alkaloids were selectively extracted and identified only in the chloroform extract (BIC). Notably, both solvent extracts tested negative for tannins, saponins, glycosides, and steroids.

Table. 1: Qualitative phytochemical estimation of extract samples

Sl No	Phytochemical tests	BIE	BIC
1	Phenols	+	+
2	Alkaloids	-	+
3	Flavonoids	+	+
4	Tannins	-	-
5	Terpenoids	+	-
6	Saponins	-	-
7	Glycosides	-	-
8	Steroids	-	-
9	Proteins	+	+
10	Carbohydrates	+	+

(Note: + **Present**, - **Absent**)

BIC = *Blepharis integrifolia* Chloroform extract

BIE = *Blepharis integrifolia* 70 % Ethanol extract

Quantitative Phytochemical Estimation

Following the qualitative identification of phytoconstituents, a quantitative spectrophotometric analysis was conducted to determine the total polyphenol and total flavonoid contents of the *B. integrifolia* extracts (**Table 2**). The contents were expressed as gallic acid equivalents (GAE) and quercetin equivalents (QE), respectively. The quantitative assessment revealed a distinct solvent-dependent variation in the concentration of these secondary metabolites. The chloroform extract (BIC) yielded a higher total polyphenol

Quantitative phytochemicals estimation:

content, recording 342.24 ± 16.126 $\mu\text{g}/\text{mg}$ GAE, compared to the 70% ethanol extract (BIE), which contained 282.94 ± 13.87 $\mu\text{g}/\text{mg}$ GAE.

Conversely, the extraction of flavonoids was notably favored by the highly polar hydroalcoholic solvent. The 70% ethanol extract (BIE) exhibited a substantially higher total flavonoid content (197.51 ± 8.41 $\mu\text{g}/\text{mg}$ QE) compared to the chloroform extract (BIC), which recorded 108.25 ± 7.88 $\mu\text{g}/\text{mg}$ QE (**Fig 2**).

Table. 2: Quantitative estimation of polyphenols and flavonoids

Sl. No.	Samples	Polyphenols ($\mu\text{g}/\text{mg}$ GAE)	Flavonoids ($\mu\text{g}/\text{mg}$ QE)
1	BIE	282.94 ± 13.87	197.51 ± 8.41
2	BIC	342.24 ± 16.126	108.25 ± 7.88

BIC = *Blepharis integrifolia* Chloroform extract

BIE = *Blepharis integrifolia* 70 % Ethanol extract

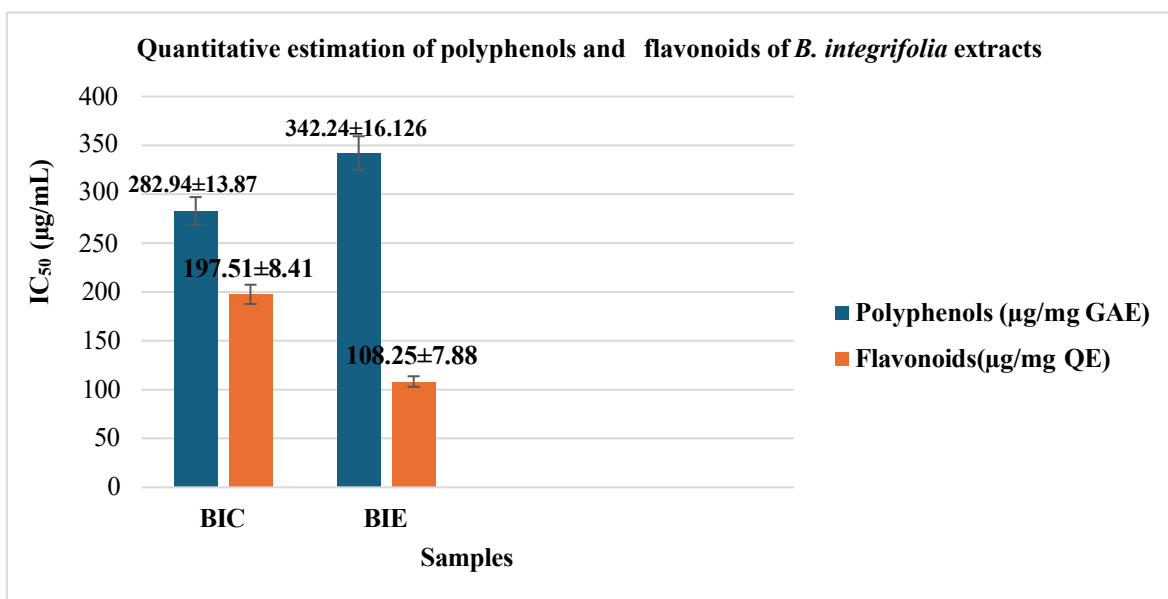


Fig 2: Quantitative estimation of polyphenols and flavonoids *B. integrifolia* extracts

Physicochemical Analysis (Moisture and Ash Content)

The physicochemical evaluation of the raw *B. integrifolia* plant powder (PA) was conducted to assess its purity and quality for subsequent extractions, with the results detailed in **Table 3**. The moisture content of the plant biomass was determined to be 2.41%. This low residual moisture level indicates that the plant material was adequately dried, which is essential to prevent microbial

degradation and enzymatic breakdown of active phytoconstituents during storage. Furthermore, the total ash content, which represents both the physiological ash (plant tissue minerals) and non-physiological ash (extraneous matter such as soil and sand), was recorded at 8.31%. These values establish important pharmacognostic baseline standards for this species.

Moisture and Ash content

Table. 3: Moisture and total ash content estimation in plant powder sample expressed in percent

Sl. No.	Samples	Total Ash	Moisture
1	Plant powder	8.31 %	2.41%

In vitro Antibacterial Activity

The antibacterial efficacy of the *Blepharis integrifolia* extracts was evaluated against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacterial strains using the agar well diffusion method. The results, quantified by measuring the diameter of the zones of inhibition (in mm), are presented in **Table 4;(Fig 3).**

Both the 70% ethanol extract (BIE) and the chloroform extract (BIC) demonstrated significant, dose-dependent antibacterial activity against the tested pathogens. The negative control (Blank) exhibited no zones of inhibition, confirming that the solvent system did not interfere with the bacterial growth.

As the concentration of the extracts increased from 1 mg to 3 mg, a proportional increase in the zone of inhibition was observed across both strains. At the highest tested concentration (3 mg), both extracts displayed comparable and potent antibacterial properties. The chloroform extract (BIC) produced inhibition zones of 15.66 ± 0.57 mm against *S. aureus* and 15.33 ± 0.57 mm against *E. coli*. Similarly, the 70% ethanol extract (BIE) yielded zones of 15.33 ± 0.57 mm and 15.66 ± 0.57 mm for *S. aureus* and *E. coli*, respectively. These findings indicate that *B. integrifolia* possesses a broad-spectrum antibacterial capability, effectively neutralizing both Gram-positive and Gram-negative bacteria.

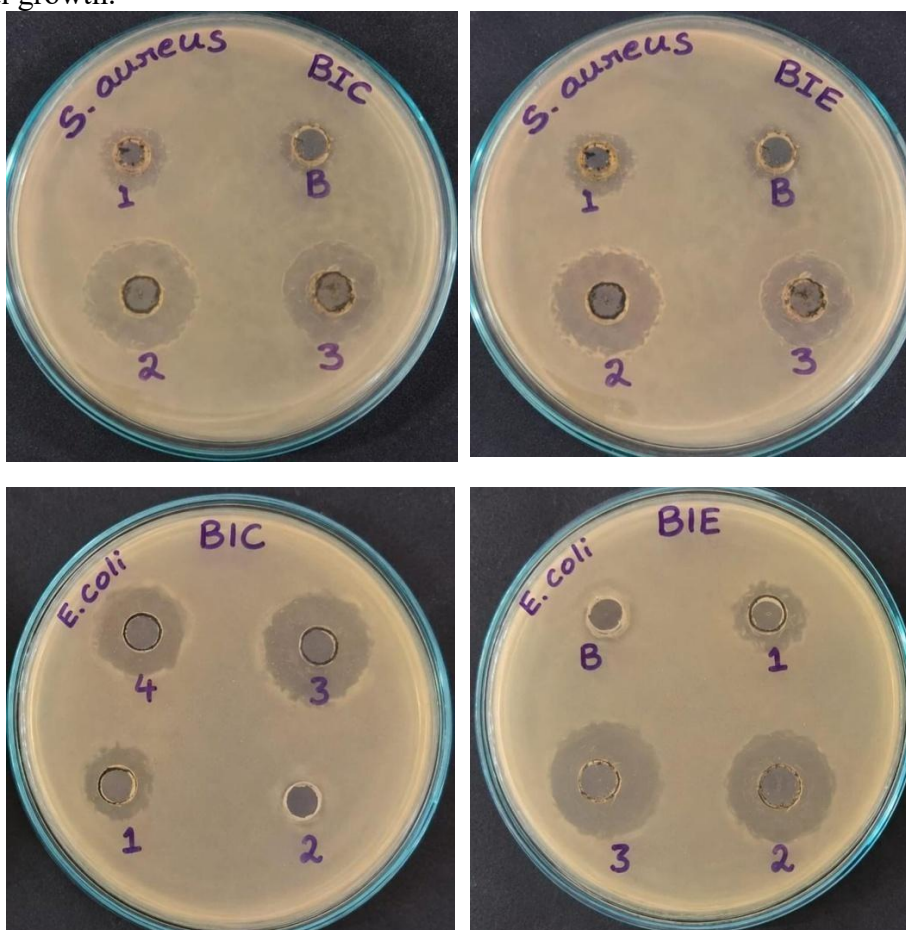


Fig 3: Antibacterial activity of *Blepharis integrifolia* Chloroform extract (BIC) and *Blepharis integrifolia* 70 % Ethanol extract (BIE)

Table 4: Antibacterial properties of samples by Agar well diffusion method in zone of inhibition mm

Sl.	Sample Name	Conc. (mg)	<i>S. aureus</i>	<i>E. coli</i>
1.	BIC	B	---	---
		1	7.33 ± 0.57	8.66 ± 0.57
		2	10.33 ± 1.15	11.33 ± 0.57
		3	15.66 ± 0.57	15.33 ± 0.57
2.	BIE	B	---	---
		1	7.33 ± 0.57	7.66 ± 1.15
		2	11.66 ± 0.57	12.33 ± 1.15
		3	15.33 ± 0.57	15.66 ± 0.57

(Note: B = Blank / Negative Control; No zone of inhibition observed)

BIC = *Blepharis integrifolia* Chloroform extract

BIE = *Blepharis integrifolia* 70 % Ethanol extract

DPPH Free Radical Scavenging Activity

The in vitro antioxidant potential of the *Blepharis integrifolia* extracts was evaluated using the DPPH free-radical scavenging assay. The scavenging capacity of the samples was quantified and expressed as the half-maximal inhibitory concentration (IC_{50}), where a lower IC_{50} value indicates a higher antioxidant potency (Table 5).

The 70% ethanol extract (BIE) demonstrated a robust free-radical scavenging ability with an IC_{50} value of 88.835 $\mu\text{g/mL}$, exhibiting significantly

greater antioxidant potential than the chloroform extract (BIC), which recorded an IC_{50} of 162.721 $\mu\text{g/mL}$. This suggests that the polar phytoconstituents extracted in the hydroalcoholic solvent contribute more significantly to the free-radical scavenging capacity of the plant. While both extracts showed notable dose-dependent scavenging activities, they were less potent compared to the standard reference, Vitamin C, which yielded an IC_{50} value of 23.596 $\mu\text{g/mL}$ (Fig 4).

Antioxidant activity by DPPH method

Table 5: Antioxidant properties of samples by DPPH method expressed in IC₅₀ value

Sl No	Samples	DPPH IC ₅₀ (µg/mL)
1.	BIC	162.721
2.	BIE	88.835
3.	Vit-C	23.596

BIC = *Blepharis integrifolia* Chloroform extract

BIE = *Blepharis integrifolia* 70 % Ethanol extract

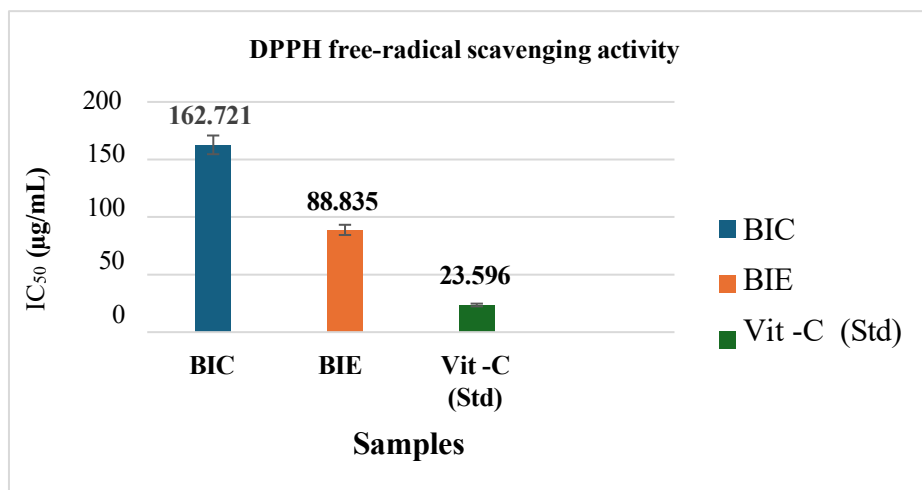


Fig 4: DPPH free-radical scavenging activity

Ferrous-Ion Chelating Activity

The metal chelating capacity of the extracts, which serves as an important secondary antioxidant mechanism by preventing transition metals from catalyzing oxidative stress, was evaluated using the ferrous-ion chelating assay (**Table 6**).

Similar to the trends observed in the DPPH assay, the polar hydroalcoholic solvent yielded a more active extract.

The 70% ethanol extract (BIE) exhibited a stronger iron-chelating ability with an IC_{50} value of 118.367 $\mu\text{g/mL}$, compared to the chloroform extract (BIC), which demonstrated an IC_{50} of 243.394 $\mu\text{g/mL}$. The standard reference utilized for this assay (EDTA) exhibited an IC_{50} of 43.555 $\mu\text{g/mL}$, indicating that while the extracts possess notable chelating properties, they are less potent than the pure standard chelator (**Fig 5**).

Table 6: Antioxidant properties of samples by Ferrous-Ion Chelating Activity expressed in IC_{50} value

Sl No	Samples	Iron chelating IC_{50} ($\mu\text{g/mL}$)
1.	BIC	243.394
2.	BIE	118.367
3.	EDTA	43.555

BIC = *Blepharis integrifolia* Chloroform extract

BIE = *Blepharis integrifolia* 70 % Ethanol extract

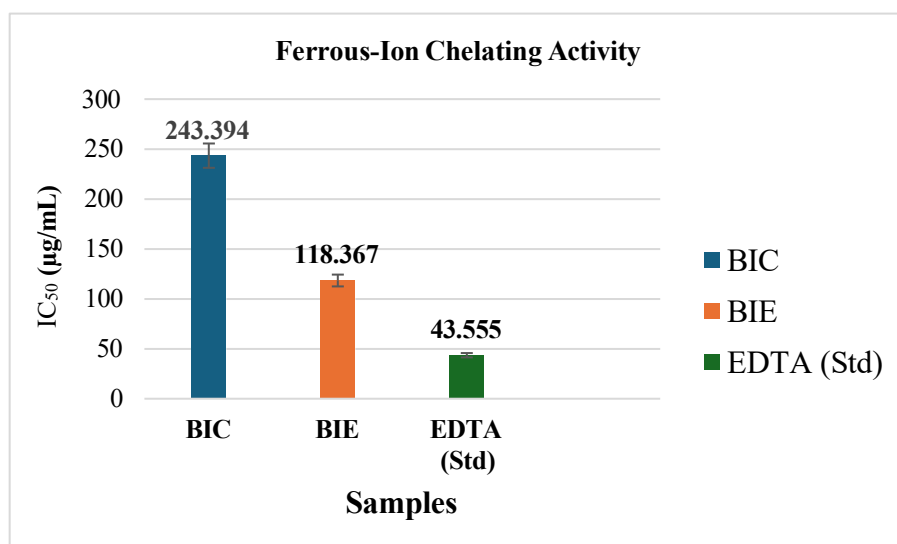


Fig 5: Ferrous-Ion Chelating Activity

In vitro Antidiabetic Activity

The in vitro antidiabetic potential of the *Blepharis integrifolia* extracts was evaluated by assessing their inhibitory effects on two key carbohydrate-hydrolyzing enzymes: α -amylase and α -glucosidase. The enzymatic inhibition was quantified and expressed as the half-maximal inhibitory concentration (IC_{50}), where a lower value indicates a higher inhibitory potency (Table 7). In the α -amylase inhibition assay, the chloroform extract (BIC) demonstrated strong inhibitory activity, yielding an IC_{50} value of 65.078 $\mu\text{g/mL}$. This was notably more potent than the 70% ethanol extract (BIE),

which recorded an IC_{50} of 85.479 $\mu\text{g/mL}$. A similar trend was observed in the α -glucosidase inhibitory assay. The chloroform extract (BIC) once again exhibited superior activity with an IC_{50} of 60.164 $\mu\text{g/mL}$, compared to the 70% ethanol extract (BIE), which produced an IC_{50} of 67.012 $\mu\text{g/mL}$ (Fig 6). Overall, while both crude plant extracts exhibited promising dose-dependent enzyme inhibition, they displayed lower potency compared to the pure clinical antidiabetic standard, acarbose, which recorded IC_{50} values of 34.596 $\mu\text{g/mL}$ and 29.53 $\mu\text{g/mL}$ for α -amylase and α -glucosidase, respectively.

Table. 7: Inhibitory activity against α -amylase and α -glucosidase

Sl No	Samples	α -Amylase IC_{50} ($\mu\text{g/mL}$)	α -Glucosidase IC_{50} ($\mu\text{g/mL}$)
1	BIC	65.078	60.164
2	BIE	85.479	67.012
3	Acarbose(std)	34.596	29.53

BIC = *Blepharis integrifolia* Chloroform extract

BIE = *Blepharis integrifolia* 70 % Ethanol extract

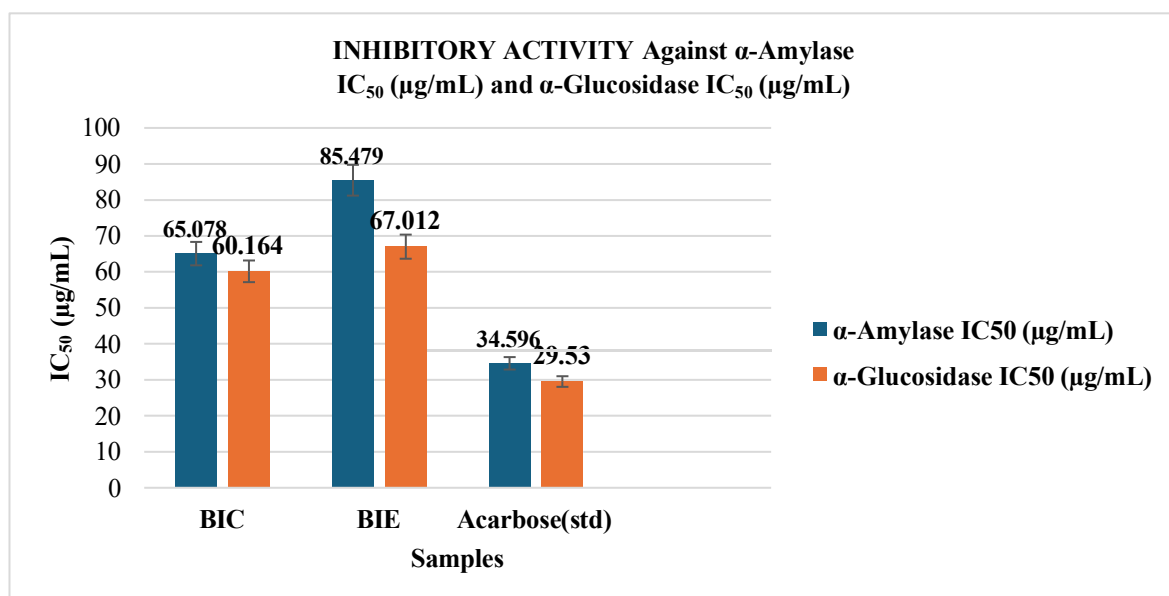


Fig 6: Inhibitory activity against α -amylase and α -glucosidase

Conclusion

The present study comprehensively validates the therapeutic potential of *Blepharis integrifolia*, establishing its distinct phytochemical profile and pharmacological efficacy. Physicochemical analysis provided foundational pharmacognostic standards, specifically moisture and total ash content, ensuring the quality and stability of the raw plant biomass. The preliminary and quantitative phytochemical screenings revealed that solvent polarity significantly influences the extraction of secondary metabolites. The hydroethanolic extract (BIE) selectively extracted terpenoids and yielded a substantially higher concentration of flavonoids. In contrast, the chloroform extract (BIC) was characterized by a higher total polyphenol content and the exclusive extraction of alkaloids. Biologically, both extracts demonstrated significant, dose-dependent, and broad-spectrum antibacterial activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) pathogens. Coupled with the robust antioxidant capacity of the polar fraction and the potent antidiabetic (α -amylase and α -glucosidase inhibitory) activity of the non-polar fraction, these

findings scientifically substantiate the traditional ethnomedicinal applications of the plant.

Ultimately, *Blepharis integrifolia* serves as a promising natural reservoir for novel therapeutic agents. Future research should prioritize the targeted chromatographic isolation, structural elucidation, and in vivo testing of the specific alkaloids and flavonoids responsible for these observed pharmacological activities to facilitate natural product-based drug discovery.

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