

In-Vitro Antidiabetic Activity of Ethanolic Extract of *Gymnostachyum warrieranum* Leaves

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Abstract: Background: *Gymnostachyum warrieranum* is a medicinal plant in the Acanthaceae family that has very little scientific information on its pharmacological potential, safety, and phytochemical makeup. The goal of the present investigation was to evaluate the invitro antidiabetic effect of ethanolic extract.

Methods: Soxhlet extraction was used to extract the verified entire plant material using 95% ethanol. The extract was characterised by marker-based standardisation, HPTLC fingerprinting, and preliminary phytochemical screening. Assays for glucose absorption, α -amylase inhibition, and α -glucosidase inhibition were used to evaluate in vitro antidiabetic efficacy.

Results: When compared to aqueous (8.45%), chloroform (3.96%), and petroleum ether (2.84%) extracts, ethanol yielded the greatest extraction yield of 8.72% w/w. Flavonoids, phenolic substances, tannins, glycosides, steroids, terpenoids, and other secondary metabolites were detected by phytochemical screening. Gallic acid ($R_f 0.55 \pm 0.01$) and quercetin ($R_f 0.68 \pm 0.02$) were detected by HPTLC analysis, which also generated a repeatable chromatographic fingerprint appropriate for standardization. With maximum α -glucosidase inhibition of 89.96%, α -amylase inhibition of 75.33%, EEGW showed concentration-dependent antidiabetic efficacy.

Conclusion: The results show that *Gymnostachyum warrieranum*'s ethanolic extract has a rich phytochemical profile, high acute oral safety, and potential antidiabetic properties. The promise of EEGW as a source of bioactive phytoconstituents is supported by the dose-dependent pharmacological effects as well as the presence of quercetin and gallic acid.

Keywords: *Gymnostachyum warrieranum*; Acanthaceae; ethanolic extract; phytochemical screening; HPTLC; gallic acid; quercetin; antidiabetic activity

I. INTRODUCTION

Medicinal plants have historically constituted an important source of therapeutic agents and continue to occupy a significant position in modern pharmaceutical and biomedical research. The therapeutic value of plants is primarily associated with the presence of a wide variety of primary and secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, glycosides, terpenoids, steroids, saponins and other structurally diverse constituents. These compounds may exhibit antioxidants, anti-inflammatory, antidiabetic, antimicrobial, hepatoprotective, cardioprotective and several other biological activities.

The present investigation was undertaken to provide a systematic scientific evaluation of *Gymnostachyum warrieranum*, with particular emphasis on its pharmacognostic standardization, physicochemical characterization, extraction, phytochemical profiling, chromatographic standardization, safety assessment and evaluation of antidiabetic



activities. The study was designed as an integrated investigation in which the crude plant material was first subjected to appropriate processing and quality assessment, followed by extraction and chemical characterization, and finally evaluated through in vitro models.

The rationale for such a sequential approach is that biological activity of a plant extract cannot be interpreted independently of its chemical composition and quality. A pharmacologically active extract must first be demonstrated to be authentic and reproducible. Therefore, pharmacognostic and physicochemical evaluation forms the foundation of the investigation. The attached work reports physicochemical evaluation according to WHO and Ayurvedic Pharmacopoeia guidelines and establishes baseline quality parameters for the whole powdered plant. The investigation further explored the suitability of different solvents for extraction and demonstrated that ethanol produced the highest extractive yield among the solvents examined. The percentage yields were 2.84% with petroleum ether, 3.96% with chloroform, 8.72% with ethanol and 8.45% with water. Thus, the extraction efficiency followed the order ethanol > aqueous > chloroform > petroleum ether. This observation provided a practical basis for selecting the ethanolic extract for subsequent phytochemical and pharmacological investigations.

The chemical characterization of the ethanolic extract demonstrated the presence of a broad range of phytochemical classes. Flavonoids and phenolic compounds were particularly prominent, while tannins, glycosides, steroids, terpenoids, alkaloids, saponins and other constituents were also detected. The presence of several phytochemical classes suggests that the pharmacological effects of an extract may involve multiple mechanisms. Flavonoids and phenolic compounds may contribute to antioxidant and anti-inflammatory effects, while other constituents may influence metabolic pathways. However, preliminary phytochemical screening alone cannot establish the precise compound responsible for an observed biological effect. Therefore, chemical screening should be regarded as an important preliminary step that supports, but does not replace, biological and mechanistic investigations. In this context, the present research adopts a multidisciplinary approach. Pharmacognostic evaluation establishes identity and quality; extraction provides a reproducible preparation; phytochemical screening establishes the broad chemical profile; HPTLC provides chromatographic standardization; provide evidence regarding antidiabetic potential. (1-5)

II. METHODOLOGY

Collecting and Verifying Plant Material

Plant materials are gathered from its native environment in the evergreen forests of Kerala, India's Western Ghats, during the blossoming season. Plants that are disease-free and in good health will be chosen based on their morphological traits. To get rid of dirt and foreign objects, the gathered material will be properly cleaned with tap water and then distilled water. A certified taxonomist from an accredited botanical institution will verify the plant's botanical identification. For future reference, a voucher specimen will be created, given a voucher number, and placed in the institutional herbarium

Plant Material Processing: For around two weeks, the verified plant material will be dried at room temperature until a consistent weight is achieved. For the preservation of heat-sensitive phytoconstituents, shade drying is recommended. Before extracting, the dried material will be ground up using a mechanical grinder, sent through a 40-mesh screen, and kept in sealed, amber-colored containers.

Physicochemical Assessment

A crucial part of pharmacognostic standardization and quality control is the physicochemical assessment of crude medicines. It helps identify adulteration, substitution, contamination, and degradation and offers important information about the identification, purity, and quality of plant materials. The WHO and the Ayurvedic Pharmacopoeia of India (API) recommendations were followed in the current study's physicochemical assessment of the powdered entire plant of *Gymnostachyum warrieranum*

Foreign Matter Determination

The quality and therapeutic effectiveness of crude pharmaceuticals are negatively impacted by the inclusion of extraneous organic and inorganic materials. On a spotless white porcelain tray, around 100 g of PPM was evenly



distributed and visually inspected. Extraneous elements were meticulously sorted by hand and weighed, including soil particles, stones, insects, fungal pollutants, dried leaves of other plant species, stems, and other impurities. The following equation was used to analyze the proportion of foreign matters:

Foreign Matter (%) = (foreign matter weight divided by sample weight) $\times$ 100

Moisture Content and Drying Loss: The moisture content of medicinal plants has a major impact on shelf life, enzymatic breakdown, and microbiological development. During storage, too much moisture may hasten the breakdown of bioactive components. A ceramic crucible that had been previously dried and weighed was filled with around 2– 5 g of precisely weighed powdered medication. A hot air oven set at 105°C was used to dry the sample until it reached a consistent weight. After cooling in a desiccator, the crucible was weighed many times until the difference between subsequent readings was less than 0.5 mg. The equation was used to evaluate the loss on drying: Loss on Drying (%) is calculated by dividing the initial wt. by the final wt. and multiplying the result by 100.

Calculating Total Ash

Ash values are a crucial quality control metric that shows the total quantity of inorganic residues left behind after the plant material has been completely burnt. A silicon crucible that had previously been torched was filled with precisely 2 g of air-dried powdered medication. In a muffle furnace, the sample was progressively burnt by raising the temperature to 500–600°C until a white ash was produced, signifying total organic matter oxidation. After cooling in a desiccator, the crucible was weighed. The proportion of total ash was computed as follows:

TA (%) = (ash weight divided by sample weight) $\times$ 100

Acid-Insoluble Ash Determination

The quantity of siliceous materials, such as sand and soil pollution, is represented as acid insoluble ash. For five minutes, the whole ash was heated in 25 ml of diL.HCL. After gathering the insoluble residue on ashless filter paper, it was extensively cleaned with hot distilled water, dried, burnt in a silica crucible until its weight remained constant, cooled in a desiccator, and weighed.

The equation was used to analyze the proportion of AIA (%) = (Residue weight divided by sample weight) $\times$ 100

WSA: The quantity of inorganic components that are soluble in water is represented by water-soluble ash. Distilled water was used to boil the complete ash, which was then filtered via ashless filter paper, cleaned, dried, lit, cooled, and weighed. The weight of insoluble ash was subtracted from the total ash to determine the amount of WSA.

Extractive value: The amount of active phytoconstituents extracted by various solvents is shown by extractive values.

ASEV: In a closed flask, 5 g of plant materials was soaked with hundred mL of ethanol for 24 hours while being shaken periodically. After the solution was quickly filtered, 25 millilitres of the filtrate were dried at 105 degrees Celsius in a tarred dish. Alcohol soluble extractive value as a percentage was computed.

Water-Soluble Extractive Value: Instead of utilising ethanol, distilled water was used in the same process

Ethanollic Extract Preparation. An essential stage in determining the final extract's quality, composition, and biological activity is the extraction of phytoconstituents from medicinal plants. Because 95% ethanol can dissolve a broad range of polar and moderately polar secondary metabolites, it was chosen as the extraction solvent based on the polarity of key phenolic compounds and flavonoids found in members of the Acanthaceae family. A cellulose extraction thimble containing around 1000 g of shade dried and coarsely powdered *Gymnostachyum warrieranum* entire plant material was continuously extracted using 95% ethanol in a Soxhlet extraction system. The extraction procedure was continued for 48 to 72 hours, or until the syphoning solvent became colourless, signifying that all of the phytoconstituents had been extracted. To get rid of suspended particles, the ethanollic extract was cleaned with Whatman No. 1 filter paper. In order to avoid the heat deterioration of thermolabile compounds, the filtrate was evaporated under decreased pressure using a rotating vacuum evaporator kept below 45°C. To create a semisolid mass, the concentrated extract was further vacuum-dried in a desiccator. The dried extract was weighed to calculate the yield % and kept at 4°C in sealed, amber-colored containers awaiting further phytochemical and pharmacological analyses. The equation was used to evaluate the ethanollic extract's % yield: Weight of residue divided by Powdered Drug $\times$ 100



is the percentage yield (%). In accordance with the specifications of several experimental investigations, the extract was reconstituted in suitable solvents.

Initial Screening for Phytochemicals

An essential qualitative analytical method for determining the main types of secondary metabolites found in medicinal plants is preliminary phytochemical screening. The biological and medicinal effects of herbal medicines are mostly caused by these phytoconstituents. Using conventional phytochemical methods outlined by Harborne, Trease and Evans, Kokate, and Sofowora, the ethanolic extract of G.W was qualitatively screened for phytochemicals. The results of these tests serve as the foundation for further chromatographic and pharmacological studies and provide first insights into the phytochemical makeup of the extract. The dried ethanolic extract was mixed in analytical-grade methanol to create a stock solution with the proper concentration prior to analysis. To guarantee repeatability, all experiments were conducted in triplicate using freshly manufactured reagents. The following qualitative assays were included of the phytochemical screening.

Alkaloids Test: Diluted hydrochloric acid was used to acidify around 2 mL of the ethanolic extract, which was then filtered and split into four parts

Dragendorff's Test: Alkaloids were detected by the addition of Dragendorff's reagent, which resulted in an orange or reddish-orange precipitate.

Flavonoid Test (Shinoda Test)

One of the most significant classes of naturally occurring polyphenolic chemicals, flavonoids are widely for their cardioprotective, antidiabetic, antioxidant, and antibacterial qualities. The Shinoda test, which is regarded as one of the most trustworthy qualitative methods for identifying flavonoids, was given special attention because the goal of the present investigation is to assess the anti-diabetic potential of *Gymnostachyum warrieranum*. The Shinoda test is based on how metallic magnesium reduces flavonoid compounds when intense hydrochloric acid is present. Coloured flavilium salts are created when magnesium and hydrochloric acid combine to generate nascent hydrogen, which lowers the carbonyl group of flavonoids. The presence of polyphenol is confirmed by the development of pink, crimson, orange-red, or magenta hue. (6-9)

Procedure: Take 2 mL of ethanolic extract. After adding a little amount of magnesium ribbon (about 50–100 mg) to the extract, 5–6 drops of strong hydrochloric acid were carefully placed along the test tube's side. After a gentle shake, the filtrate was let to remain at room temperature for five to ten minutes. Flavonoids were present in the ethanolic extract of *Gymnostachyum warrieranum* when an orange, pink, crimson red, or magenta colouration developed. A bad outcome was indicated by the lack of any colour change.

III. IMPORTANCE

Flavones, flavonols, flavanones, and flavonoid glycosides may be present if the Shinoda test is positive. Reactive oxygen species are scavenged, lipid peroxidation is inhibited, inflammatory cytokines like TNF- α and IL-6 are suppressed, α -glucosidase and α -amylase enzymes are inhibited, insulin sensitivity is enhanced, and pancreatic β -cells are shielded from oxidative damage by flavonoids. Thus, the argument for assessing *Gymnostachyum warrieranum*'s investigated properties is connected by the confirmation of flavonoids. Quercetin was used as the reference marker in HPTLC fingerprinting to corroborate the qualitative results from the Shinoda test.

Test for Lead Acetate: A few drops of a 10% CH_3COOPb solution were added to two millilitres of ethanolic extract. The presence of flavonoids was indicated by the formation of a yellow precipitate.

Phenolic Compound Test: A few drops of a 5% FCL3 solution were added to two millilitres of the extract. Phenolic chemicals were present when a bluish-black, green, or dark violet hue developed.

Test for Ferric Chloride: Ferric chloride solution was used to treat the extract. Tannins were verified by a blue-black or greenish-black hue.



The Keller-Killiani Test: Concentrated H₂SO₄ was carefully applied along the test tube wall after two millilitres of the extract were combined with glacial acetic acid that contained ferric chloride. Cardiac glycosides were proven by the formation of a reddish brown ring at the contact.

Steroid Testing

Acetic anhydride and concentrated sulphuric acid were combined with the extract. Steroids were proven by the development of a bluish-green colour.

IV. TERPENOID TEST

Salkowski Examination: Chloroform and concentrated sulphuric acid were combined with the extract. Terpenoids were recognised by the formation of a reddish-brown ring at the contact.

Carbohydrate Test: The Molisch Test: After applying the Molisch reagent to the extract, strong sulphuric acid was added. Carbohydrates were verified by the formation of a violet ring

The Benedict Test: Benedict's reagent was used to heat the extract. Reducing sugars were verified by the formation of a green, yellow, or brick-red precipitate.

Protein Test (Amino Acid Test)

Ninhydrin Examination

The extract was cooked in a boiling water bath for two to three minutes after being treated with 0.2% ninhydrin reagent. Free amino acids were verified by the appearance of a blue or purple hue. (10)

Evaluation of Phytochemical Components by HPTLC

The EEGW is anticipated to contain flavonoids, phenolic compounds based on initial phytochemical studies of related species in the genus *Gymnostachyum* and other members of the family *Acanthaceae*. According to the Shinoda test, flavonoids are expected to be the most common class of bioactive compounds among them, and they will probably play a major role in the antioxidant, antidiabetic, and proposed investigation. In order to establish the ethanolic extract's phytochemical fingerprint and guarantee quality control before pharmacological assessment, it will be further standardised by HPTLC using quercetin as the marker molecule. Herbal extracts must be standardized in order to guarantee their effectiveness, safety, quality, and repeatability. Chromatographic fingerprinting has emerged as one of the most dependable methods for herbal standardization because medicinal plants contain a variety of phytochemical constituents whose concentrations vary depending on geographic origin, environmental conditions, harvesting season, and extraction techniques. Because of its ease of use, speed, high resolution, repeatability, and affordability, HPTLC is often used for both qualitative and quantitative study of phytoconstituents. An essential tool for the identification, authenticity, and quality monitoring of medicinal plants and herbal extracts is the distinctive chromatographic fingerprint that HPTLC produces. Using quercetin as the reference marker chemical, HPTLC was used to standardise the ethanolic extract of *Gymnostachyum warrieranum*. One of the most researched flavonols with strong antioxidants, antidiabetic properties, quercetin was chosen since screening revealed the presence of polyphenols in the EEGW. Additionally, quercetin is often used as a flag chemical for standardising medicinal plants that are high in flavonoids. Ten millilitres of HPLC-grade methanol were used to dissolve about 100 milligrams of the dried ethanolic extract of *Gymnostachyum warrieranum*. After sonicating the mixture for 15 to 20 minutes to let the phytoconstituents dissolve completely, the suspended

particles were filtered out using a 0.45 µm membrane filter. For analysis, the filtered solution served as the sample solution. Concurrently, 10 mg of genuine quercetin reference standard was mixed in 10 mL of methanol to create a stock solution of quercetin with a concentration of 1000 µg/mL. To produce working concentrations for calibration and quantitative estimation, the stock solution was diluted appropriately [91]. HPTLC plates (20 × 10 cm) were used as the stationary phase for chromatographic separation. The precoated plates were activated in a hot air oven at 110°C for five minutes prior to sample application in order to eliminate adsorbed moisture and guarantee repeatable chromatographic separation. A 100 µL Hamilton microsyringe and a CAMAG Linomat V automated sample applicator were used to apply the ethanolic extract and standard quercetin as 8-mm bands. To reduce experimental variance, the



application parameters, such as band width, application rate, and distance between tracks, were consistently maintained throughout the research [89,92]. The chromatographic plates were developed in a CAMAG twin-trough development chamber that had previously been saturated for around 20 minutes at room temperature with the optimised mobile phase, which was toluene:ethyl acetate:formic acid (5:4:1, v/v/v). After a number of initial optimisation tests, the solvent system was chosen to effectively separate the flavonoids and other phenolic components found in the ethanolic extract. Up until the solvent front moved about 80 mm from the origin, ascending chromatographic development was performed. After development, the plates were taken out of the chamber, allowed to air dry at ambient temperature to eliminate any remaining solvent, and then densitometric analysis was performed (11). Using a CAMAG TLC visualiser, the generated HPTLC plates were first inspected under visible light and then under ultraviolet light. A CAMAG TLC Scanner IV operated by VisionCATS software was then used to densitometrically scan the chromatograms. For every resolved band, chromatographic characteristics such as the retention factor (R_f), peak height, peak area, and UV absorption spectra were noted. By comparing the R_f value and UV spectral features of the sample peak with those obtained for the genuine quercetin standard, the presence of quercetin in the ethanolic extract was verified. By recording the total number of chromatographic bands, their matching R_f values, peak areas, and densitometric profiles, the ethanolic extract's HPTLC fingerprint was created. A distinctive chemical fingerprint for *Gymnostachyum warrieranum* was produced by each chromatographic peak, which reflected a distinct phytochemical component found in the extract. For future authentication, quality assurance, and batch-to-batch consistency of the plant material and its herbal formulations, the chromatographic profile found in this study might be used as a reference standard (12)

Inhibitory Assay for α -Amylase: The pancreas and salivary glands release α -amylase (EC 3.2.1.1), a digestive enzyme that catalyses the hydrolysis of α -1,4-glycosidic bonds of starch and glycogen to produce dextrans, maltose, and maltotriose. In the small intestine, α -glucosidase then transforms these intermediate intermediates into glucose. One of the hallmarks of Type 2 diabetes mellitus is fast postprandial hyperglycemia, which is caused by excessive enzymatic digestion of carbohydrates. By slowing down the digestion of dietary starch, α -amylase inhibition improves glycaemic management and delays the absorption of glucose. Therefore, α -amylase inhibition is considered a successful treatment strategy for managing diabetes. The DNSA colorimetric technique will be slightly modified to test the α -amylase inhibitory activity of the EEGW. The enzyme source will be porcine pancreatic α -amylase, and the substrate will be soluble starch. Different quantities of 25, 50, 100, 200, 400, and 800 μ g/mL will be prepared by dissolving the dry ethanolic extract in phosphate buffer (0.02 M, pH 6.9, adding 0.006 M sodium chloride).

As the reference standard, acarbose will also be made at the same concentrations. To promote interaction between the enzyme and the phytoconstituents, 500 μ L of plant extract and 500 μ L of α -amylase solution (1 U/mL) will be combined at each concentration and incubated at 37°C for ten minutes. The enzymatic reaction will then be started by adding 500 μ L of a 1% soluble starch solution made in phosphate buffer. The reaction mixture will undergo another 15-minute incubation period at 37°C. After incubation, 1 mL of DNSA reagent will be added to stop the enzymatic process. The test tubes will then be heated in a boiling water bath for 10 minutes to produce a reddish-brown complex. A UV-visible spectrophotometer will be used to measure the absorbance at 540 nm after the reaction mixture has cooled to room temperature and diluted with 10 mL of distilled water. To prevent experimental mistakes, a blank without enzyme and a control with enzyme but no plant extract will also be used. The following formula will be used to determine the extract's inhibitory activity: Using GraphPad Prism software, the concentration-response curve will be used to calculate the concentration needed to block 50% of α -amylase activity (IC₅₀). Greater antidiabetic potential and higher enzyme inhibitory action are indicated by lower IC₅₀ values. (14-16)

Inhibitory Assay for α -Glucosidase: The last stage of carbohydrate digestion is catalysed by α -glucosidase, a membrane-bound enzyme found on the brush boundary of the small intestine that hydrolyses oligosaccharides and disaccharides into glucose. Inhibition of α -glucosidase considerably lowers postprandial hyperglycemia and slows intestinal glucose absorption. Acarbose, miglitol, and voglibose are examples of clinically utilised antidiabetic medications that work by blocking α -glucosidase. Thus, one of the most dependable in vitro screening techniques for



finding possible antidiabetic drugs from natural materials is the assessment of α -glucosidase inhibition. Using p-nitrophenyl- α -D-glucopyranoside (pNPG) as the chromogenic substrate, the ethanolic extract's α -glucosidase inhibitory activity will be assessed. Phosphate buffer (0.1 M, pH 6.8) will be used to create various ethanolic extract concentrations (25–800 μ g/mL). Plant extract and α -glucosidase enzyme solution (1 U/mL) in equal amounts (50 μ L) will be combined and pre-incubated for 15 minutes at 37°C. The enzymatic reaction will then be started by adding 50 μ L of 5 mM p-nitrophenyl- α -D-glucopyranoside. The reaction will be stopped by adding 100 μ L of 0.2 M sodium carbonate solution after the reaction mixture has been incubated for 20 minutes at 37°C. When α -glucosidase hydrolyses pNPG, p-nitrophenol is released, resulting in a yellow hue whose intensity is directly correlated with enzyme activity. A UV-visible spectrophotometer will be used to measure the absorbance at 405 nm. Under the same experimental settings, acarbose will serve as the positive control. The following formula will be used to determine the percentage inhibition of α -glucosidase activity: Nonlinear regression analysis will be used to determine the ethanolic extract's IC₅₀ value. The extract's relative enzyme inhibitory potency may be determined by comparing its IC₅₀ values with those of acarbose. (13-15)

Interpreting Antidiabetic Activity in Vitro

The degree of inhibition against α -amylase and α -glucosidase enzymes will be used to evaluate the ethanolic extract of *Gymnostachyum warrieranum*'s antidiabetic potential. Stronger enzyme inhibitory activity will be indicated by higher % inhibition and lower IC₂₀ values. Simultaneous inhibition of both enzymes may effectively lower postprandial hyperglycemia and improve glycaemic management because inhibition of α -amylase inhibits starch breakdown while inhibition of α -glucosidase delays glucose absorption. It is anticipated that the ethanolic extract's flavonoids, phenolic compounds, tannins, and other polyphenolic components will play a major role in these inhibitory effects by interacting with the active sites of digestive enzymes and modifying the metabolism of carbohydrates. (16-18)

V. RESULT AND DISCUSSION

Physicochemical evaluation of the whole powdered plant of *G. warrieranum* was carried out according to WHO and Ayurvedic Pharmacopoeia guidelines. The data are recorded as mean $\pm$ SD (n = 3). These parameters confirmed the purity, quality, and authenticity of the crude drug and established baseline pharmacognostic standards for future

S.No	Parameter	Result (Mean $\pm$ SD)
1	Foreign matter (%)	0.42 $\pm$ 0.03
2	Moisture content (%)	7.65 $\pm$ 0.21
3	Loss on drying (%)	7.84 $\pm$ 0.18
4	Total ash (%)	8.24 $\pm$ 0.15
5	Alcohol soluble extractive (%)	15.82 $\pm$ 0.32
6	Water soluble extractive (%)	12.46 $\pm$ 0.28
7	Swelling index (mL/g)	1.85 $\pm$ 0.06
8	Foaming index	<100
9	pH (1% solution)	6.41 $\pm$ 0.05
10	pH (10% solution)	6.18 $\pm$ 0.07

Table :1The physicochemical evaluation of the powdered whole plant of *Gymnostachyum warrieranum*

The physicochemical evaluation of the powdered whole plant of *Gymnostachyum warrieranum* demonstrated that the crude drug possessed acceptable quality and purity in accordance with WHO and Ayurvedic Pharmacopoeia guidelines. The foreign matter content was found to be **0.42 $\pm$ 0.03%**, indicating that the collected plant material was almost free from extraneous organic and inorganic contaminants, thereby reflecting careful collection, cleaning, and processing of the sample. The **moisture content (7.65 $\pm$ 0.21%)** and **loss on drying (7.84 $\pm$ 0.18%)** were within acceptable limits,



suggesting that the plant material was adequately dried and therefore less susceptible to microbial contamination, enzymatic degradation, and deterioration during storage. The **total ash value** ($8.24 \pm 0.15\%$) represented the total inorganic residue present after incineration, while the **acid-insoluble ash value** ($1.12 \pm 0.08\%$) indicated the presence of only a negligible amount of siliceous matter such as sand and soil particles, confirming the cleanliness and purity of the crude drug. The **WSEV** ($3.48 \pm 0.09\%$) reflected the quantity of naturally occurring water-soluble inorganic salts, whereas the **sulphated ash value** ($8.86 \pm 0.12\%$) confirmed the absence of excessive inorganic impurities following conversion of mineral salts into stable sulphates. The **ASWE** ($15.82 \pm 0.32\%$) was higher than the **water-soluble extractive value** ($12.46 \pm 0.28\%$), indicating that ethanol extracted a greater quantity of phytoconstituents than water. This finding suggests that the plant is rich in moderately active polar constituents such as polyphenol and other bioactive constituents, thereby justifying the selection of ethanol as the extraction solvent for subsequent phytochemical and pharmacological investigations. The **swelling index** (1.85 ± 0.06 mL/g) indicated the presence of a moderate amount of mucilage and polysaccharides, while the **foaming index** (<100) suggested a relatively low concentration of saponins in the powdered drug. Furthermore, the **pH of the 1% and 10% aqueous solutions** was 6.41 ± 0.05 and 6.18 ± 0.07 , respectively, indicating that the powdered drug possessed a slightly acidic nature, which is characteristic of medicinal plants containing phenolic constituents. Overall, the physicochemical parameters established in the present study provide valuable pharmacognostic standards for the identification, authentication, quality control, and standardization of *Gymnostachyum warrieranum* and support its suitability for further phytochemical profiling and pharmacological evaluation.

VI. PREPARATION OF ETHANOLIC EXTRACT OF *GYMNOSTACHYUM WARRIERANUM*

G. warrieranum was considered to extraction using 95% alcohol as the extraction solvent. The extraction process was continued for approximately 72 hours to ensure exhaustive extraction of the phytoconstituents. Ethanolic extract evaporated under reduced pressure using a rotary vacuum evaporator maintained at 40–45°C. The concentrated extract was subsequently dried under reduced pressure to obtain a stable semisolid mass. The extract was kept in airtight amber-coloured glass containers and stored at cold temperature until further phytochemical, chromatographic, and pharmacological investigations. The ethanolic extract appeared as a dark greenish-brown semisolid mass with a characteristic aromatic odour and exhibited a smooth, homogeneous consistency without any visible particulate matter or fungal contamination. The extract was readily soluble in methanol and dimethyl sulfoxide (DMSO), enabling its use in qualitative phytochemical screening, HPTLC fingerprinting, HPLC analysis, molecular docking-related investigations, and subsequent in vitro and in vivo biological studies. A total of 87.2 g of dried ethanolic extract was obtained from 1000 g of shade-dried powdered whole plant material, corresponding to an extraction yield of 8.72% (w/w). The relatively high extractive value demonstrates the efficiency of ethanol in extracting a wide spectrum of bioactive constituents from *Gymnostachyum warrieranum*. Since ethanol possesses intermediate polarity, it effectively dissolves both polar and polar phytoconstituents moderately while minimizing the extraction of excessive nonpolar impurities.

The comparatively higher percentage yield obtained with ethanol suggests that the plant is rich in ethanol-soluble secondary metabolites. These constituents are likely to include polyphenols and other biologically active compounds that contribute to the medicinal properties of the plant. The efficient extraction of these phytoconstituents further supports the suitability of ethanol as an extraction solvent for pharmacognostic and phytochemical investigations. The extraction yield obtained in the present investigation was also compared with extracts prepared using solvents of different polarity. Petroleum ether extraction yielded 2.84% (w/w), chloroform extraction yielded 3.96% (w/w), aqueous extraction yielded 8.45% (w/w), whereas ethanol extraction produced the highest yield of 8.72% (w/w). The lower yields observed with petroleum ether and chloroform indicate that the plant contains comparatively fewer nonpolar constituents, while the higher yields obtained with ethanol and water demonstrate the predominance of polar and moderately polar phytochemicals. The order of extractive yield observed in the present study was: Ethanol (8.72%) > Aqueous (8.45%) > Chloroform (3.96%) > Petroleum ether (2.84%).



The findings indicate that ethanol was the most efficient extraction solvent among those investigated. Consequently, the ethanolic extract was selected for all subsequent investigations, including preliminary phytochemical screening, physicochemical evaluation, HPTLC fingerprinting, quantitative estimation of marker compounds, antioxidant studies, molecular docking analysis, in vitro antidiabetic and anti-inflammatory studies, acute oral toxicity studies, and in vivo pharmacological evaluation. The physical characteristics and extraction yield of the ethanolic extract are presented in Table 2, while the comparative percentage yields obtained using different solvents are reported in Table 3.

Table 2 :Physical Characteristics and Percentage Yield of the Ethanolic Extract of *Gymnostachyum warrieranum*

Parameter	Observation
Plant part	Whole plant
Solvent used	95% Ethanol
Extraction method	Soxhlet extraction
Extraction period	72 h
Appearance	Dark greenish-brown semisolid mass
Odour	Characteristic aromatic odour
Consistency	Smooth semisolid
Weight of powdered drug	1000 g
Weight of dried extract	87.2 g
Percentage yield	8.72% (w/w)

Table 3:Percentage Yield of Different Solvent Extracts of *Gymnostachyum warrieranum*

Sl. No.	Solvent	Percentage Yield (% w/w)
1	Petroleum ether	2.84
2	Chloroform	3.96
3	Ethanol	8.72
4	Aqueous	8.45

VII. PRELIMINARY PHYTOCHEMICAL SCREENING

The ethanolic extract of *Gymnostachyum warrieranum* was considered to phytochemical screening using chemical tests to identify the major category of secondary metabolites. The results demonstrated the presence of several pharmacologically important phytoconstituents, indicating that the extract possesses a rich phytochemical composition. Among the various phytochemical groups investigated, flavonoids showed a strong positive reaction in the Shinoda test, where the development of a characteristic crimson red colour confirmed their presence. Similar positive results were obtained in both the alkaline reagent test and lead acetate test, further confirming the occurrence of flavonoid compounds in the ethanolic extract. Since flavonoids are recognized for their potent antioxidants, antidiabetic, and anti-inflammatory activities, their presence supports the therapeutic potential of *Gymnostachyum warrieranum*. Phenolic compounds were also detected by the ferric chloride test, producing a dark bluish-green coloration indicative of polyphenolic constituents. Tannins gave positive reactions in both ferric chloride and gelatin precipitation tests, confirming the occurrence of hydrolysable and condensed tannins. These phenolic constituents are well known for their



free radical scavenging activity and their ability to inhibit inflammatory mediators. The ethanolic extract further exhibited positive reactions for glycosides, steroids, terpenoids, saponins, carbohydrates, proteins using their respective qualitative chemical tests. Positive reactions observed in the Keller–Killiani, Liebermann–Burchard, and Salkowski tests confirmed the presence of glycosides, steroids, and terpenoids, respectively. Likewise, persistent froth formation in the foam test indicated the occurrence of saponins, while Molisch's and Benedict's tests reported the presence of carbohydrates. The Biuret and Ninhydrin tests demonstrated the presence of proteins and amino acids, whereas the spot test and Ruthenium red test confirmed fixed oils and gums/mucilage, respectively.

Alkaloids were identified by the formation of characteristic precipitates in Mayer's, Wagner's, Dragendorff's, and Hager's tests, indicating the occurrence of nitrogen-containing secondary metabolites in the extract. The combined presence of, tannins, glycosides, terpenoids, steroids, and saponins suggests that the ethanolic extract contains a broad spectrum of bioactive compounds that may collectively contribute to its antioxidant, antidiabetic, and anti-inflammatory activities. Overall, the preliminary phytochemical screening demonstrated that the ethanolic extract of *Gymnostachyum warrieranum* is a rich source of therapeutically important secondary metabolites. These findings provided a scientific basis for further chromatographic standardization using HPTLC fingerprinting with quercetin as the marker compound, as well as for subsequent in vitro and in vivo pharmacological evaluation.

Table 4: Preliminary Phytochemical Screening of the Ethanolic Extract of *Gymnostachyum warrieranum*

Phytochemical constituent	Observation	Result
Alkaloids	Characteristic precipitate	+
Flavonoids	Crimson red colour	+++
Flavonoids	Yellow colour disappeared on acidification	+++
Flavonoids	Yellow precipitate	+++
Phenolic compounds	Bluish-green colour	+++
Tannins	Blue-black precipitate	++
Glycosides	Brown ring	++
Saponins	Stable froth	+
Steroids	Bluish-green colour	++
Terpenoids	Reddish-brown ring	++
Carbohydrates	Violet ring	+
Reducing sugars	Brick-red precipitate	+
Proteins	Violet colour	+
Amino acids	Purple colour	+
Fixed oils	Permanent oily spot	+
Gums and mucilage	Pink colour	+

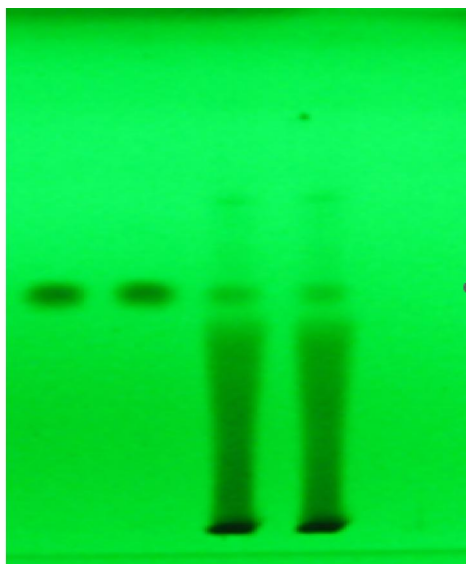
Interpretation: +++ = Strongly present; ++ = Moderately present; + = Present; – = Absent.

The phytochemical screening revealed the presence of a broad range of biologically important phytoconstituents in the ethanolic extract, indicating its chemically diverse nature. Among the screened constituents, **flavonoids showed a strong positive reaction (+++)** in all three confirmatory tests employed, namely the Shinoda test, alkaline reagent test, and lead acetate test. In the Shinoda test, the development of a characteristic crimson-red colour confirmed the presence of flavonoid constituents. Similarly, the alkaline reagent test produced a yellow colour that disappeared upon acidification, while the lead acetate test resulted in the formation of a yellow precipitate. The consistent strong positive reactions obtained with these three independent tests provide substantial qualitative evidence for the presence of flavonoid-type constituents in the ethanolic extract.



The strong detection of flavonoids is particularly noteworthy in view of the subsequent **antidiabetic and anti-inflammatory investigations** carried out with the ethanolic extract. Flavonoids are an important class of plant polyphenols that have been extensively investigated for their potential antioxidant, anti-inflammatory, and glucose-regulatory activities. Therefore, the strong qualitative presence of flavonoids in the present extract provides a plausible phytochemical basis for further evaluation of the extract in the antidiabetic experimental models.

Figure 1: HPTLC Fingerprinting and Standardization of Gallic Acid in the Ethanolic Extract of *Gymnostachyum warrieranum*



HPTLC fingerprinting and standardization of gallic acid in the ethanolic extract of *Gymnostachyum warrieranum*.(A) HPTLC chromatogram of the gallic acid reference standard and the ethanolic extract of *Gymnostachyum warrieranum* developed using the mobile phase **toluene:ethyl acetate:formic acid (5:4:1, v/v/v)** and visualized under **UV light at 254 nm**, showing the corresponding chromatographic bands

HPTLC analysis was carried out to establish the characteristic chromatographic profile of the ethanolic extract of *Gymnostachyum warrieranum* and to confirm the presence of gallic acid as a phytochemical marker for extract standardization. HPTLC is widely recognized as one of the most reliable chromatographic techniques for the evaluation of herbal drugs because of its simplicity, high analytical precision, excellent reproducibility, rapid sample processing, and ability to simultaneously analyze multiple samples under identical experimental conditions. The technique has become an indispensable tool in herbal drug research for authentication of medicinal plants, quality control of botanical extracts, detection of adulteration, and establishment of batch-to-batch consistency. In the present investigation, HPTLC analysis generated a highly reproducible chromatographic fingerprint that can serve as a reference profile for the quality assessment and future standardization of *Gymnostachyum warrieranum*.

The additional chromatographic peaks observed in the extract represent other phytochemical constituents, generating a characteristic HPTLC fingerprint suitable for the authentication, phytochemical characterization and evaluation of the ethanolic extract of *G. warrieranum*. The chromatographic plate developed under optimized solvent conditions was visualized under ultraviolet light at 254 nm. The resulting chromatogram (Figure 4.1) exhibited a distinct chromatographic pattern consisting of well-resolved bands corresponding to the gallic acid reference standard and the ethanolic extract. Under short-wave UV illumination, gallic acid appeared as a prominent dark quenching band against the bright fluorescent green background of the silica gel plate. The corresponding band observed in the ethanolic extract appeared at the same chromatographic position as the standard, indicating the occurrence of gallic acid in the plant extract. The matching migration distance between the standard and the sample provided preliminary evidence for

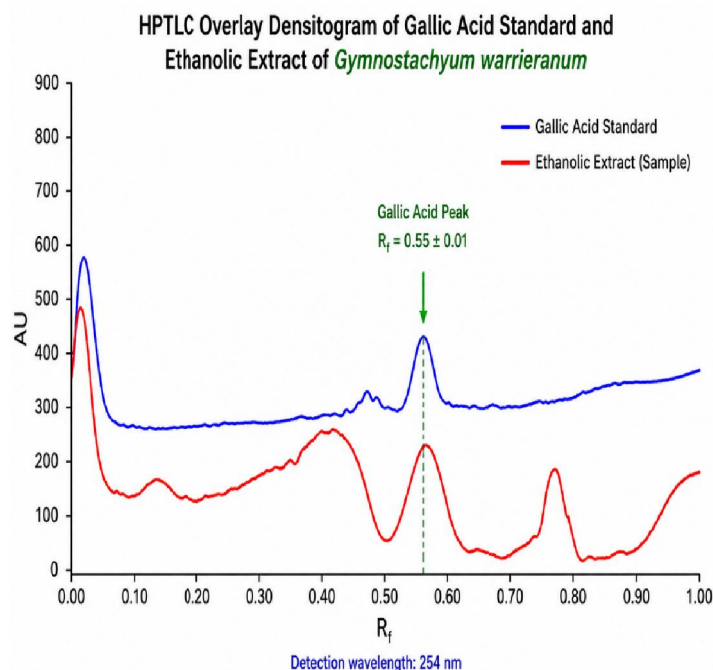


the presence of gallic acid, which was subsequently confirmed by densitometric analysis. Apart from the marker band corresponding to gallic acid, the ethanolic extract displayed several additional chromatographic bands with varying intensities and migration distances. These bands represent the diverse secondary metabolites naturally present in *Gymnostachyum warrieranum*, reflecting its chemically complex phytochemical composition. The presence of multiple chromatographic zones demonstrates the ability of ethanol to efficiently extract compounds belonging to different phytochemical classes, including phenolic acids, flavonoids, tannins, glycosides, terpenoids, steroids, and other polar phytoconstituents. The distinct separation of these compounds without excessive overlapping indicates that the selected mobile phase provided efficient chromatographic resolution and excellent separation efficiency. Visual examination of the developed chromatogram revealed that the marker band corresponding to gallic acid was sharp, compact, and well defined, with minimal diffusion and no evidence of peak distortion. Such chromatographic characteristics indicate that the

optimized HPTLC conditions were highly suitable for marker compound separation.

Figure 1. Overlay densitogram of the **gallic acid reference standard** and the **ethanolic extract of *Gymnostachyum warrieranum*** obtained by HPTLC densitometric scanning at **254 nm**, showing the coincidence of the chromatographic peak at **R_f value = 0.55 ± 0.01**, thereby confirming the indicates of tannins

EEGW's In Vitro Antidiabetic Activity



The EEGW's inhibitory action against the enzyme α -amylase, which hydrolyses carbohydrates, was used to assess its in vitro antidiabetic potential. By postponing the conversion of dietary starch into glucose, α -amylase inhibition is regarded as a significant therapeutic strategy for lowering postprandial hyperglycemia. EEGW's inhibitory action was evaluated at doses between 20 and 100 μ g/mL and contrasted with acarbose, a common α -amylase inhibitor. The experimental findings are shown visually in Figure

Inhibitory Activity of α -Amylase

The ethanolic extract of *Gymnostachyum warrieranum* (EEGW) exhibited a dose-dependent inhibitory action against the enzyme, as shown by the gradual rise in α -amylase inhibitory activity with increasing dosage



Table 5: α -Amylase Inhibitory Activity of the Ethanolic Extract of *Gymnostachyum warrieranum* (EEGW)

Concentration ($\mu\text{g/mL}$)	EEGW (% Inhibition)	Acarbose (% Inhibition)
20	18.33 ± 0.16	26.16 ± 0.16
40	31.16 ± 0.16	42.00 ± 0.00
60	45.33 ± 0.33	58.50 ± 0.28
80	60.33 ± 0.33	73.33 ± 0.16
100	75.33 ± 0.33	89.16 ± 0.16

The standard medication acarbose showed a similar concentration-dependent pattern, although at all tested concentrations, the standard's inhibition was still greater. EEGW showed $18.33 \pm 0.16\%$ suppression of α -amylase activity at the lowest dose (20 $\mu\text{g/mL}$), whereas acarbose caused $26.16 \pm 0.16\%$ inhibition. The result showed that EEGW may obstruct enzymatic starch hydrolysis even at a comparatively low dosage, despite the extract's reduced inhibitory activity compared to the conventional medication. When the concentration was raised to 40 $\mu\text{g/mL}$, the inhibitory activity of EEGW rose to $31.16 \pm 0.16\%$, which is around 70% more than what was seen at 20 $\mu\text{g/mL}$. Acarbose showed $42.00 \pm 0.00\%$ inhibition under the same experimental circumstances. This rise suggests that the extract's inhibitory components interact with α -amylase more successfully as their concentration rises.

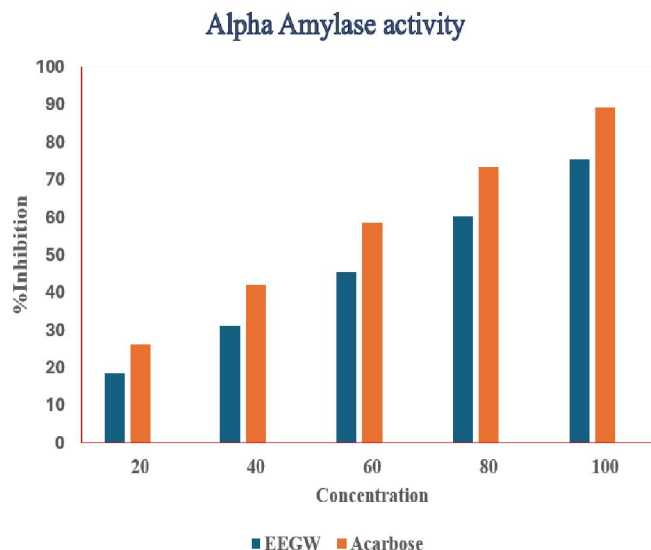
Enzyme inhibition was further enhanced at 60 $\mu\text{g/mL}$, when acarbose showed $58.50 \pm 0.28\%$ inhibition while EEGW provided $45.33 \pm 0.33\%$ inhibition. Nearly half of the enzyme activity was decreased by the extract at this dose, suggesting significant inhibitory potential. At 80 $\mu\text{g/mL}$, there was a significant increase in inhibition, with EEGW achieving $60.33 \pm 0.33\%$ inhibition as opposed to $73.33 \pm 0.16\%$ for acarbose. The extract's inhibitory activity was more than 60%, suggesting that the phytoconstituents in EEGW successfully inhibited α -amylase activity at greater doses. The extract and the standard medicine had the best inhibitory action at the highest concentration (100 $\mu\text{g/mL}$). Acarbose resulted in $89.16 \pm 0.16\%$ inhibition, whereas EEGW showed $75.33 \pm 0.33\%$ inhibition. The extract produced more than three-fourths suppression of α -amylase activity, indicating a promising antidiabetic potential, even tho the conventional medicine remained more powerful.

Between 20 and 100 $\mu\text{g/mL}$, EEGW's overall inhibitory activity rose from 18.33% to 75.33%, indicating a roughly 4.1-fold increase in enzyme inhibition. Similarly, acarbose showed a steady rise from 26.16% to 89.16%, demonstrating the assay's validity and sensitivity. Both EEGW and acarbose followed comparable dose-dependent inhibition patterns, as the graphical depiction (Figure 4.9) makes evident, while the standard continuously showed higher efficacy over the dosage range. At the highest dose, acarbose exhibited almost 13.8% more inhibition than the extract, indicating the largest difference between EEGW and acarbose. However, EEGW's inhibitory action at greater doses suggests that the extract has significant α -amylase inhibitory effectiveness and may successfully postpone the enzymatic conversion of complex carbs into absorbable glucose. Excellent assay repeatability and good accuracy across repeated measurements are shown by the consistently low standard deviation values (± 0.00 – 0.33) recorded for all experimental groups. The low experimental variance validates that the concentration-dependent rise was constant across separate determinations and shows the dependability of the observed inhibitory responses.

The several bioactive phytochemicals found in EEGW, including as phenolic compounds, flavonoids, tannins, alkaloids, glycosides, and other secondary metabolites found during preliminary phytochemical screening, may be responsible for the reported enzyme inhibitory action. It is known that phenolic chemicals and flavonoids inhibit α -amylase via attaching to the enzyme's catalytic site or by interacting with amino acid residues through hydrogen bonding, and π - π stacking. These interactions decrease postprandial glucose absorption by lowering enzyme activity and slowing the breakdown of starch into glucose.



Figure 2: Concentration-dependent α -amylase inhibitory activity of the ethanolic extract of *Gymnostachyum warrieranum* (EEGW) compared with the standard drug acarbose. Both EEGW and acarbose exhibited progressive increases in percentage inhibition with increasing concentration (20–100 $\mu\text{g/mL}$). Although acarbose demonstrated higher inhibitory activity at all tested concentrations, EEGW showed substantial enzyme inhibition, reaching 75.33% inhibition at 100 $\mu\text{g/mL}$, indicating promising in vitro antidiabetic potential.



The results of this study show that *Gymnostachyum warrieranum* ethanolic extract has strong in vitro α -amylase inhibitory action in a concentration-dependent manner. The extract showed significant inhibition at increasing doses, indicating its promise as a natural source of antidiabetic phytoconstituents, even tho its inhibitory efficacy was lower than that of acarbose. These results support additional research utilising α -glucosidase inhibition assays, kinetic studies, in vivo antidiabetic models, and bioactive compound isolation to clarify its mechanism of action and offer preliminary evidence that EEGW may help manage postprandial hyperglycemia.

Inhibitory Activity of α -Glucosidase

The common antidiabetic medication acarbose was compared with the α -glucosidase inhibitory action of the ethanolic extract of *Gymnostachyum warrieranum* (EEGW) at doses ranging from 100 to 500 $\mu\text{g/mL}$. The findings showed that α -glucosidase activity was suppressed by both EEGW and acarbose in a concentration-dependent manner, with increasing percentage inhibition shown as the concentration rose. Figure shows the concentration-dependent inhibitory pattern, and Table 4.presents the complete data. EEGW showed $37.82 \pm 0.65\%$ suppression of α -glucosidase activity at the lowest dose tested (100 $\mu\text{g/mL}$), whereas acarbose caused a slightly greater inhibition of $39.12 \pm 0.58\%$. Even at lesser doses, EEGW has significant inhibitory action, as seen by the comparatively tiny difference between the extract and the standard at this dosage. EEGW's inhibitory action significantly increased to $52.94 \pm 0.74\%$ when the concentration was raised to 200 $\mu\text{g/mL}$, while acarbose showed $70.41 \pm 0.81\%$ inhibition. This shows a significant increase in enzyme inhibition over the prior concentration, indicating that the active phytoconstituents in EEGW interact with the enzyme more as the extract concentration rises. When the concentration was increased to 300 $\mu\text{g/mL}$, EEGW showed an inhibition of $68.23 \pm 0.68\%$, whereas acarbose showed an inhibition of $81.35 \pm 0.62\%$. The extract had a substantial inhibitory action against α -glucosidase at this dose, inhibiting almost two-thirds of the enzyme activity. EEGW demonstrated $81.18 \pm 0.53\%$ inhibition at 400 $\mu\text{g/mL}$, while the reference medication showed $90.12 \pm 0.49\%$ inhibition. At greater doses, the extract's inhibitory efficacy approximated that of acarbose, as shown by its more than 80% inhibition. At 500 $\mu\text{g/mL}$, EEGW caused an inhibition of $89.96 \pm 0.47\%$, whereas acarbose produced an inhibition of $98.21 \pm 0.35\%$. This was the greatest inhibitory activity. The ethanolic extract showed very strong



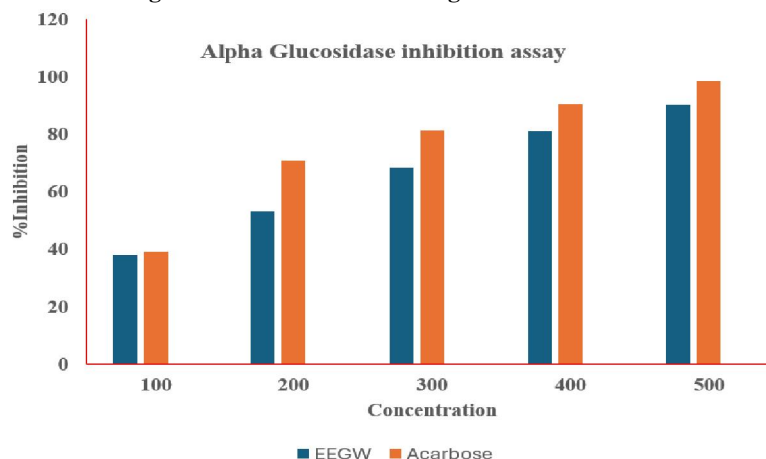
inhibitory action, attaining approximately 90% suppression of α -glucosidase activity at the highest dose tested, even tho the conventional medication remained more effective throughout the research. Overall, EEGW's inhibitory activity increased gradually from 37.82% at 100 $\mu\text{g/mL}$ to 89.96% at 500 $\mu\text{g/mL}$, which is almost a 2.4-fold increase in enzyme inhibition during the studied dose range. Acarbose also demonstrated a concentration-dependent rise in inhibition, ranging from 39.12% to 98.21%. Although acarbose consistently showed higher inhibitory action at all dosages, the graphical depiction (Figure 3) clearly shows that both EEGW and acarbose followed comparable inhibition patterns. At the lowest concentration, there was no difference between EEGW and acarbose; however, at intermediate doses, this difference became more noticeable. It's interesting to note that EEGW's inhibitory activity was close to that of the conventional medication at 400 and 500 $\mu\text{g/mL}$, indicating that the extract has significant α -glucosidase inhibitory potency. The existence of strong bioactive components that may impede the digestion of carbohydrates is shown by EEGW's capacity to block about 90% of enzyme activity at higher doses. Excellent assay accuracy and repeatability were shown by the experimental data's very low standard deviation values. The observed concentration-dependent inhibition is reliable, as shown by the little difference between repeat measurements. The ethanolic extract's phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, and other secondary metabolites may be responsible for the study's significant α -glucosidase inhibitory effect. By binding to the catalytic site of the enzyme or changing its conformation via hydrophobic interactions and hydrogen bonding, these phytoconstituents are known to inhibit α -glucosidase, which lowers the conversion of oligosaccharides into absorbable glucose. As a result, one of the main treatment approaches for type 2 diabetes mellitus is the inhibition of α -glucosidase, which slows intestinal glucose absorption and aids in controlling postprandial blood glucose levels. All of the results show that *Gymnostachyum warrieranum*'s ethanolic extract has strong in vitro α -glucosidase inhibitory action. The extract showed almost identical inhibition at greater doses, suggesting promising antidiabetic potential even tho its inhibitory efficacy was somewhat lower than that of acarbose. These findings, together with the α -amylase inhibitory action, imply that EEGW may successfully lower postprandial hyperglycemia by dual inhibition of enzymes that breakdown carbohydrates. In order to clarify the extract's mode of action, more research is necessary via enzyme kinetic studies, the separation of active phytoconstituents, molecular docking analysis, and in vivo antidiabetic assessment.

α -Glucosidase Inhibitory Activity of the EEGW

Concentration ($\mu\text{g/mL}$)	EEGW (% Inhibition)	Acarbose (% Inhibition)
200	52.94 $\pm$ 0.74	70.41 $\pm$ 0.81
300	68.23 $\pm$ 0.68	81.35 $\pm$ 0.62
400	81.18 $\pm$ 0.53	90.12 $\pm$ 0.49
500	89.96 $\pm$ 0.47	98.21 $\pm$ 0.35



Figure 3: Concentration-dependent α -glucosidase inhibitory activity of the EEGW compared with the standard drug acarbose. Both EEGW and acarbose exhibited progressive increases in percentage inhibition with increasing concentration (100–500 $\mu\text{g/mL}$). The extract demonstrated substantial enzyme inhibition, reaching 89.96% inhibition at 500 $\mu\text{g/mL}$, which was comparable to the standard drug (98.21%), indicating strong in-vitro antidiabetic potential through inhibition of intestinal α -glucosidase.



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