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BIOMEDICAL POTENTIAL OF *STROBILANTHES GLUTINOSUS*: EXPLORING ANTIMICROBIAL, ANTIOXIDANT, AND ENZYME INHIBITION

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Abstract: Strobilanthes glutinosus Nees, is a useful medicinal herb owing to its bioactive secondary metabolites. In this study the biomedical applications of leaf and stem extracts of S. glutinosus such as antimicrobial, antioxidant, antidiabetic, and anti-melanin activities (in vitro) and antiinflammatory activity (in vivo) were explored. Antibacterial and antifungal activities showed dose-dependent response against selective bacterial and fungal strains. The leaves and stem extracts showed better DPPH scavenging activity with IC_{50} value of 0.54 and 0.49 mg/ml respectively in comparison to standard ascorbic acid (0.76 mg/ml). Similarly, ABTS scavenging values of leaves, stem, and standard Trolox were 0.85, 0.32, and 2.55 µg/ml respectively. The S. glutinosus sample showed comparatively better α-amylase inhibition potential having IC_{50} values of 0.06 and 0.46 µg/ml by leaves and stem extract respectively vs acarbose (0.745 µg/ml). In the case of Tyrosinase inhibitory assay, the IC_{50} values of leaves, stem and standard Kojic acid were 5.924, 7.46 and 3.166 µg/ml. The leaf and stem extracts of S. glutinosus showed better anti-inflammatory activity in rabbits. Thus, S. glutinosus possesses great biomedical applications.

Keywords: Antiinflammatory, Antioxidant. Medicinal plants, Antimicrobial, Enzyme Inhibition

1. Introduction

The interest to explore novel bioactive phytochemical from plants with medicinal value has gained substantial consideration currently owing to their growing demand for effective therapeutic agents

with minimal adverse effects. One particular plant with rich traditional medicinal value is the *Strobilanthes. glutinosus* Nees. *S. glutinosus Nees*, commonly known as "mukunuwenna" or "sticky wicky," is a perennial herb of family Acanthaceae which is predominantly found in Southeast Asia, including Sri Lanka, India and Pakistan [1]. With its diverse phytochemical composition and reported pharmacological activities, *S. glutinosus* is a great source of active biocompounds for various therapeutic applications [2, 3].

The Acanthaceae family, specifically the Ruellieae: Strobilanthinae subgroup, is among the largest angiosperm families, comprising 346 genera and approximately 4,300 species. In the subcontinent alone, it is estimated that there are around 150 Strobilanthes species, with 59 of them distributed in peninsular areas [4]. Several species within the Strobilanthes genus have garnered attention due to their pharmacological significance based on their phytochemical composition and biomedical activities [5]. For instance, *S. cusia*, a traditional Chinese medicinal plant, has been extensively utilized by local communities for treating severe acute respiratory syndrome (SARS) [6], sore throat [7], influenza [8] coronavirus NL63 [9], inhibition of glycolytic metabolism [10], renal cancer [11], colon cancer [12], breast and prostate cancer [13]. Furthermore, *S. callosus* has shown potential for the treatment of acute and chronic inflammation [7], viral pneumonia, epidemic cerebrospinal meningitis, mumps, and encephalitis B [14].

S. glutinosus Nees has long been recognized for its culinary and medicinal properties in traditional systems of medicine [15]. However, the scientific community has paid limited attention to investigating the composition of its crude extracts' phytoconstituents and assessing its antibacterial and antioxidant activities [16]. Therefore, the scarcity of research on *S. glutinosus* Nees plant not only limits our understanding of its therapeutic properties but also hampers the development of effective pharmaceutical interventions derived from this plant. In order to fully unlock its potential, it is crucial to bridge this knowledge gap by identifying and characterizing the bioactive compounds present in its crude extracts. Additionally, the evaluation of its antibacterial and antioxidant activities is essential for evaluating its potential efficacy and safety as a medicinal agent. By unraveling the unique bioactive compounds present in *S. glutinosus* and understanding their mechanisms of action, we can uncover new possibilities for its utilization in healthcare and the development of novel therapeutic interventions [17].

Type II diabetes (T2D) is a metabolic disorder with 463 million patients worldwide [18]. According to recent study, 16.93% Pakistani population is affected with T2D [19]. The causes of T2D include pancreatic beta-cell dysfunction, impaired secretory function of adipocytes, and diminished insulin function in liver [20, 21].

Alpha-amylase (1,4-glucan-4glucanohydrolases) is a calcium metalloenzyme produced by the pancreas and salivary glands that helps in breaking down internal αlpha-1,4-glycosidic bonds of starch to form maltose and glucose [22, 23]. Then glucose is absorbed in intestine and spread into the body. Inhibition of α-amylase and α-glucosidase results in the increased carbohydrates absorption and decreases blood glucose level [24]. Therefore, inhibition of these enzymes might be a promising therapeutic approach to treat hyperglycemia. Moreover, amylase inhibitors have a remedial worth and are used to manage diabetes [22, 25]

Tyrosinase enzyme inhibition is a critical area of research in biochemistry and pharmacology. Tyrosinase, a copper-containing enzyme, is present in animals, plants, and fungi which is involved in melanin synthesis. Melanin is the a skin pigment which is involved in protection of human skin from ultraviolet radiations as well as responsible for giving skin, hair, and eye color [26]. When there is an overproduction and deposition of melanin, it can result in conditions like solar melanosis or age spots, ephelides, melasma, senile lentigos, and post-inflammatory hyperpigmentation [27]. On the other hand, when exposed to UV radiation, the body produces too much Reactive-Oxygen Species (ROS) which can interact with lipids, proteins, and DNA and changes cellular processes causing age-related diseases or melanogenesis. The existing treatments for skin conditions including melasma, senile lentigo, and ephelides have not yielded satisfactory outcomes. Additionally, they are linked with severe effects including high toxicity and mutagenicity, limited skin penetration, and instability

of the formulation. It is necessary to develop novel medications with enzyme-inhibitory action [28]. Inhibition of tyrosinase activity can lead to a reduction in melanin production, making it an essential mechanism for skin lightening and treating hyperpigmentation.

The goal of this study was to assess the biomedical potential of *S. glutinosus* such as antimicrobial, antioxidant and enzyme inhibition of amylase and tyrosinase *in vitro* as well as antiinflammatory activities *in vivo*. Understanding the mechanisms underlying its effects will provide valuable insights into the therapeutic potential of *S. glutinosus* as a natural remedy for metabolic and skin disorders.

2. Materials and methods

2.1. Plant sampling

The sampling of *S. glutinosus* Nees was done from Kotli, Azad Jammu and Kashmir (AJK), Pakistan at 33.48˚N and 73.32˚E during flowering season. Sample specimen (voucher number MUST. BOT. 5356) of *S. glutinosus* was identified by an expert botanist.

2.1.1. Plant extraction

The fresh parts of *S. glutinosus* were washed and shade dried and subsequently converted into fine powder. Then plant powder (0.5 kg) was kept in 75 % of methanol in water for 2 days and shaking was done many times during daytime for good mixing. The plant material was filtered with muslin cloth, while leftover residue was subjected to two additional extractions with fresh solvent. The plant mixture was concentrated in a rotary-evaporator at 40°C. Semi-liquid extracts of leaves (SGLE) and stems (SGSE) were allowed to dry and then kept in refrigerator for further use.

2.2. In vitro studies

2.2.1. Antibacterial screening

Crude extracts obtained from SGLE and SGSE against various Gram-positive bacteria included *Methicillin Resistance Staphylococcus aureus* (MRSA), *Enterococcus faecalis*, *Enterococcus aerogenes*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus subtilis*, and *Micrococcus leteus* and Gram-negative bacterial strains included *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter aerogenes*. The antimicrobial assay was performed using disc diffusion procedure [18]. The bacteria were cultured overnight in nutrient broth at 36°C. Then, 100 µl of inoculum (108 CFU/ml) was dispersed on nutrient-agar plates. Sterile discs of a diameter of 6 mm were filled with 20 µl of plant sample (a concentration of 50 µg/µl). These discs were placed onto the agar plates containing the bacterial cultures. As a positive control, the standard antibiotic Rifampicin was used. Each plate was kept overnight at 37 °C. The samples were run in triplicates for each bacterium. The resulting inhibition zones formed around the filter discs were measured in millimeters. According to established criteria [29, 30], inhibition zone values ranging from 8 to 13 mm indicate low antibacterial activity, 14 to 19 mm indicate moderate activity, and a zone size of 20 mm or greater indicates high antibacterial activity

2.2.2. Antifungal screening

The antifungal screening of SGLE and SGSE was conducted through previously reported disc-diffusion procedure [29]. Prior to the experiment, culturing of fungal strains was done at 30°C in Sabouraud-Dextrose Agar media for 20 hours. Then, 100 µl of fungal suspension in distilled water was placed onto SDA plates. The fungal strains *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium solani*, and *Mucor indicus* were used in this study. A sterile disc with a diameter of 6 mm was loaded with 1000 µg of the test sample and placed on the medium plate. Following a 28-hours incubation, antifungal activity was measured through zones of inhibition. The procedure was conducted in triplicates, and the interpretation of results was as follows: a zone of inhibition measuring ≥ 20 mm indicated high inhibition, 14-19 mm indicated moderate inhibition, and 8-13 mm indicated low inhibition [31].

2.2.3. Antioxidant activity

The antioxidant potential of the SGLE and SGSE was measured using DPPH and ABTS scavenging methods.

2.2.3.1. DPPH method

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method was utilized for screening antioxidant potential of SGLE and SGSE [30, 32]. Methanolic solutions of test samples SGLE and SGSE of various concentrations of 150, 250, 500, and 1000 mg/ml were prepared. A 0.2 mM solution of DPPH in methanol was also prepared. In the dark, 3 ml of the different concentrations of SGLE and SGSE test samples were mixed with DPPH solution (1 ml) and kept in dark for about 30 minutes followed by absorbance measurement at 517 nm using an ELISA microplate reader (ELx808). For the control, 1 ml DPPH solution was mixed with 3 ml of methanol, and ascorbic acid used as the standard. Absorbance measurements were taken under the same conditions as for the plant extracts. The scavenging ability was determined using following formula:

$$\%\ inhibition\ = \frac{ODcontrol - ODsample}{\text{ODcontrol}}\ \times\ 100$$

2.2.3.2. The ABTS assay

The ABTS scavenging method was conducted with a few modifications based on [30]. To prepare the $ABTS^{\bullet+}$ solution, 7 mM ABTS solution was reacted with 2.45 mM of potassium persulfate. The resultant $ABTS^{++}$ solution kept in the dark at RT for 12-16 hours. Later, absorbance of $ABTS^{++}$ adjusted to 0.7 at 734 nm with methanol. 20 µl of the test sample or reference standard Trolox was taken in test tubes and then 2 ml of $ABTS^{\bullet+}$ was added. Test tubes were vortexed and kept at 25°C for 25 min. The absorbance was taken at 734 nm using an ELISA microplate reader (ELx808). The % inhibition was calculated with a specific formula:

$$\text{Inhibition}\ \% = \frac{ODstandard - ODsample}{\text{ODstandard}}\ \times 100$$

2.2.4. Enzyme inhibition assay

2.2.4.1. α-Amylase inhibition activity

To assess α-amylase inhibitory potential of the SGLE and SGSE, 3,5-dinitrosalicylic acid (DNSA) procedure was adopted [33]. First, the SGLE and SGSE samples were dissolved in DMSO (10%) and then in a buffer containing 0.02 M Na_2HPO_4/NaH_2PO_4, 0.006 M NaCl having pH 6.9. Various concentrations ranging from 250 to 1000 µg/ml were prepared. In each test tube, 200 µl of sample was added in 200 µl of α-amylase solution (2 units/ml), followed by 10 minutes incubation at 30°C. Afterwards, 200 µl of aqueous starch (1%) was mixed in each tube and kept for 3 min. This mix was warmed at 85-90 °C in water bath for 10 mins., and then 200 µl of DNSA reagent was added to stop the reaction. Distilled water (5 ml) was added and allowed to cool to RT followed by absorbance at 540 nm. For blank reaction, the crude extract was replaced with 200 µl of buffer. Acarbose (100 µg/ml) was used as control. The enzyme inhibition potential was determined using the provided equation and expressed as a percentage. By plotting the percentage of α-amylase inhibition against the SGLE and SGSE concentration, the IC50 values were calculated from the graph.

$$\%\ inhibition = \frac{\text{ODblank}\ -\ \text{ODsample}}{OD\text{blank}}\ \times\ 100$$

2.2.4.2. Tyrosinase enzyme inhibitory potential

The tyrosinase potential of plant samples was determined following a procedure with slight modifications [34, 35]. The mixtures containing 112.5 µl of 50 mM KH_2PO_4 buffer (pH 6.5), Kojic acid as the reference standard, and various concentrations of the test samples (5, 10, 25, 50, 100 µl)

were prepared. Additionally, 85 µl of deionized water and 2.5 µl of enzyme (5600 units/ml) were mixed to above mixture and kept at 37℃ for 8 minutes. Subsequently, 50 µl of L-Dopa (4.25 mM) was added as a substrate, mixed for 10 minutes, and maintained at 37℃. Then, absorbance was recorded at 475 nm with an ELISA microplate reader (ELx808). A control reaction was conducted without adding the test sample. The entire reaction was carried out in a 96-well plate, and three replicates were taken. Inhibition (%) was calculated using the provided equation.

$$\%\ inhibition = \frac{\text{ODblank} - \text{ODsample}}{\text{ODblank}} \times 100$$

2.3. In vivo studies

Male domestic rabbits of about 1 kg were obtained from market of Mirpur, AJK, Pakistan. All procedures pertaining to the care and use of rabbits were in line with NIH, USA Publication No. 18–23, 1985. Approval of all animal research was taken from MUST Animal Care and Use Committee (MUST-ACUC) at Mirpur University of Science and Technology (MUST) through notification number MUST-2021-08.

2.3.1. Anti-inflammatory potential

The anti-inflammatory potential of SGLE and SGSE was evaluated by inducing edema in hind paws of the experimental rabbits using carrageenan [32]. Animals were distributed into four groups. Each experimental group contained three rabbits (n = 3). Prior to the induction of paw edema, the rabbits were fasted overnight. Two treatment groups received the leaves and stem herbal extracts, at a dosage of 100 mg/kg dissolved in 0.1% sodium CMC. The negative control (third group) was given normal saline, while the positive group (fourth group) was administered Ibuprofen (5 mg/kg). After 60 minutes of the respective treatments, 0.1 ml of 1% carrageenan in saline was injected into intraplanar area of right back paw of experimental rabbit. The decrease in edema was measured at 30-minute intervals for a total of 3 hours using a plethysmometer. The reduction (% protection) was determined with following formula:

$$\%\ Protection = \frac{\text{Reading of control} - \text{Reading of sample}}{\text{Reading of control}} \times 100$$

2.4. Statistical analysis

The collected data was evaluated using GraphPad Prism 9 and Microsoft Excel 2010. The Mean and Standard Error of the Mean (Mean ± SEM) values were calculated.

3. Results

The therapeutic efficacy of *S. glutinosus* was assessed with various *in vitro* as well as *in vivo* bioassays such as antibacterial, antifungal, antioxidant, α-amylase and tyrosinase enzyme inhibition assays, and anti-inflammatory activity. *In vitro* as well as *in vivo* studies have demonstrated promising therapeutic properties of *S. glutinosus*.

3.1. Antibacterial assay

Leaves and stem extracts of *S. glutinosus* (SGLE and SGSE) showed significant inhibitory effect (20.4 ± 1.2 mm and 19.3 ± 0.6 mm respectively) against Gram-positive bacterial strain *E. faecalis* while for MRSA and *M. luteus* zone of inhibition were 10.5 ± 0.14, 8.5 ± 0.78 and 12 ± 0.07, 9.5 ± 0.42 respectively (Fig. 1a). The least antimicrobial potential was exhibited against *Bacillus subtilis* by SGLE (9.2 ± 0.92 mm) and SGSE (6.0 ± 0.35 mm). The SGLE and SGSE showed significantly improved antibacterial potential against the most sensitive Gram-negative bacterial strain *P. aeruginosa* with a zone of inhibition of 20.3 ± 0.42 mm and 20.3 ± 1.06 mm respectively while for *E. aerogenes* zone of inhibition were 8.5 ± 0.4 mm and 10.5 ± 0.5 mm respectively (Fig 1b). The zones of inhibition against Gram -ve bacteria *E. coli* and *K. pneumonia* exhibited were 11 ± 1.06 mm, 8.5 ± 1.41 mm and 15.3 ± 1.2 mm, 9.2 ± 0.85 mm by SGLE and SGSE respectively.

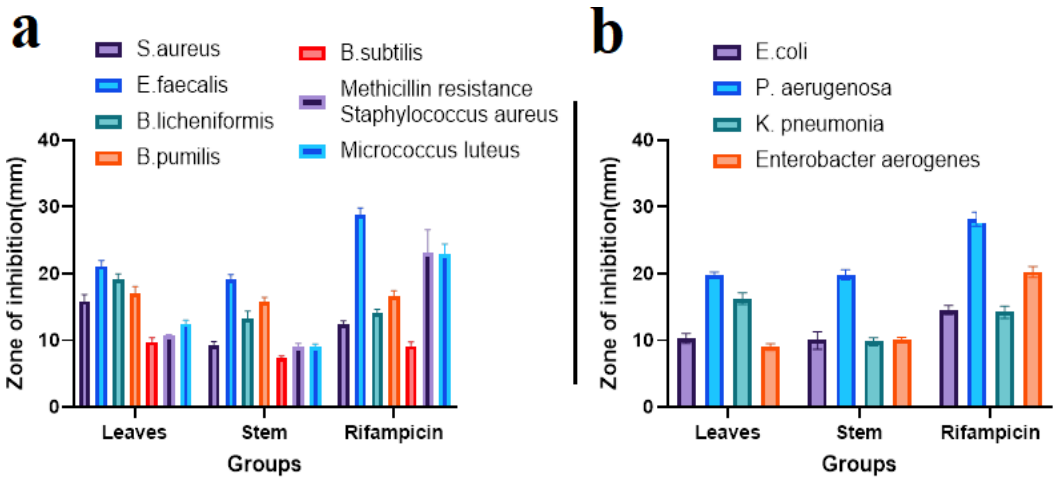

Figure 1. Antibacterial activity of *S. glutinosus* leaves and stem extracts: (a) against Gram-positive bacterial strains; (b) against Gram-negative bacterial strains. The results show zones of inhibition of SGLE, SGSE and reference standard Rifampicin. All values are expressed as Mean ± SEM and experiments are repeated thrice.

3.2. Antifungal assay

The antifungal assay of SGLE and SGSE were screened against four fungal strains viz. *Aspergillus fumigatus*, *Fusarium solani*, *Aspergillus niger* and *Mucor indicus*, Terbinafine was used as reference antifungal antibiotic. SGLE and SGSE showed moderate inhibition against selected fungal strains. The zones of inhibition were observed for SGLE against *Mucor indicus* (12 ± 0.14 mm), *A. fumigatus* (9.5 ± 0.07 mm), *F. solani* (9.9 ± 0.07 mm) and *A. niger* (10.9 ± 0.07 mm) as shown in Fig. 2. Similarly, SGSE showed zones of inhibition against *Mucor indicus* (9.5 ± 0.14 mm), *A. fumigatus* (10.5 ± 0.07 mm), *F. solani* (9.5 ± 0.07 mm) and *A. niger* (8.2 ± 0.14 mm) as shown in Fig. 2.

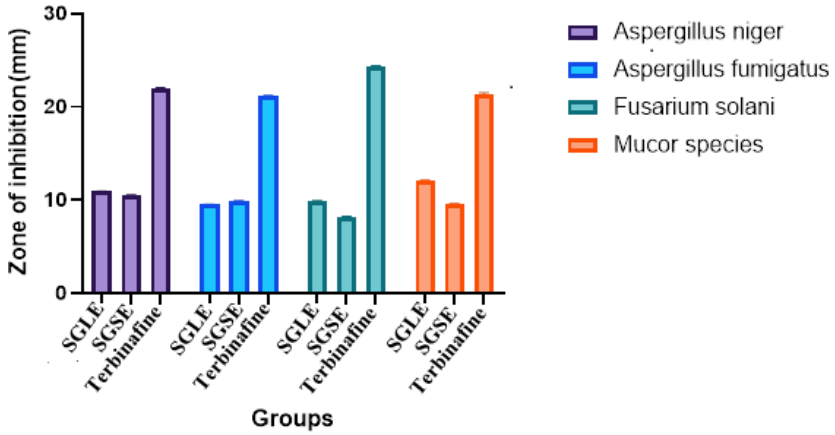

Figure 2. Antifungal activity of S. glutinosus leaves (SGLE) and stem (SGSE) extracts and positive control Terbinafine. The zones of inhibition of SGLE, SGSE, and Terbinafine against the selective model fungal strains. The procedure was carried out in triplicates. Results are represented as Mean ± SEM.

3.3. Antioxidant activity

The antioxidant assay of SGLE and SGSE was assessed by DPPH and ABTS *in vitro*. Both extracts SGLE and SGSE showed promising antioxidant activity, attributed to the existence of abundant secondary metabolites in *S. glutinosus*.

SGLE and SGSE exhibited IC50 values of 0.54 and 0.49 mg/ml, respectively, in comparison to the standard reference ascorbic acid (0.76 mg/ml). The IC values were calculated using a linear equation

derived from the plotted graphs of percentage inhibition against different concentrations of the test samples as shown in Fig. 3a. The antioxidant potential of SGLE and SGSE were also studied using the ABTS radical cation scavenging. The observed IC50 values for SGLE and SGSE were 0.85 and 0.32 µg/ml, respectively, when compared to the reference Trolox (2.55 µg/ml). These values were calculated using the linear equation provided in Fig. 3b.

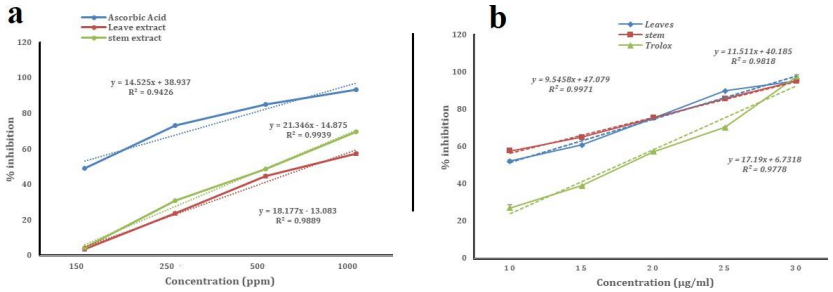

Figure 3. Antioxidant activity of Strobilanthes glutinosus Nees leaves (SGLE) and stem (SGSE) extracts and standard ascorbic acid: (a) by DPPH radical scavenging assay; (b) ABTS scavenging assay. Each value represents the mean ± standard error of the mean (SEM) and each experiment in triplicates.

3.4. Enzyme inhibition assays

3.4.1. α-amylase inhibitory studies

To assess the antidiabetic potential of leaves (SGLE) and stem (SGSE) extracts of *S. glutinosus*, α-amylase inhibition assay was performed *in vitro*. The IC-50 values were determined from the equation obtained from the graph given in Fig. 4a. The plant extracts showed strongest α-amylase inhibitory action at concentration of 1000 µg/ml. SGLE and SGSE exhibited IC-50 value of 0.06 and 0.46 µg/ml respectively in comparison to reference Acarbose (0.745 µg/ml).

3.4.2. Tyrosinase inhibition assay

To assess the potential of *S. glutinosus* for applications in cosmetics such as skin whitening, the tyrosinase inhibitory activity of plant samples SGLE and SGSE were carried out *in vitro*. The graph drawn shown in Fig. 4b by plotting % inhibition against different concentrations of SGLE, SGSE and positive control kojic acid is used to calculate IC-50 values. The IC-50 values of SGLE and SGSE was 5.924 and 7.46 µg/ml respectively in comparison to kojic acid (3.166 µg/ml). The maximum inhibition shown by SGLE was 42.25 % and by SGSE was 30.13 % at the concentration of 100 µg/ml. According to results, the plant extracts inhibit tyrosinase activity in a dose-dependent manner.

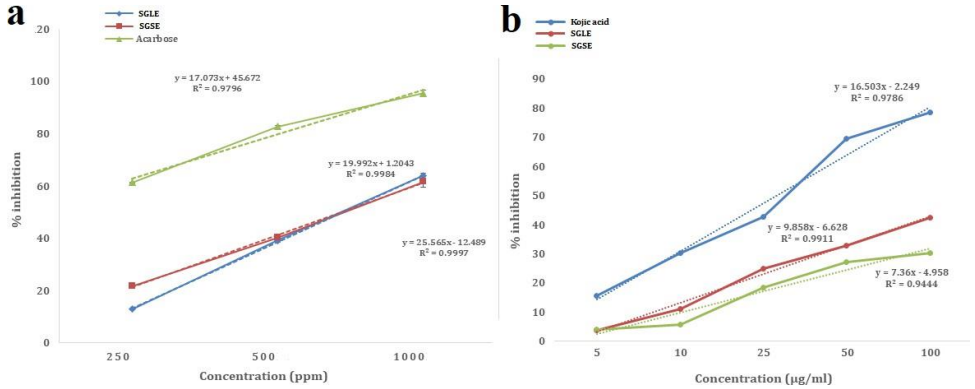

Figure 4. Enzyme inhibition assay: (a) α-Amylase inhibition activity S. glutinosus leaves (SGLE) and stem (SGSE) extracts and reference standard Acarbose; (b) Tyrosinase inhibi-tion activity of S. glutinosus leaves and stem extracts and positive control kojic acid. The values represent the mean ± SEM and all the experiments are performed in triplicates.

3.5. Anti-inflammatory activity

Paw edema induced by Carrageenan is a common model used to evaluate anti-inflammatory action by measuring the reduction in paw swelling. In this study, the % decrease in paw inflammation caused by the SGLE and SGSE (300 mg/kg) was assessed and compared to the standard anti-inflammatory drug Ibuprofen (5 mg/kg), as shown in Fig. 5. The results revealed that both SGLE and SGSE exhibited significantly higher anti-inflammatory activities compared to the reference drug Ibuprofen, which is a COX-1 inhibitor. After the 3rd hour of carrageenan administration, SGLE showed a percentage decrease in paw inflammation of 87.0 ± 0.22%, while SGSE exhibited a % decrease of 98.0 ± 0.18%. In contrast, Ibuprofen demonstrated a decrease of 77.0 ± 0.08%.

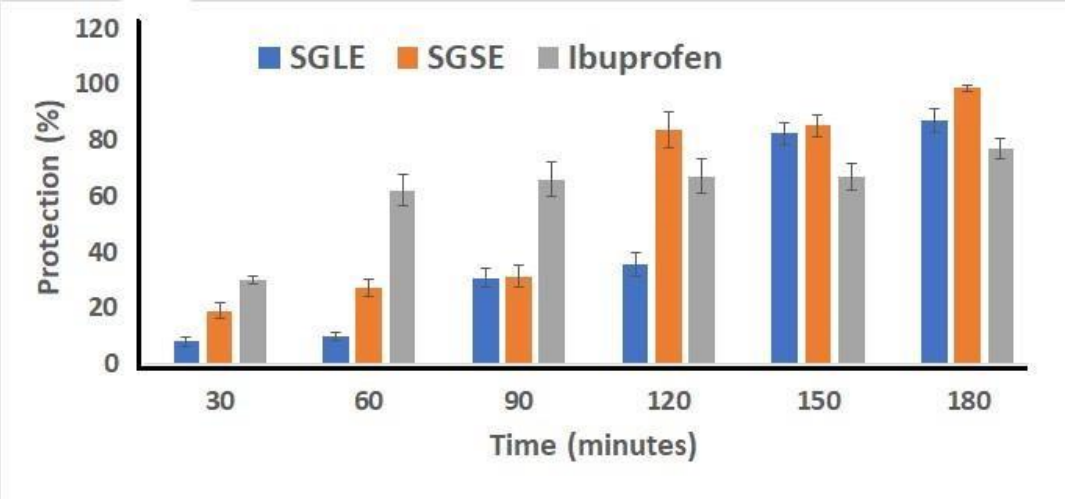

Figure 5. Anti-inflammatory activity of *S. glutinosus* leaves (SGLE) and stem (SGSE) extracts, standard Ibuprofen, and negative control (saline). Data points reflect the average result with standard error of the mean (Mean ± SEM, n = 3).

4. Discussion

Plants and herbs are a natural source of large number of active phytochemicals and play a significant role in the advancement of pharmaceutical industry [32]. According to an estimate, about 25000 secondary metabolites produced by plants are known for their promising therapeutic use of which over 8000 are polyphenols [28,36]. The therapeutic efficacy of plants is often due to presence of high polyphenolic compounds in them [37]. In this study, we explored the therapeutic potential of SGLE and SGSE through a series of bioassays. Despite its traditional use as a medicinal herb, scientific reports on the therapeutic activities of *S. glutinosus*, such as its anticoagulant, antifungal, and anti-inflammatory effects. Thus, our investigation aimed to shed light on the natural products derived from *S. glutinosus* for potential drug discovery. The choice of extraction solvent plays a fundamental role in deciding the presence of therapeutic compounds in plant materials.

While aqueous solvents have been traditionally used, methanolic extracts offer greater efficacy and versatility in dissolving a wide range of plant-based metabolites [38]. Notably, a recent study highlighted the significant phenolic and flavonoid contents found in the methanolic extract of *S. glutinosus* [39].

Our research revealed the robust antibacterial and antifungal effects of the crude extracts. SGLE and SGSE demonstrated moderate to high inhibitory effects against various Gram-positive and Gram-negative bacteria as shown in Fig.1, as well as fungal strains in Fig. 2. These findings align with previous studies that identified important biological compounds, including alkaloids, fatty acids, phenols, flavonoids, alkanes, monoterpenoids, di-terpenes, sesquiterpenoids, and sterols, in *S. glutinosus*.[1, 40] which might be responsible for the significant antimicrobial and antifungal activities of the plant [41, 42]. These phytoconstituents contribute to the potent antioxidant activity of *S. glutinosus*, as evidenced by the DPPH and ABTS scavenging assays conducted in our study (Fig. 3).

The antidiabetic and skin whitening potential of SGLE and SGSE is investigated by evaluating its α-amylase inhibition and tyrosinase inhibition activities *in vitro*. Our results demonstrated significant inhibition of α-amylase and tyrosinase by SGLE and SGSE, indicating their potential as alternative α-amylase inhibitors and skin whitening agents (Fig. 4). Inhibition of α-amylase is crucial for lowering blood glucose levels, as seen in currently available antidiabetic drugs. These drugs have unwanted side-effects such as hepatotoxicity and gastro-intestinal problems [43]. Therefore, *S. glutinosus* can be a promising alternative to α-amylase inhibitors (antidiabetic drugs) owing to its significant α-amylase inhibition. Additionally, the strong tyrosinase inhibition activity of SGLE and SGSE suggests their potential application in the treatment of pigmentation disorders and certain manifestations of skin cancer [44-46]. Inhibition of this biosynthesis step may be utilized to modify pigmentation of skin in certain pigmentation disorders. In addition, alternations in this biosynthesis step are also included in escalating certain histopathological manifestations of malignant metastatic melanomas, so this inhibition may have utility in treating skin cancer [45, 47].

We evaluated the anti-inflammatory activity of *S. glutinosus* using the carrageenan-induced paw edema assay, a commonly employed method to assess the anti-inflammatory Our findings revealed significant anti-edematous effects of SGLE and SGSE, suggesting their ability to inhibit key mediators involved in the inflammatory response, such as kinins and prostaglandins [48,49]. These results align with comparable studies that have reported the anti-inflammatory properties of *S. glutinosus* and support its traditional use in the treatment of inflammatory conditions. In addition to its anti-inflammatory activity, SGLE and SGSE exhibited notable gastric protective effects in an acetic acid-induced ulcer model. This model is widely used to evaluate the efficacy of various anti-secretory, H2 blockers, and cytoprotective agents [50, 51].

5. Conclusions

The diverse therapeutic potential of *S. glutinosus* extracts including antibacterial, antifungal, antioxidant, antidiabetic, and anti-inflammatory activities was observed. These findings highlight the valuable role of plants and herbs as sources of active phytochemicals with promising therapeutic applications. By providing novel insights into the pharmacological effects of SGLE and SGSE, our study contributes to the existing body of knowledge. The novelty of our study lies in the comprehensive assessment of multiple therapeutic activities of SGLE and SGSE extracts using rigorous biological assays. We explored the potential of SGLE and SGSE as alternative treatments for various conditions, including diabetes, skin whitening, and inflammation. These findings underscore the potential of SGLE and SGSE as natural and effective therapeutic interventions. The presence of bioactive compounds, particularly polyphenols and flavonoids, in the extracts likely contributes to their pharmacological effects. However, further research is needed to identify and isolate the specific bioactive compounds responsible for the observed activities and elucidate their mechanisms of action. Future studies should focus on validating the therapeutic applications of these extracts through *in vivo* studies and clinical trials, ultimately advancing their potential in the management of various diseases.

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Data Availability

The datasets supporting the outcomes of the study are accessible from the corresponding author.

Conflicts of Interest

The authors declare that there is no conflict of interest.
All authors have reviewed and consented to the published version of the manuscript.

References

1. Aziz, M., et al., Comprehensive Biological Potential, Phytochemical Profiling Using GC-MS and LC-ESI-MS, and In-Silico Assessment of Strobilanthes glutinosus Nees, An Important Medicinal Plant. Molecules, 2022. 27(20): p. 6885.
2. Javed, R., et al., Phytochemical-Mediated Biosynthesis of Silver Nanoparticles from Strobilanthes glutinosus: Exploring Biological Applications. Micromachines, 2023. 14(7): p. 1372.
3. Ab Aziz, N.A., et al., Extraction, anti-tyrosinase, and antioxidant activities of the collagen hydrolysate derived from Rhopilema hispidum. Prep Biochem Biotechnol, 2021. 51(1): p. 44-53.
4. Sasidharan, N., Biodiversity documentation for Kerala part 6: flowering plants. KFRI handbook, 2004. 17.
5. Ahmad, N.M., et al., Amperometric Biosensor Based on Zirconium Oxide/Polyethylene Glycol/Tyrosinase Composite Film for the Detection of Phenolic Compounds. Biosensors (Basel), 2016. 6(3).
6. Zawawi, N. and M. Ismail, Strobilanthes crispus Extract Reduces Respiratory Exchange Ratio in Obese Mice Fed High Fat and Low Fat Diets. Malays J Med Sci, 2018. 25(6): p. 46-58.
7. Kumar, S., et al., Acute and chronic inflammation studies of Strobilanthes callosus leaves extract on rat model. Inflammopharmacology, 2013. 21(3): p. 233-9.
8. Gu, W., et al., A novel isocoumarin with anti-influenza virus activity from Strobilanthes cusia. Fitoterapia, 2015. 107: p. 60-62.
9. Tsai, Y.C., et al., Antiviral Action of Tryptanthrin Isolated from Strobilanthes cusia Leaf against Human Coronavirus NL63. Biomolecules, 2020. 10(3).
10. Muhammad, S.N.H., et al., Antiglycolytic Activities of Strobilanthes crispus Active Fraction and its Bioactive Components on Triple-Negative Breast Cancer Cells In Vitro. Anticancer Agents Med Chem, 2021.
11. Iqbal, M., et al., Strobilanthes crispus attenuates renal carcinogen, iron nitrilotriacetate (Fe-NTA)-mediated oxidative damage of lipids and DNA. Mol Cell Biochem, 2010. 341(1-2): p. 271-7.
12. Al-Henhena, N., et al., Chemopreventive effects of Strobilanthes crispus leaf extract on azoxymethane-induced aberrant crypt foci in rat colon. Sci Rep, 2015. 5: p. 13312.
13. Yaacob, N.S., et al., Anticancer activity of a sub-fraction of dichloromethane extract of Strobilanthes crispus on human breast and prostate cancer cells in vitro. BMC Complement Altern Med, 2010. 10: p. 42.
14. Samuelsson, G., et al., Inventory of plants used in traditional medicine in Somalia. I. Plants of the families Acanthaceae-Chenopodiaceae. J Ethnopharmacol, 1991. 35(1): p. 25-63.
15. Aggarwal, B.B., A. Kumar, and A.C. Bharti, Anticancer potential of curcumin: preclinical and clinical studies. Anticancer research, 2003. 23(1/A): p. 363-398.
16. Dudhatra, G.B., et al., A comprehensive review on pharmacotherapeutics of herbal bioenhancers. The Scientific World Journal, 2012. 2012.
17. Haq, S.M., et al., Human-driven disturbances change the vegetation characteristics of temperate forest stands: A case study from Pir Panchal mountain range in Kashmir Himalaya. Trees, Forests and People, 2021. 6: p. 100134.
18. Hameed H, Waheed A, Sharif MS, Saleem M, Afreen A, Tariq M, Kamal A, Al-onazi WA, Al Farraj DA, Ahmad S, et al. Green Synthesis of Zinc Oxide (ZnO) Nanoparticles from Green Algae and Their Assessment in Various Biological Applications. Micromachines. 2023; 14(5):928. https://doi.org/10.3390/mi14050928
19. Kazeem, M.I., J.O. Adamson, and I.A. Ogunwande, Modes of inhibition of alpha -amylase and alpha -glucosidase by aqueous extract of Morinda lucida Benth leaf. Biomed Res Int, 2013. 2013: p. 527570.
20. BHISE, M.A.B.a.S.B., IN VITRO ASSAY OF ALPHA AMYLASE INHIBITORY ACTIVITY OF SOME INDIGENOUS PLANTS Int. J. Chem. Sci, 2012. 10(1): p. 457-462.

21. Takahashi, N., Deciding to Seek Help among Family Members Caring for Veterans with Diabetes Mellitus and Co-morbid Illnesses. 2016, UCLA.
22. Chelladurai, G.R.M. and C. Chinnachamy, Alpha amylase and Alpha glucosidase inhibitory effects of aqueous stem extract of Salacia oblonga and its GC-MS analysis. Brazilian Journal of Pharmaceutical Sciences, 2018. 54(1).
23. Selby, A., et al., Pathophysiology, differential diagnosis, and treatment of diabetic diarrhea. Digestive Diseases and Sciences, 2019. 64: p. 3385-3393.
24. Kumar, S., et al., Crystal structure determination and inhibition studies of a novel xylanase and alpha-amylase inhibitor protein (XAIP) from Scadoxus multiflorus. FEBS J, 2010. 277(13): p. 2868-82.
25. Ali Asgar, M., Anti-Diabetic Potential of Phenolic Compounds: A Review. International Journal of Food Properties, 2012. 16(1): p. 91-103.
26. Vaibhav1, S. and a.K. Lakshaman2, Tyrosinase Enzyme Inhibitory Activity of selected Indian Herbs International Journal of Research in Pharmaceutical and Biomedical Sciences, 2012. 3 (3).
27. Gholamhoseinian, A. and Z. Razmi, Screening the methanolic extracts of some plants for tyrosinase inhibitory activity. Toxicological & Environmental Chemistry, 2012. 94(2): p. 310-318.
28. Acosta-Estrada, B.A., J.A. Gutiérrez-Uribe, and S.O. Serna-Saldívar, Bound phenolics in foods, a review. Food chemistry, 2014. 152: p. 46-55.
29. Ali, S., et al., Phytochemical investigation and antimicrobial appraisal of Parrotiopsis jacquemontiana (Decne) Rehder. BMC complementary and alternative medicine, 2018. 18(1): p. 1-15.
30. Afreen, A., et al., Phytochemical-assisted biosynthesis of silver nanoparticles from Ajuga bracteosa for biomedical applications. Materials Research Express, 2020. 7(7): p. 075404
31. Ambrosio, C.M., et al., Antimicrobial activity of several essential oils on pathogenic and beneficial bacteria. Industrial Crops and Products, 2017. 97: p. 128-136.
32. Zulfiqar S., et al., Synthesis, crystal structure, anti-cancer, anti-inflammatory anti-oxidant and quantum chemical studies of 4-(pyrrolidine-2, 5-dione-1-yl) phenol. Journal of Molecular Structure, 2021. 1224: p. 129267
33. Ambreen S., et al., Anticoagulant potential and total phenolic content of six species of the genus Ficus from Azad Kashmir, Pakistan. Tropical Journal of Pharmaceutical Research, 2019. 18(6): p. 1245-51
34. Ali, A., et al., Influence of plasma-activated compounds on melanogenesis and tyrosinase activity. Sci Rep, 2016. 6: p. 21779.
35. Iida, K., et al., Potent inhibitors of tyrosinase activity and melanin biosynthesis from Rheum officinale. Planta medica, 1995. 61(05): p. 425-428.
36. Patel, P.R. and T. Rao, Antibacterial activity of underutilized fruits of Jamun (Syzygium cumini L. Skeels). Int J Curr Pharmac Res, 2012. 4: p. 36-9.
37. Newman, D.J. and G.M. Cragg, Natural products as sources of new drugs over the last 25 years. Journal of natural products, 2007. 70(3): p. 461-477..
38. Andriani, Y., et al., Phytochemical analysis, antioxidant, antibacterial and cytotoxicity properties of keys and cores part of Pandanus tectorius fruits. Arabian Journal of Chemistry, 2019. 12(8): p. 3555-3564.
39. Ajaib, M., et al., Antimicrobial and antioxidant analysis of Strobilanthes glutinous: an unexplored medicinal plant. Bioscience Research, 2020. 17(2): p. 1521-1534.
40. Aziz, M., et al., Phytochemical, pharmacological, and In-silico molecular docking studies of Strobilanthes glutinosus Nees: An unexplored source of bioactive compounds. South African Journal of Botany, 2021.
41. Baydar, N.G., et al., Determination of antibacterial effects and total phenolic contents of grape (Vitis vinifera L.) seed extracts. International journal of food science & technology, 2006. 41(7): p. 799-804.

42. Figueiredo-Gonzalez, M., et al., The involvement of phenolic-rich extracts from Galician autochthonous extra-virgin olive oils against the alpha-glucosidase and alpha-amylase inhibition. Food Res Int, 2019. 116: p. 447-454.
43. Etxeberria, U., et al., Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. Expert Opin Ther Targets, 2012. 16(3): p. 269-97.
44. Baurin, N., et al., Preliminary screening of some tropical plants for anti-tyrosinase activity. Journal of Ethnopharmacology, 2002. 82(2-3): p. 155-158.
45. Cheraif, K., et al., Chemical composition, antioxidant, anti-tyrosinase, anti-cholinesterase and cytotoxic activities of essential oils of six Algerian plants. Molecules, 2020. 25(7): p. 1710.
46. Cheraif, K.; Bakchiche, B.; Gherib, A.; Bardaweel, S.K.; Çol Ayvaz, M.; Flamini, G.; Ascrizzi, R.; Ghareeb, M.A. Chemical Composition, Antioxidant, Anti-Tyrosinase, Anti-Cholinesterase and Cytotoxic Activities of Essential Oils of Six Algerian Plants. Molecules 2020, 25, 1710. https://doi.org/10.3390/molecules25071710
47. Fiocco, D., et al., Lavender and peppermint essential oils as effective mushroom tyrosinase inhibitors: A basic study. Flavour and fragrance journal, 2011. 26(6): p. 441-446.
48. Ismail, H. and B. Mirza, Evaluation of analgesic, anti-inflammatory, anti-depressant and anti-coagulant properties of Lactuca sativa (CV. Grand Rapids) plant tissues and cell suspension in rats. BMC complementary and alternative medicine, 2015. 15(1): p. 1-7.
49. Khan, I., et al., Anti-inflammatory activities of Sieboldogenin from Smilax china Linn.: experimental and computational studies. Journal of ethnopharmacology, 2009. 121(1): p. 175-177.
50. Okabe, S. and K. Amagase, An Overview of Acetic Acid Ulcer Models—The History and State of the Art of Peptic Ulcer Research—. Biological and Pharmaceutical Bulletin, 2005. 28(8): p. 1321-1341.
51. Tsukimi, Y., C. Nozue, and S. Okabe, Effects of leminoprazole, omeprazole and sucralfate on indomethacininduced delayed healing of kissing gastric ulcers in rats. Journal of gastroenterology and hepatology, 1996. 11(4): p. 335-340.