Journal of Chemical Health Risks

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JCHR (2025) 15(6), 2139-2150 | ISSN:2251-6727

Exploring the Phytochemical Elements and Therapeutic Potential of *Stephenia Hernandifolia* Leaf Extract: Evaluation of Antioxidant and Anti-Inflammatory Properties

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(Received: 27 October 2025 Revised: 05 November 2025 Accepted: 04 December 2025)

KEYWORDS

Stephenia hernandifolia, Phytochemical screening, Therapeutic potential, Antioxidant activity, Anti-inflammatory activity.

ABSTRACT:

Introduction: *Stephania hernandifolia* (S. hernandifolia) is widely distributed across northeastern India, including Assam, Mizoram, Tripura, Arunachal Pradesh, Sikkim, Manipur, Nagaland, and Meghalaya. It holds great economic importance in the perfume industry, traditional medicine, and incense production, though its phytochemical composition varies with geography. Objectives: This study aimed to perform phytochemical screening and evaluate the antioxidant and anti-inflammatory activities of *S. hernandifolia* leaves collected from Ikishe and Tenyiphe villages, Chumoukedima district, Nagaland.

Methods: Leaf samples were subjected to qualitative phytochemical tests and GC-MS analysis of methanolic extracts. Antioxidant activity was determined using the DPPH radical scavenging assay, while anti-inflammatory potential was assessed through the egg albumin denaturation assay. **Results:** Phytochemical screening revealed the presence of phenols, flavonoids, and saponins in both extracts, whereas alkaloids, tannins, and terpenoids were absent in the Tenyiphe sample. GC-MS identified major constituents such as n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z), and Strophanthidin. The Ikishe extract exhibited stronger antioxidant and anti-inflammatory activities. **Conclusion:** The methanolic extract of *S. hernandifolia* from Ikishe village demonstrates significant antioxidant and anti-inflammatory potential, suggesting its usefulness as a natural source of bioactive compounds for food and pharmaceutical applications.

1. Introduction

A plant with multiple parts utilized in traditional medicine, some of which are effective in treating various health conditions. The use of medicinal plants supports scientific research and pharmaceutical development due to the presence of important bioactive compounds. Furthermore, these plants have played a vital role in the progress of human societies across the world [1-3]. Herbs refer to any part of a plant-such as the roots, stigmas, leaves, barks, stems, seeds and fruit-as well as non-woody plants, and they are utilized for even in certain spiritual practices, perfumery, flavoring, medicinal applications and culinary purposes. In contemporary times, scientists are isolating bioactive compounds from medicinal plants to develop new pharmaceutical drugs and formulations. The outlook for medicinal plants is highly promising, with approximately 500,000 species existing worldwide-many of which remain unexplored for their therapeutic potential. These plants may offer significant advantages for ongoing and future research efforts [4, 5]. Different bioactive compounds derived from natural sources have played a crucial role in the development of new medicines as well as in traditional healing practices in the modern pharmaceutical era [6]. Owing to trust in traditional remedies, their proven efficacy, cultural significance, and especially the limited access to

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JCHR (2025) 15(6), 2139-2150 | ISSN:2251-6727

modern medical services, traditional medicine serves as an affordable and effective from of primary healthcare in developing nations such as India [7]. With the global growth of traditional medicine and the rising interest in herbal remedies, the use of medicinal plants has also seen a significant increase [8]. Medicinal plants have been studied for a long time, particularly to determine their therapeutic value. Initially, organoleptic methods were employed to evaluate food based on its appearance, aroma, and taste. Gradually, these approaches evolved into more sophisticated instrumental techniques [9, 10].

Stephania hernandifolia, Initial phytochemical screening revealed that the ethanolic extract contains alkaloids, carbohydrates, steroids, saponins, tannins, phenolic compounds, flavonoids, and lignin, while the aqueous extract showed carbohydrates, saponins, tannins, phenolic compounds, flavonoids, and lignin [11]. In this is a continuation of the research on S. hernandifolia phytochemical profile and its anti-inflammatory and antioxidant properties using methanolic leaf extract. This is advantageous since it provides information on the regions where plant is grown.

2. Materials and Methods

Collection of plant and preparation of plant extract

The green leaf samples of *Stephenia hernandifolia* were collected from Ikishe Model village (IMV) and Tenyiphe village, Chumoukedima District, Nagaland, India. Phytochemical analyses were done at the Biochemistry Laboratory, Rain Forest Research Institute, Jorhat. The samples were then shade dried for (10-12) days in moisture free area inside the biochemistry laboratory. The samples were cut into small pieces using scissors and pulverized the same in mortar and pestle, and the fine powder was packed in sealed air tight pouches for further analysis. Using electronic balance 5 grams of powdered samples were taken into two beakers and it dissolved with 50 ml methanol, the crude was filtered with Whatman No. 1 filter paper and the filtrates were kept in falcon tubes (Maker- Eppendorf) for phytochemical tests.

Phytochemical screening

Using the methanolic leaf extract of *S. hernandifolia* and the standard methodology of Harborne 1973 [12], a qualitative test was performed to validate the presence of various phytochemical components, such as phenols, tannins, alkaloids, terpenoids, flavonoids, and saponins. Preliminary screening test confirms the presence of phytochemicals such as Alkaloid [12], Flavonoid [13], Tannin, Saponin [14], Terpenoid and Phenol [15] in the leaves of *Stephenia hernandifolia* and hence quantification tests also need to be carried out to find the potential leaf extracts at different locations.

GC–MS analysis

A Perkin-Elmer Clarus 680 system (Perkin-Elmer, Inc. U.S.A.) with a 30 m long, 250 µm diameter, and 0.25 µm thick capillary column filled with Elite-5MS was used for the GC–MS study of *S. hernandifolia* leaf extracts. At a flow rate of 1 mL/min, helium gas, which is 99.99% pure, was used as the transport gas. An electron ionisation method with an ionisation energy of 70 eV, a scan time of 0.2 seconds, and fragment bands from 40 to 600 m/z was used to find the GC–MS spectrum. The split ratio used was 10:1, and the injection amount was 1 L. The temperature of the injector was set to 250°C. The temperature of the column oven was set at 50°C for three minutes, then raised at a rate of 10°C per minute until it reached 280°C, and finally raised to 300°C for ten minutes. The phytochemicals were found by making a comparison between the test samples' retention times (in minutes), peak areas, peak intensities, and mass spectral patterns and those in the databases.

Antioxidant activity

The purpose of the antioxidant test was to slow down the oxidation process of DPPH free radicals in the plant extract methanol solution, which caused the color of DPPH to shift from purple to yellow. To determine the IC_{50} value, which represents the total quantity of antioxidant required to reduce the initial DPPH radical concentration by 50%, 1 ml of extract solution with varying concentrations (12.5 to 500 µg/ml) was combined with 1 ml of 0.1 mg of DPPH in methanol. For half an hour, the mixture was left to stand at room temperature in the dark. 1 ml of DPPH and 1 ml of methanol were combined to create the blank solution. Using a UV-vis spectrophotometer [16], the mixture absorbance was measured at 517 nm. As a positive control, ascorbic acid was utilized as the standard antioxidant. The following formula was used to

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JCHR (2025) 15(6), 2139-2150 | ISSN:2251-6727

calculate the percentage of scavenging, and the standard curve was created by graphing the proportion of DPPH scavenging against concentration:

$$\text{Scavenging effect (\%)} = [1- \frac{\text{Absorbance(sample) at 517 nm}}{\text{Absorbance (Control) at 517nm}}] \times 100$$

The IC_{50}, or sample concentration required to block 50% of the DPPH free radical, was found using a log dose inhibition curve.

The reaction mixture contained 2 ml of varying doses (50-400 µg/ml) of plant extract, 2 ml of phosphate buffered saline (pH 6.4), and 0.2 ml of fresh hen's egg albumin, according to the technique outlined [17]. After 15 minutes of incubation at 37°C±2°C in a biological oxygen demand incubator, the mixture was heated for five minutes at 70°C. Double-distilled water in the same volume was used as a control. Once they cooled, their absorbance at 660 nm was determined. To calculate absorbance, diclofenac sodium (50-400 µg/ml) was utilized as a reference and handled in the same way. One can calculate the percentage of inhibition towards inflammation using the formula below:

$$\text{Percentage of inhibition} = \frac{Absorbance of the control - Absorbance of the sample}{Absorbance of the contro} \text{ x } 100$$

A dose-response curve was used to calculate the plant extract concentration for 50% inhibition (IC_{50}).

Statistical analysis

The SPSS software package, version 16.0, was used for all analyses. All data were calculated as the mean ± standard error using GraphPad PRISM 6. According to technique [18], and Tukey's HSD test was used to differentiate the means ($P < 0.05$).

3. Results and Discussion

Phytochemical analysis was conducted on fresh leaves of *S. hernandifolia* to the obtain information of secondary metabolite compounds present in it and conforms the presence of are chemical compounds alkaloids (Mayer's and Wagner's test) (Fig 1A,B), flavonoids (Lead acetate test) (Fig 1C), phenols (Ferric chloride test) (Fig 1D), saponins (Froth test) (Fig 1E), tannins (Ferric chloride test) (Fig 1F) and terpenoids (Salkkowski test) (Fig 1G) obtained from IMV. However, the leaves obtained from Tenyiphe village shows difference on chemical compounds such as saponin (Fig 2A), flavonoid (Fig 2B) and Phenol (Fig 2C), i.e., some compounds present on leaf of IMV location is missing and the same reported in Table 1 and Fig. 1 A, B, C. triterpenoid/steroid, glycoside, tannin, and flavonoids are all present in the hot water extraction of *S. hernandifolia* leaves. In a similar manner, the primary chemical constituents of the *Caryophyllaceae* family include phenylpropanoids, phytoecdysteroids, benzenoids, saponins, and nitrogenous compounds [19]. The phytopharmacology stages, which involve the first selection of the plant and its testing to establish the presence of specific chemical components related to its biological activity, can benefit from the use of screening techniques [20]. According to the plant has been reported to possess a diverse range of secondary metabolites, including terpenoids, phenolic compounds, saponins, tannins, flavonoids and alkaloids, and these constituents have demonstrated cytotoxic, sinusitis-relieving, blood sugar-lowering, pain-relieving, antibacterial, cough-suppressing, antioxidant and anti-anxiety properties [21]. The phenolic compounds present in this plant have been shown to possess oocyst-inhibitory activity in vitro [22]. The tannins identified in this study may also disrupt the life cycle of coccidia, as indicated by the decreased sporulation of oocysts [23].

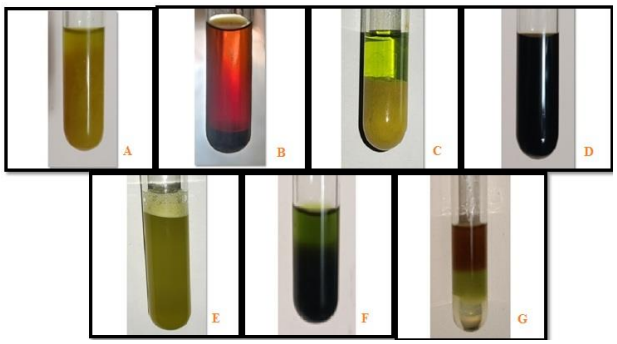

Figure 1. Qualitative tests for leaf extract of *S. hernandifolia* from RFRI, Ikishe village, (A & B- Alkaloid test; C- Flavonoid test; D- Phenol test; E- Saponin test; F- Tannin test; G- Terpenoid test).

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JCHR (2025) 15(6), 2139-2150 | ISSN:2251-6727

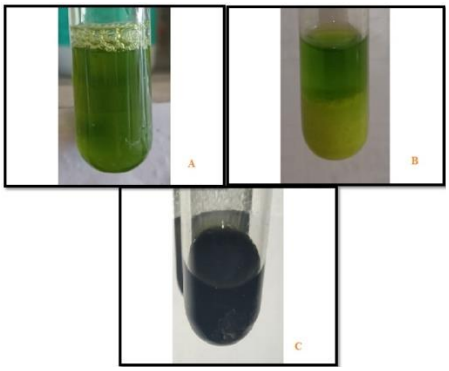

Figure 2. Qualitative tests for leaf extract of *S. hernandifolia* from RFRI, Ikishe village, (A- Saponin test; B- Flavonoid test; C- Phenol test).

Total alkaloid content present in the leaves of *S. hernandifolia* was determined and results. The results conform the total alkaloid content 4.376% in the leaf powder collected from IMV but not in leaves of Tenyiphe (Fig 3A, B). Thus, medicinally valuable plants exhibit pharmacological properties owing to the presence of bioactive phytochemicals synthesized within plant tissues as primary and secondary metabolites [24]. Since a saponin molecule is one type of secondary metabolite, growth rate can also boost the manufacture of secondary metabolites [25], with Antihelmintic and antidiarrheal activity has been found for saponin [26]. The amount of total saponins in the leaves of *S. hernandifolia* was quantified. *S. hernandifolia* leaves obtained from IMV and Tenyiphe village were estimated to contain 4.515% and 1.310% saponins, respectively (Fig 4 A, B). The aqueous extract of *S. hernandifolia* exhibits certain activities, potentially through the suppression of one or more mediators such as histamine, serotonin, kinins, and prostaglandins [27].

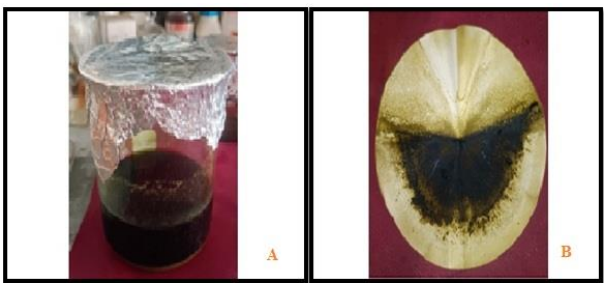

Figure 3. Quantitative test for alkaloids (A- 10% Acetic Acid in ethanolic extract of leaf sample; B- Dried residue)

Figure 4. Quantitative test for saponin (A- separation using diethyl ether; B- dried residue)

To determine the total flavonoids content in the extracts, sodium nitrite was reacted with aluminium chloride in an alkaline environment to produce a coloured flavonoids-aluminium complex that was spectrophotometrically detected at a maximum wavelength of 510 nm. Fig. 5A present the results of the calibration curve plotting of quercetin at different concentrations (0.1 to 5 mg/ml) using Microsoft Office Excel 2007. Using a standard calibration curve ($y=0.211x-0.060$, $R^2= 0.997$) to determine the total flavonoids content of the methanol extract of the leaves of both locations, it was discovered that sample 1 contained 270.36 mg of quercetin/g and sample 2 contained 247.48 mg of quercetin/g (Fig 5B). Flavonoids are known to possess anti-inflammatory effects [28].

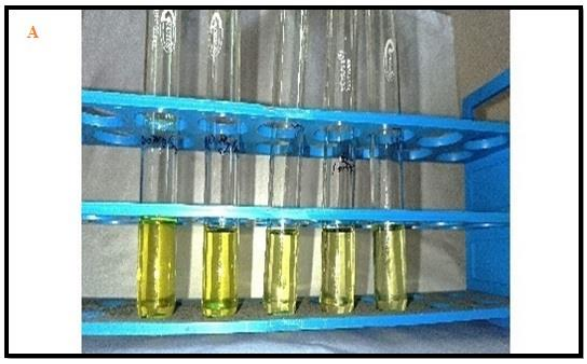

Figure 5: Quantitative test for flavonoids

Flavonoids are the most abundant class of phenolic chemicals in nature. A few flavonoids have a bitter taste to help them resist against specific kinds of caterpillar. Flavonoids are also found in plants parts, including fruit, pollen, and root as glycosides [29]. The number of flavonoids in tea brewed from young seagrass (*Enhalus acoroides*) leaves is lower (0.0888%); put another way, the total average value of the tea drink is lower than that

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of the young leaves [30]. Furthermore, the amount and kind of polyphenols found in the leaves of gambier plants are influenced by the general age of the leaves [31]. The dichloromethane fraction contained a high level of flavonoids, measured at 123.27 µg QE [32].

The standard gallic acid equivalent of phenols was used to assess the total phenolic content of the methanolic extract of *S. hernandifolia* leaves at the two sites. Using Microsoft Office Excel 2007, a calibration curve for gallic acid at different concentrations (10 to 100 µg/ml) was plotted (Fig 6 A, B). The results are shown in Fig 6C. The total phenolic contents were determined to be 45.69 mg of GAE/g for sample 1 and 39.84 mg of GAE/g for sample 2, using the following linear equation based on the calibration curve of gallic acid ($y=0.003x+0.021$, $R^2= 0.992$). According to research by Rice-Evans *et al.,* [33], the redox characteristics, hydrogen donors, and singlet oxygen quenchers of phenol are the primary causes of their antioxidant activity. Phenolic chemicals are significant plant antioxidants that have strong antiradical scavenging properties. As a result, a sample's phenolic compounds can be primarily responsible for its antioxidant capacity [34-36].

Figure 6. Quantitative test for phenols (A &B)

Perspicacious antioxidants, as phenolic compounds are efficient donors of hydrogen [33]. In a similar vein, naturally occurring phenolics were reported to have antioxidant qualities by Shahidi and Naczk, [37]. This suggests that the presence of phenolic chemicals in the extract may contribute to some of the antioxidant capacity of the leaf extract of *S. hernandifolia*. The antioxidant activity of ascorbic acid (97.37±0.86 µM Fe (II)/g) was comparable to that of the *Sarcocephalus latifolius* root extract (86.21±4.28 µM Fe (II)/g), with no significant difference observed ($P>0.05$) [38]. Numerous studies have demonstrated that plant-derived secondary metabolites, including polysaccharides, tannins, flavonoids, and polyphenols, can disturb the oxidant-antioxidant balance across the oocyst membranes [39-41].

The GC-MS (Fig.7) study of the aqueous extract from *S. hernandifolia* leaves identified a variety of compounds. The chromatogram generally displays many peaks, each representing distinct chemicals, with their retention durations reflecting their volatility and stability. Various chemicals can be recognized from the mass spectra analysis based on their mass-to-charge ratios (m/z). The methanolic extract of *S. hernandifolia* contains a total of 24 components, with the predominant constituents being 3-(1,3-Dihydroxyisopropyl)-1,5,8,11-tetraoxacyclotridecane (28.06%), 4-Piperidinamine, N,1-dimethyl (16.86%), n-Hexadecanoic acid (5.92%), 9,12-Octadecadienoic acid (Z,Z) (5.58%), 3-Isopropyl-6a,10b-dimethyl-8-(2-oxo-2-phenyl-ethyl)-dodecahydro-benzo[f]chromen-7-one (5.19%), Strophanthidin (5.16%), Ergosta-5,22-dien-3-ol, acetate, (3á,22E) (4.85%), and Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate (4.02%). 3-(1,3-Dihydroxyisopropyl)-1,5,8,11-tetraoxacyclotridecane (28.06%) (D6), is a cyclic siloxane having diverse biological characteristics that have been explored in recent studies. 3-(1,3-Dihydroxyisopropyl)-1,5,8,11-tetraoxacyclotridecane (28.06%) displays several biological features, including minimal toxicity, potential antibacterial actions, and concerns regarding endocrine disruption. D6 has demonstrated potential in biomedical applications owing to its biocompatibility, especially in drug delivery systems and tissue engineering [42]. Heptasiloxane, referred to as heptamethylcyclotrisiloxane or octamethylcyclotetrasiloxane (D7), is a cyclic siloxane having diverse biological properties that have been examined. Heptasiloxane is typically regarded as having low acute toxicity. Research suggests that it presents negligible hazards regarding skin and ocular irritation. Nonetheless, there are apprehensions regarding prolonged exposure and its possible implications for human health and the environment. Heptasiloxane has many biological features, such as low toxicity, possible antibacterial activity, and biocompatibility for biomedical applications [43]. Octadecanoic acid, also referred to as stearic acid, is a saturated fatty acid present in numerous animal and plant lipids.

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Octadecanoic acid (stearic acid) possesses multiple therapeutic features, including advantages for cardiovascular health, anti-inflammatory actions, and antibacterial characteristics.

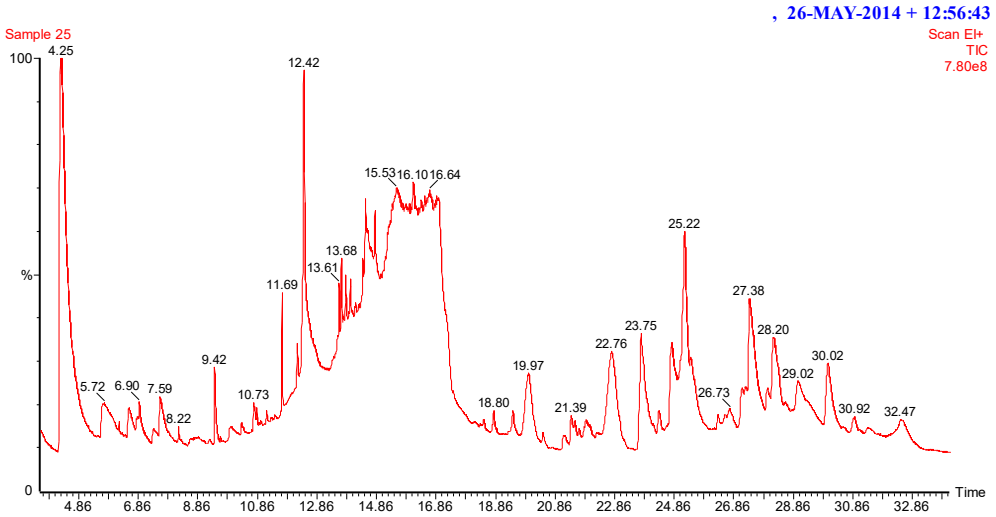

Figure 7. GC-MS Chromatogram analysis of methanolic leaf extract of *S. hernandifolia*

It is essential to acknowledge that although these qualities are promising, additional research is required to comprehensively grasp the scope of its medical uses [44]. Benzoic acid is a fundamental aromatic carboxylic acid possessing numerous Hexadecanoic acid, or palmitic acid, is a saturated fatty acid present in diverse animal and plant lipids. Hexadecanoic acid exhibits antibacterial action against several infections. Its occurrence in specific natural products may assist in suppressing the proliferation of bacteria and fungus [45]. pharmacological and health-related attributes. Benzoic acid demonstrates antibacterial efficacy against bacteria, fungus, and yeast. It is frequently utilized as a preservative in food and pharmaceutical products to impede microbial proliferation [46]. Benzofuran is an organic molecule comprising a fused benzene and furan ring structure. It demonstrates numerous therapeutic characteristics and has been the focus of investigation in multiple domains. Benzofuran derivatives exhibit antioxidant capabilities that may mitigate oxidative stress and offer protection against numerous diseases, including cancer and neurological disorders [47].

The evaluation and comparison of the DPPH radical scavenging activity of leaves from the methanolic extract of *S. hernandifolia* at both sites, with ascorbic acid used as a control. Microsoft Office Excel 2007 was used to compute and plot the percentage inhibition at different concentrations of the methanolic extract leaves of *S. hernandifolia* of both sites along with standard ascorbic acid in Fig. 8A. Based on the graph, the IC_{50} values for ascorbic acid were determined to be 27.43µg/ml, while sample 1 and sample 2 had IC_{50} values of 55.20 µg/ml and 90.29µg/ml, respectively (Fig 8B). Likewise, *Arenaria rubra* extract exhibits strong DPPH radical scavenging activity and functions as an antioxidant [48]. Free radicals are molecules or atoms with unpaired electrons, which gives them a strong attraction to other compounds electrons [49]. In an earlier study, certain plant extracts demonstrated strong DPPH radical scavenging activity along with notable ferric-reducing power [50]. Moreover, high antioxidant activity has been associated with elevated total phenolic content [51].

Strong antioxidant activity of shown by *Wikstroemia tenuiramis* ethanol and water extracts. Using ethanol solvent and hot water, the IC_{50} values for the fresh leaves are 27.78 and 28.80 µg/ml, respectively, while for rotting leaves, they are 25.35 and 28.59 µg/ml, respectively [52]. The antioxidant activity of *Saccharopolyspora spinosa* was evaluated using the DPPH radical assay, revealing free radical scavenging effects in acetone, methanol, and dichloromethane extracts, with IC_{50} values ranging from 33.66 to 230.15 µg/ml [53]. The methanol extract of stem roots demonstrated significant free radical scavenging

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activity (68.30%, 75.20%, and 81.26%) at concentrations of 10, 50, and 100 µg/ml, respectively [54].

In contrast, an IC_{50} value of 72.25±0.72 ppm was found in mature leaves of the agarwood species *A. beccariana*, which was proposed as a possible antioxidant [55]. The amount of total phenolics and flavonoids in pomegranate leaves was substantially linked with their antioxidant activity. During pomegranate leaf growth and development, every bioactive chemical detected from the leaves increased [56]. Strong antioxidant activity was demonstrated by two isolates: Diaporthe sp. (P1DS1(C), IC_{50} 76.65 mg/mL) and *Apodus oryzae* (R2MC3A, IC_{50} 60.92 mg/mL) [57]. In different species, the extract of *Silene spergulifolia* exhibited the highest radical scavenging activity, followed by *Silene gynodioca* [58].

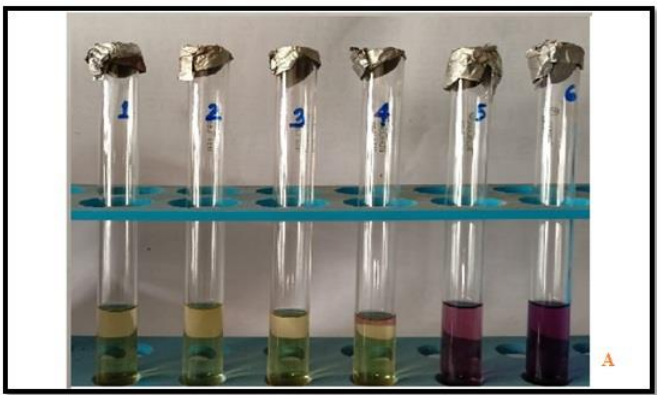

Figure 8. DPPH radical scavenging activity of methanolic extract of leaves of *S. hernandifolia*

The results of in-vitro denaturation of egg albumin of methanolic extract *S. hernandifolia* leaves of both sites. It was observed that inhibition in protein denaturation activity was positively correlated to the concentration of the extract. Sample 1 had shown higher inhibition in protein denaturation activity than sample 2. Both the sample showed a marked inhibition in protein denaturation with an IC_{50} value 274.91 and 368.89 µg/ml (Fig 9A, B), respectively. In-vitro research was done on the anti-inflammatory properties of both the extract and the isolated components. Aquilarinenside E, luteolin-7,3′,4′-trimethyl ether, luteolin-7,4′-dimethul ether, acacetin, iriflophenone-2-O-α-Lrhamnopyranoside and iriflophenone-3-C-β-glucoside were among the fractions that were identified [59]. Macrophages have the ability to produce and release more nitric oxide (NO) when they are stimulated by the bacterial endotoxin lipopolysaccharide (LPS) [60]. The findings indicated that the methanol extract of *Euclea crispa* exhibited strong antioxidant activity, with a half-maximal inhibitory concentration (IC_{50}) of 1.42 µg/ml, which was similar to ascorbic acid at concentrations ranging from 62.5 to 250 µg/ml [61]. The outcomes are in line with a prior investigation on *A. sinensis* leaves, in which the ethanol extract with an IC_{50} of 80.4 µg/ml inhibited the formation of NO in LPS-stimulated RAW264.7, with hydrocortisone serving as the positive control (IC_{50}= 0.1 µg/mL). With IC_{50} values of 21.76 µM, the inhibitory actions of epifriedelanol,5-hydroxy-7,4′-dimethoxyflavone, luteolin-7,3′,4′-trimethyl ether, and 5,7-dihydroxy-4′-methoxyflavone (acacetin) were similar to the findings of other investigations [62], 24.5 µM [63], 23.3 µg/mL [64], and 7.23 µM [65], respectively. Cytotoxicity assays revealed that both *Lactobacillus kimchicus-Elaeocarpus sylvestris* and Lactobacillus plantarum- *Elaeocarpus sylvestris* exhibited selective toxicity toward cancer cells, underscoring their potential as therapeutic agents [66]. The anticancer potential of the synthesized TiO_2NPs was evaluated against MCF-7, HeLa, PC-3, and A549 cell lines using MTT and MTS assays [67].

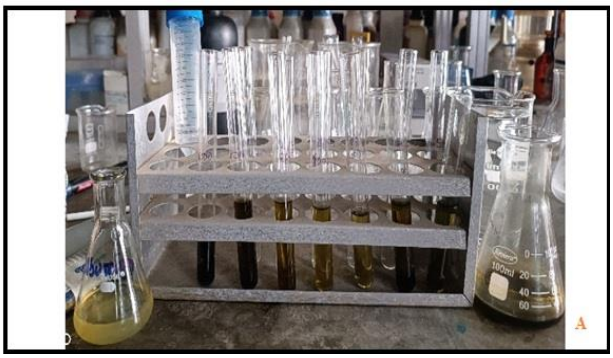

Figure 9. Egg albumin denaturation assay of methanolic extract of leaves of *S. hernandifolia*

Conclusion

In conclusion, the analysis of phytochemicals, antioxidant, and anti-inflammatory properties of *S. hernandifolia* leaves from two different grown sites in Nagaland, India has provided valuable insights into the potential medicinal applications of this plant. Our study's findings suggest that *S. hernandifolia* leaves have a considerable concentration of phytochemicals

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that have anti-inflammatory and antioxidant qualities that may be utilized to treat a range of illnesses. The research highlights the importance of recognizing the medicinal potential of indigenous flora and encourages further investigation into the diverse phytochemical properties of these plants. However, further research is needed to fully understand the mechanisms behind these properties and to explore their potential applications in traditional medicine. By leveraging these natural resources, we can pave the way for the development of novel drugs, nutraceuticals and complementary medicines, thereby contributing to both human health and the conservation of valuable plant species.

Acknowledgment

This study was supported by the Department of Zoology laboratory and management also.

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