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*Original Research Article*

# Study of the Antioxidant and Antidiabetic Properties of the Alga *Halopteris Scoparia*: *In Vitro* and *In Vivo* Evaluation in Alloxan-Induced Diabetic Mice

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ABSTRACT

*Halopteris scoparia* is an edible brown algae seaweed with significant biological activities. This study aimed to explore the biological and toxicological activities of the methanol extract of *Halopteris scoparia*. The study included a comprehensive evaluation of the phytochemical composition of the extract, as well as its antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging, and Ferric reducing antioxidant power (FRAP) assays. Antidiabetic activity was evaluated *in vitro* using α-amylase and α-glucosidase inhibitory assays, and *in vivo* in alloxan-induced diabetic mice. Toxicological effect was investigated *in vivo* by acute toxicity and sub-acute toxicity tests in mice. Phytochemical analysis revealed a rich phytochemical profile of *Halopteris scoparia*, with phenolic and flavonoid contents of 55.47 ± 0.70 mg GAE /g and 147.67 ± 1.53 mg QE/g, respectively. LC-MS analysis revealed various bioactive compounds. *Halopteris scoparia* extract showed remarkable antioxidant potential, with $IC_{50}$ values of 1.213 ± 0.290 mg/mL and 2.671 ± 0.210 mg/mL in the DPPH and ABTS radical scavenging assays, respectively, and FRAP value of 82.215 ± 0.970 mg AAE/g. *Halopteris scoparia* extract exhibited promising antidiabetic activity by demonstrating significant α-amylase and α-glucosidase inhibitory activities *in vitro*, and potent hypoglycaemic effect *in vivo* comparable to that of glibenclamide. In addition, *Halopteris scoparia* extract significantly decreased malondialdehyde, and increased the levels of antioxidant enzymes in the pancreas, liver and kidneys of diabetic mice. These results highlight the potentials of *H. scoparia* extract as a relatively safe natural antioxidant and alternative source of antidiabetic agent(s).

*Keywords: Halopteris scoparia*, Alloxan-induced diabetic mice, Anti-diabetic, Antioxidant, Oxidative stress, Antioxidant enzymes.

## Introduction

The medical field has witnessed significant advancements in the treatment of various diseases, particularly those with severe impacts such as diabetes, cancer, and cardiovascular diseases. However, these treatments are not universally accessible, often come with side effects, and can be prohibitively expensive. This necessitates the search for more effective alternatives that are devoid of adverse effects and can be made accessible to a broader population.[1]

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The increasing nutritional and health demands of the expanding human population have drawn significant focus towards both marine and terrestrial botanical resources.

Particularly, marine algae, abundant in potent chemical compounds, have been long utilized across various domains including food, pharmaceuticals, medical, and the cosmetics industry since ancient eras.[2]

Organisms residing in oceanic environments contain distinct compounds setting them apart from their terrestrial counterparts. Recent discoveries from marine life, particularly from sponges and polychaetes, as well as extracts from seaweed, have garnered attention as promising raw materials for cosmetic development. These extracts contain representative compounds like tannins, terpenes, phenols, halogens, and catechins, known for their physiological activities within marine organisms.[3]

Macroalgae, often known as seaweed, are an important ocean resource that humans have understood and used for millennia.[4,5] Despite its long history of use, seaweed has not been as widely utilized as other marine and terrestrial resources. Its use and applications have been mostly limited to specific locations, most notably Asia, where it has long been

prized as a food source.[5] In fact, seaweeds are now employed for a variety of purposes, including food, particularly sushi and other traditional cuisines; biofertilizer; colloids supply; and extraction of bioactives with antibacterial, anticancer, and other biological activities. The majority of the world's seaweed production is cultivated.[5]

Seaweed extracts comprise natural compounds with inherent antioxidant, antibacterial, anti-cancer, anti-inflammatory, anti-coagulant, and immune-regulatory properties. Consequently, they stand out as a prominent resource for the development of novel drugs, offering potential applications in disease prevention and treatment.[6]

However, the biological activities of algae have emerged as a promising source of natural compounds with remarkable therapeutic potential, extending beyond their well-established antioxidant properties. Numerous studies have delved into the anticancer effects of algae compounds, demonstrating encouraging results in both *in vivo* and *in vitro* cancer intervention studies.[7] Aside from their anticancer qualities, marine algae have received a lot of interest for their anticoagulant and anti-inflammatory characteristics. Many algae chemicals have shown promise anticoagulant and anti-inflammatory properties, leading to their inclusion in a variety of medicinal and pharmaceutical applications.[8]

The *Halopteris scoparia* is an edible brown algae seaweeds from *Sphacelariales* order (Family *Stypocaulaceae*),[2,9] with significant biological activities, and it's served as a salad in the East.[2] Due to its diverse antibacterial compounds, *Halopteris scoparia* can be employed on a lesser scale as a supplement in meals as well as for pharmaceutical purposes.

The purpose of this research was to estimate the content of secondary metabolites (flavoinoids and polyphenols) of the methanol extract of *H. scoparia* collected from the coast of Al-Hoceima, Morocco. Moreover, the pharmaceutical and medicinal applications of the tested seaweed extract were documented by evaluating the antioxidant, antidiabetic, acute and subacute toxicity.

## Materials and Methods

*Seaweed source, collection and preparation*

*H. scoparia* was collected in the Mediterranean Sea at shallow depth, in an area of high light intensity, in the coastal region of Al-Hoceima, Cala iris, Morocco (Coordinates: 35°9'0" N and 4°21'36" W). The algae was identified by Professor Abou Oualid, Laboratory of Aquatic Systems: Marine and Continental Environments, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco, with the aid of "The Guide to British Algae" and "Marine Algae: Biodiversity, Taxonomy, Environmental Assessment, and Biotechnology".[11,12]

The sample was transported to the laboratory while immersed in seawater, then washed three times with tap water to remove salt, epiphytes and sand adhering to the surface, then rinsed well with distilled water and stored in the refrigerator at 20°C.

*Preparation of extract*

The algae powder was extracted by maceration in methanol (80%) at room temperature for 24 h. The extract was centrifuged at 5,000 g for 10 min, then filtered through a Whatman filter paper. The solvents were evaporated in a rotary steamer to recover the methanol, then a Freeze dryer was used to remove the water, and the dried extract was stored in the refrigerator until used for further analysis.

*Determination of total phenolic content*

The total phenolic content of the *H. scoparia* extract was determined using the Folin-Ciocalteu method, as described by Drioua *et al*. (2024).[13] Briefly, 0.3 mL of extract was mixed with 1.5 mL diluted Folin–Ciocalteu reagent (1:10) and 1.2 mL sodium carbonate (7.5%), vortexed, and incubated in the dark for 30 min at room temperature. Absorbance was measured at 750 nm using a spectrophotometer. Gallic acid was used as standard, and results were expressed as mg gallic acid equivalents per g dry weight (mg GAE/g DW).

To determine the total phenolic contents, *H. scoparia* extract was tested at three different concentrations (1 mg/mL, 5 mg/mL, and 10 mg/mL). The 5 mg/mL concentration demonstrated optimal performance and was subsequently employed in the assay.

*Determination of total flavonoid content*

The flavonoid content was determined using the method described by Huang *et al*. (2005)[14] with some modifications. In brief, 40 µL of the *H. scoparia* extract was mixed with 10 µL of potassium acetate (1 M) and 10 µL of aluminium chloride (10%). Thereafter, 100 µL of methanol (50%) was added, and the final volume was increased to 400 µL with distilled water. The absorbance of the mixture was measured spectrophotometrically at 415 nm. Quercetin was used as standard. For the determination of total flavonoid content, three concentrations of *H. scoparia* extract (1 mg/mL, 5 mg/mL, and 10 mg/mL) were initially tested. Among these, the concentration of 5 mg/mL was found to be optimal and was therefore used in the assay.

*HPLC-DAD-ESI/MS analysis*

Analysis of polyphenolic compounds from the methanol extract of *H. scoparia* was carried out using HPLC-DAD-ESI/MS on a Shimadzu (Kyoto, Japan) liquid chromatography system. The same protocol of separation and quantification has already been published in the work of Kabach *et al*. (2023).[15]

*Determination of Antioxidant activity*

*DPPH radical scavenging assay*

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a purple-coloured synthetic free radical. The purple DPPH is reduced to yellow $DPPH_2$ (2,2-diphenyl-1-picrylhydrazine) in the presence of a free radical scavenger.[16] Briefly, 1.25 mL of the *H. scoparia* extract (of different concentrations 0.31, 0.62, 1.25, 2.5, 5 mg/mL, was mixed with 0.25 mL of methanol-prepared DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was then measured at 517 nm. The percentage of antiradical activity was calculated using equation 1:

$$\text{Scavenging effect (\%)} = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100 \quad \text{..... (1)}$$

Where;

$A_S$ is the absorbance of the extract; $A_{DPPH}$ is the absorbance of the DPPH solution.

*Ferric reducing antioxidant power (FRAP) assay*

The FRAP assay is based on measuring the ability of the extract to reduce ferric iron to ferrous iron. Briefly, 0.5 mL of the extract at various concentrations (0.5, 1, 2, 4, 8 mg/mL) was mixed with 1.25 mL of phosphate buffer solution (0.2 M, pH 6.6) and 1.25 mL of potassium ferricyanide (1%). This mixture was incubated at 50°C for 20 minutes. After incubation, 1.25 mL of trichloroacetic acid (10%) was added to quench the reaction. The mixture was centrifuged at 3000 rpm for 10 minutes. Then, 1.25 mL of the supernatant was mixed with 1.25 mL of distilled water and 0.25 mL of ferric chloride (0.1%). The absorbance of the mixture was measured at 700 nm. The blank was prepared by replacing the extract with distilled water. Results were expressed as mg ascorbic acid equivalent per g extract.[17]

*ABTS radical scavenging assay*

The radical scavenging potential of the extract derived from *H. scoparia* was evaluated against the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^+$) radical using the method described by Re *et al*. (1999).[18] The $ABTS^+$ radical was generated using potassium persulfate. Prior to conducting the assay, the stock solution of $ABTS^+$ was diluted with methanol until it reached an absorbance of 0.700 ± 0.020 at 734 nm. Subsequently, 185 µL of the diluted $ABTS^+$ solution was mixed with 15 µL of different concentrations of the *H. scoparia* extract (0.31, 0.62, 1.25, 2.5, 5 mg/mL). The absorbance was read after 10 min at 734 nm. The extract was replaced by methanol in the control. The $IC_{50}$ was determined following the method published by Kabach *et al*. (2019).[1]

*Determination of antidiabetic activity*

*α-amylase inhibitory assay*

The evaluation of *H. scoparia* extract inhibition potential against α-amylase was conducted following the method outlined by Mrid *et al*. (2022).[19] Briefly, 100 µL of different concentrations (62.5, 125, 250, 500, 1000 ug/mL) of *H. scoparia* extract solubilized in dimethyl sulfoxide (3%), was combined with 100 µL of α-amylase solution (0.1 U/mL) prepared in phosphate buffer (pH 6.9) and allowed to incubate at 37°C for 30 min. After the pre-incubation period, 100 µL of starch solution (0.25%) in phosphate buffer (pH 6.9) was added to each tube. The reaction proceeded at 37°C for an additional 30 min. Subsequently, the reaction was stopped by adding of 200 µL of the DNS reagent.

The test tubes were placed in a boiling water bath for 5 min and then cooled to room temperature before measuring the absorbance at 540 nm using a spectrophotometer. Acarbose served as the positive control for comparison purposes. The α-amylase inhibitory activity was determined using equation 2:

$$\text{Inhibition } (\%) = [((\text{Ac} - \text{Acb}) - (\text{Ae} - \text{Aeb}))/((\text{Ac} - \text{Acb}))] \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where;

Ac is the absorbance of control; Acb refers to the absorbance of control blank (without enzyme). Ae and Aeb are the absorbance of extract and extract blank (inhibitor without enzyme), respectively.

*α-glucosidase inhibition assay*

The assessment of *H. scoparia* extract inhibitory effect against α-glucosidase was conducted following previously established procedures reported by Mrid *et al*. (2022).[19] Various concentrations (62.5, 125, 250, 500, 1000 ug/mL) of the extract (100 µL) were mixed in a test tube with 110 µL of α-glucosidase enzyme (0.1 U/mL) prepared in sodium phosphate buffer (0.1M; pH 6.7) was added to 165 µL of the sample and then incubated at 37°C for 10 min. After incubation, 220 µL of 4-Nitrophenyl *β*-D-glucopyranoside was added to the reaction and incubated at 37°C for 30 min. In the end, a volume of 605 µL of $Na_2CO_3$ was added to stop the reaction. The absorbance was measured at 405 nm. The result was calculated and presented in the same manner as α-amylase inhibition.

*Experimental animals*

Swiss albino mice of both sexes, weighing 20 - 30 g, were obtained from the Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco. Before the start of the experiment, the animals were allowed an acclimatization period of 7 days under standard laboratory conditions (12 h light/dark cycle, 25 ± 2°C, 50–60% humidity) with free access to commercial pellet diet and water *ad libitum*. All experimental procedures complied with the organization for economic cooperation and development (OECD) guidelines.[20] At the end of the study, mice were humanely sacrificed under ether anaesthesia.

*Ethical approval*

All experimental procedures involving animals were conducted in accordance with international guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco. Ethical approval was granted by the Pharmacological Research Committee, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco. All experiments adhered to the guidelines for laboratory animal use set by the Pharmacological Research Committee.

*Acute toxicity study*

The acute toxicity test followed the protocols outlined in Guideline 425 of the OECD.[20] The animals were divided into two groups, each consisting of five mice. One group was administered *H. scoparia* extract at a dose of 2000 mg/kg orally, while the other group was not given the extract, and served as the control group. Mortality and any behavioural changes in the mice were closely monitored for a period of 14 days.

*Sub-acute toxicity study*

The subacute oral toxicity study was conducted over a period of 28 days. The study adhered to OECD Test Guideline No. 407.[21] Fifteen mice were divided into two groups, each comprising five mice. They were individually housed in sterile polypropylene cages under the following conditions: the first group served as the control and did not receive any treatment, while the remaining group was administered *H. scoparia* extract at 200 mg/kg once daily for 28 days. Throughout, the mice were weighed weekly to monitor any fluctuations and observe potential abnormalities. On the 29th day of the experiment, the mice were fasted for 4 hours before being anesthetized for blood collection. Subsequently, they were humanely euthanized using a surgical blade to extract vital organs such as the spleen, lungs, liver, kidneys, and pancreas.

*Diabetes induction and mice grouping*

The mice were fasted for 18 h but with free access to water. Thereafter, the mice were intraperitoneally injected with alloxan monohydrate solubilized in cold PBS (0.1 M; pH 5) at a dose of 180 mg/kg body weight. After 4 days, a measurement of the blood glucose of each mouse was performed. All mice with a blood glucose level above 200 mg/dL were considered diabetic and designed for the study.[22] The mice were divided into 5 groups, each group consisting of 5 mice; Group 1 (normal control): normal mice treated with distilled water. Group 2 (diabetic control): diabetic mice treated with distilled water. Groups 3 and 4: diabetic mice treated with *H. scoparia* extract at 250 and 500 mg/kg, respectively. Group 5: diabetic mice treated with glibenclamide at 10 mg/kg. All mice were treated by oral gavage once a day for 3 weeks.

*Body weight assessment and measurement of fasting blood glucose level*

The body weight of each mouse was recorded using a scale at a regular 7-day interval throughout the experimental period. Blood glucose was measured after 12 hours of fasting on days 0, 7, 14, and 21 of treatment. Blood was collected from the tail vein and measured using the AccuChek® active blood glucose meter.

*Collection of blood samples and organs*

At the end of the study, the mice were sacrificed under ether anesthesia, and blood samples were collected via cardiac puncture. The blood samples were placed in tubes with no anticoagulant, left at room temperature for 30 min, and centrifuged at 3,000 g for 15 min at 4°C. The supernatant was collected for analysis of serum biochemical parameters.[23] The liver, pancreas and kidneys were harvested from each mouse for biochemical assays.

*Measurements of serum biochemical parameters*

The serum levels of aspartate aminotransferase (AST), cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) were measured using COBAS c 502 (ROCHE diagnostics).

*Determination of malondialdehyde (MDA) content*

The quantification of malondialdehyde (MDA) content involves measuring the malondialdehyde-thiobarbituric acid (MDA-TBA) complex in acidic and hot conditions, following the method previously described by Zouaoui *et al*. (1979).[24] Briefly, 0.125 mL of cellular homogenate was mixed with 0.250 mL of 20% trichloroacetic acid (TCA) and 0.250 mL of 0.67% thiobarbituric acid (TBA). The mixture was heated at 100°C for 1 hour. The mixture was cooled in an ice-cold, followed by the addition of 1 mL of n-butanol, then centrifuged at 1200x g for 12 min. The absorbance was measured at 535 nm.

*Antioxidant enzyme assay*
The antioxidants enzyme activities were determined by spectrophotometry. The superoxide dismutase (SOD) and glutathione (GSH) activities were assayed according to the method described by El Omari *et al*. (2016).[25] The activity of catalase (CAT) was measured following the method of Aebi (1984).[26]

*Statistical analysis*
Statistical analyses were performed using GraphPad Prism software (version 8). Results were presented as the mean ± standard deviation (SD).

## Results and Discussion

The study on the methanol extract of *Halopteris scoparia* (HSEM) provides comprehensive insights into its multifaceted bioactivity and safety profile, as observed in various assays and toxicity studies. This extract showcased notable antioxidant capacity, as evidenced by its effective DPPH, ABTS, and FRAP activities.[27] HSEM radical scavenging potential, with $IC_{50}$ values comparable to standards like quercetin, catechin, and ascorbic acid, signifies its competence in mitigating oxidative stress.[10]
Moreover, HSEM exhibited intriguing enzyme inhibitory effects, notably against α-amylase and α-glucosidase, hinting at potential antidiabetic properties by modulating enzymes crucial in carbohydrate metabolism.[28]
Critical safety assessments in animal models indicated a favorable safety profile of HSEM at doses up to 2000 mg/kg, complying with OECD guidelines. Stable body weight and organ weights observed in sub-chronic toxicity studies affirm its minimal impact on physiological parameters over an extended period, crucial for potential pharmaceutical or therapeutic applications.[2]

*Compounds identified from the HPLC-DAD-ESI/MS analysis*
The HPLC-DAD-ESI/MS analysis result presented in Table 1 offers a comprehensive overview of the composition of the methanol extract of *H. scoparia*, revealing a range of compounds in the plant material studied. This method enabled the identification of several important compounds, showing their retention times, molecular ion masses ($[M-H]^-$) and various fragmentation patterns by MS/MS.

**Table 1:** Compounds identified in *H. scoparia* methanol extract by HPLC-DAD-ESI/MS

| | Metabolite | Cosine | m/z error (ppm) | Mass diff. | Precursor m/z | Formula |
|---|---|---|---|---|---|---|
| 1 | 12-hydroxy stearic acid | 0.74 | 3330 | 1.797 | 539.503 | $C_{34}H_{66}O_4$ |
| 2 | Juniperoside III | 0.84 | 597 | 0.186 | 311.114 | $C_{15}H_{20}O_7$ |
| 3 | 1-(3,4-dimethoxyphenyl)-3-[(1-methylbenzimidazol-6-yl)amino]propan-1-one | 0.76 | 2745 | 0.934 | 340.166 | $C_{19}H_{21}N_3O_3$ |
| 4 | 14-deoxy-11,12-dihydroandrographolide | 0.71 | 5377 | 1.910 | 355.19 | $C_{20}H_{28}O_4$ |
| 5 | 1-*O*-trans-cinnamoyl-beta-D-glucopyranose | 0.80 | 6153 | 1.902 | 309.098 | $C_{15}H_{18}O_7$ |
| 6 | Avocadyne acetate | 0.91 | 190 | 0.062 | 325.238 | $C_{19}H_{34}O_4$ |
| 7 | 2-ethyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid | 0.80 | 4818 | 1.099 | 228.099 | $C_{12}H_{15}NO_2$ |
| 8 | N-cis-p-Coumaroyltyrosine | 0.74 | 3670 | 1.197 | 326.103 | $C_{18}H_{17}NO_5$ |
| 9 | (2Z)-4,6-dihydroxy-2-[(3-hydroxy-4-methoxyphenyl)methylidene]-1-benzofuran-3-one | 0.75 | 6546 | 1.971 | 301.071 | $C_{16}H_{12}O_6$ |
| 10 | N-4-quinazolinylvaline | 0.71 | 4160 | 1.024 | 246.124 | $C_{13}H_{15}N_3O_2$ |
| 11 | (4-oxido-2,3,5,6,7,8-hexahydro-1H-pyrrolizin-4-ium-1-yl)methyl 2,3-dihydroxy-3-methylpentanoate | 0.74 | 2779 | 0.862 | 310.162 | $C_{14}H_{25}NO_5$ |
| 12 | 5,6,2'-Trimethoxyflavone | 0.88 | 668 | 0.208 | 311.092 | $C_{18}H_{16}O_5$ |
| 13 | [5-acetyloxy-3-(hydroxymethyl)-2-oxo-6-propan-2-ylcyclohex-3-en-1-yl] 3-methylpentanoate | 0.71 | 238 | 0.081 | 339.181 | $C_{18}H_{28}O_6$ |
| 14 | 6-amino-9-[2-(4-methylphenoxy)ethyl]purine-8-thiol | 0.79 | 2671 | 0.807 | 302.107 | $C_{14}H_{15}N_5OS$ |
| 15 | 7-[(2E,5E)-7-hydroxy-3,7-dimethylocta-2,5-dienoxy]chromen-2-one | 0.74 | 3017 | 0.945 | 313.145 | $C_{19}H_{22}O_4$ |
| 16 | (8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl) (Z)-2-methylbut-2-enoate | 0.86 | 5285 | 1.803 | 341.103 | $C_{19}H_{18}O_6$ |
| 17 | Aceroside VII | 0.73 | 4080 | 1.882 | 461.218 | $C_{25}H_{34}O_8$ |
| 18 | Apigenin 6,8-digalactoside | 0.75 | 86 | 0.051 | 593.151 | $C_{27}H_{30}O_{15}$ |
| 19 | Beta-uridine | 0.71 | 3894 | 1.040 | 267.060 | $C_9H_{12}N_2O_6$ |
| 20 | Catalpol | 0.79 | 49 | 0.020 | 407.120 | $C_{15}H_{22}O_{10}$ |
| 21 | D-Mannitol | 0.77 | 1270 | 0.230 | 181.07 | $C_6H_{14}O_6$ |
| 22 | Docosanol | 0.93 | 454 | 0.148 | 325.348 | $C_{22}H_{46}O$ |
| 23 | Dodecylbenzenesulfonic acid | 0.85 | 356 | 0.116 | 325.184 | $C_{18}H_{30}O_3S$ |

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| | | | | | | |
|---|---|---|---|---|---|---|
| 24 | Eicosanoic acid | 0.70 | 2910 | 0.906 | 311.294 | $C_{20}H_{40}O_2$ |
| 25 | Ethylenediaminetetra acetic acid | 0.76 | 5440 | 1.583 | 291.083 | $C_{10}H_{16}O_8N_2$ |
| 26 | Fluoxetine | 0.70 | 3482 | 1.073 | 308.127 | $C_{17}H_{18}F_3NO$ |
| 27 | GalCer(d18:1/23:0) | 0.79 | 1589 | 1.267 | 796.667 | $C_{47}H_{90}N_1O_8$ |
| 28 | Holostyligone | 0.73 | 5515 | 1.970 | 357.170 | $C_{21}H_{24}O_5$ |
| 29 | Knemolic acid B | 0.73 | 441 | 0.143 | 325.143 | $C_{20}H_{22}O_5$ |
| 30 | Albuterol | 0.76 | 3997 | 0.960 | 240.160 | $C_{13}H_{21}NO_3$ |
| 31 | Canrenone | 0.93 | 303 | 240.16 | 339.197 | $C_{22}H_{28}O_3$ |
| 32 | Bisoprolol | 0.81 | 2859 | 0.933 | 326.233 | $C_{18}H_{31}NO_4$ |
| 33 | 4-(undecan-5-yl)benzenesulfonic acid | 0.82 | 221 | 0.069 | 311.169 | $C_{17}H_{28}O_3S$ |
| 34 | 6-(4-sulfophenyl)octanoic acid | 0.86 | 6335 | 1.895 | 299.095 | $C_{14}H_{20}O_5S$ |
| 35 | Citric acid | 0.89 | 1989 | 0.380 | 191.020 | $C_6H_8O_7$ |
| 36 | 3,4-Dihydroxymandelic acid | 0.83 | 10382 | 1.900 | 183.000 | $C_8H_8O_5$ |
| 37 | Acacetin | 0.90 | 491 | 0.139 | 283.061 | $C_{16}H_{12}O_5$ |
| 38 | Fortunellin | 0.75 | 3092 | 1.828 | 591.172 | $C_{28}H_{32}O_{14}$ |
| 39 | Saikosaponin B2 | 0.93 | 2345 | 1.936 | 825.464 | $C_{42}H_{68}O_{13}$ |
| 40 | Saikosaponin D | 0.77 | 2345 | 1.936 | 825.464 | $C_{42}H_{68}O_{13}$ |
| 41 | Saikosaponin A | 0.77 | 198 | 0.164 | 825.464 | $C_{42}H_{68}O_{13}$ |
| 42 | 13S-hydroxy-9Z,11E,15Z-octadecatrienoic acid | 0.75 | 641 | 0.188 | 293.212 | $C_{18}H_{30}O_3$ |
| 43 | 9-keto-octadeca-10E,12Z-dienoic acid | 0.76 | 641 | 0.188 | 293.212 | $C_{18}H_{30}O_3$ |
| 44 | Phosphatidylcholine lyso 16:0 | 0.78 | 624 | 0.300 | 480.1 | $C_{24}H_{50}NO_7P$ |
| 45 | Mulberroside C | 0.92 | 4007 | 1.840 | 459.16 | $C_{24}H_{26}O_9$ |
| 46 | N-Acetylneuraminic Acid | 0.75 | 3589 | 1.113 | 310.113 | $C_{11}H_{19}NO_9$ |
| 47 | Parthenolide | 0.73 | 705 | 0.163 | 231.137 | $C_{15}H_{20}O_3$ |
| 48 | Phenergan | 0.81 | 6817 | 1.944 | 285.144 | $C_{17}H_{21}ClN_2S$ |
| 49 | Pinolidoxin | 0.92 | 884 | 0.300 | 339.0 | $C_{18}H_{26}O_6$ |
| 50 | p-methoxycinnamic acid ethyl ester | 0.75 | 9174 | 1.900 | 207.100 | $C_{12}H_{14}O_3$ |
| 51 | Psoralidin | 0.71 | 5932 | 2.000 | 337.1 | $C_{20}H_{16}O_5$ |
| 52 | Quercetin-7-*O*-beta-D-glucopyranoside | 0.78 | 4300 | 2.000 | 465.100 | $C_{21}H_{20}O_{12}$ |
| 53 | Scutellarioside II | 0.80 | 3645 | 1.849 | 507.151 | $C_{24}H_{28}O_{12}$ |
| 54 | Benzenesulfonic acid | 0.74 | 456 | 0.142 | 311.158 | $C_{17}H_{28}O_3S$ |
| 55 | Decylbenzenesulfonic acid | 0.89 | 535 | 0.159 | 297.141 | $C_{16}H_{26}O_3S$ |
| 56 | Ricinoleic acid methyl ester | 0.97 | 6641 | 1.961 | 295.261 | $C_{19}H_{36}O_3$ |
| 57 | Thymol-beta-D-glucoside | 0.83 | 482 | 0.150 | 311.15 | $C_{16}H_{24}O_6$ |
| 58 | Uridine | 0.92 | 666 | 0.162 | 243.062 | $C_9H_{12}N_2O_6$ |

The methanol extract of *H. scoparia* reveals a rich chemical diversity, underlining its pharmacological potential. Among the classes of compounds identified are:

Fatty acids and their derivatives were represented by 12-hydroxystearic acid (1), eicosanoic acid (24), and ricinoleic acid methyl ester (56). Flavonoids and glycosides included Acacetin (37), apigenin 6,8-digalactoside (18), and quercetin-7-O-β-D-glucopyranoside (52). Saponins, such as saikosaponin A (41), saikosaponin B2 (39), and saikosaponin D (40), were also identified, all of which are triterpenoids known for their bioactivity. Phenolic compounds were represented by p-methoxycinnamic acid ethyl ester (56) and psoralidin (51), while nitrogen-containing compounds included N-4-quinazolinylvaline (10) and fluoxetine (26). Sulfur-containing metabolites, such as dodecylbenzenesulfonic acid (23) and 4-(undecan-5-yl)benzenesulfonic acid (33), were also detected. Additionally, nucleosides such as uridine (58) and β-uridine (19) were present. This chemical diversity highlights the potential of *H. scoparia* as a valuable source of bioactive molecules for pharmaceutical and nutraceutical applications.

The presence of phenolic compounds, flavonoids and saponins in *H. scoparia* suggests antioxidant, anti-inflammatory or antimicrobial properties, often associated with these chemical classes. For example, flavonoids such as acetin and quercetin-7-*O*-glucoside are known for their antioxidant effects, while saikosaponins are recognized for their immunomodulatory and hepatoprotective activities. The detection of unexpected compounds, such as the antidepressant fluoxetine (26), could be the result of contamination or misallocation, requiring further verification.

The chemical profiling of the methanol extract of *H. scoparia* using HPLC-DAD-ESI/MS revealed a complex and diverse composition of secondary metabolites. The identified compounds belong to different

classes, including flavonoids, phenolic acids, saponins, alkaloids, fatty acids, and phenylpropanoids. Analytical parameters, such as cosine similarity scores and mass errors, broadly confirm the reliability of the identifications, although some high mass error values suggest the need for further validation. These results underline the potential of *H. scoparia* as a source of bioactive metabolites, meriting further study to explore their therapeutic applications. For a more precise interpretation, chromatographic peak analysis (Figure 1) and confirmation of redundant or atypical compounds would be necessary.

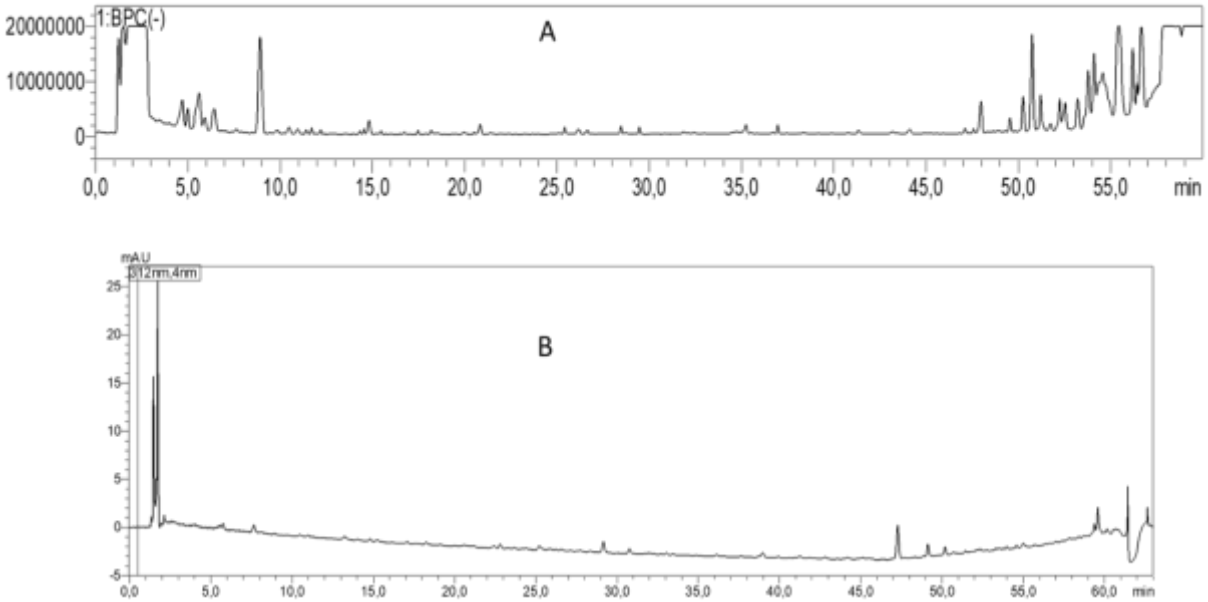


**Figure 1:** HPLC Chromatogram of phenolic compounds in *H. scoparia* methanol extract. A: Base Peak Chromatogram (BPC), B: UV chromatogram. Conditions: C18 column, mobile phase water/acetonitrile with gradient elution, detection at 280 nm.

The presence of these bioactive metabolites aligns with previous reports on the phytochemical richness of brown algae and reinforces the potential pharmacological value of *H. scoparia*.[2,6] The high abundance of flavonoids and saponins, known for their antioxidant and metabolic regulatory effects, may explain the observed antioxidant, antidiabetic, and antiglycation activities of the extract.[7,8,15]

The methanol extract of *H. scoparia* contains diverse secondary metabolites whose synergistic and antagonistic interactions may influence its biological activity and toxicity. Synergistic interactions may enhance antioxidant activity, particularly through the combination of flavonoids and phenolic acids, which are well-known for their free radical scavenging properties. Studies have shown that specific phenolic compound combinations exhibit significant synergistic effects in antioxidant assays.[29] Additionally, saponins when combined with flavonoids, may exhibit enhanced anti-inflammatory effects by modulating inflammatory pathways more effectively than individual compounds.[29] However, antagonistic effects are also possible. Metabolite interactions can influence compound bioavailability, as certain flavonoid combinations have been reported to reduce the absorption and efficacy of other metabolites.[30] Furthermore, some interactions may increase toxicity; for instance, specific combinations of fatty acids and alkaloids have been associated with reduced antioxidant activity and potential cytotoxic effects.[29] These interactions highlight the complexity of *H. scoparia* and their potential pharmacological implications.

Chemical analysis reveals HSEM rich composition of phenolic compounds and flavonoids, including gallic acid, quinic acid, catechin, and kaempferol derivatives, known for their antioxidant and anti-inflammatory properties. These compounds potentially mediate the observed bioactivities, warranting further exploration into their individual or synergistic roles in facilitating the extract's therapeutic effects.[10]

*Polyphenol and total flavonoid contents*

The bioactive compounds such as polyphenols and flavonoids of *H. scoparia* methanol extract were determined, and the results are shown in Table 2.

*H. scoparia* extract shows a notable content of total phenolic compounds (TPC) with a value of 55.47 ± 0.7 mg GAE/g extract. This concentration, expressed as gallic acid equivalent (GAE), indicates a rich content of polyphenols, which are recognized for their antioxidant properties. Comparatively, the total flavonoid content (TFC) was even higher, reaching 147.67 ± 1.53 mg EQ/g extract (quercetin equivalent). This value suggests that flavonoids, an important subclass of polyphenols, make up a significant proportion of the extract's bioactive compounds, probably contributing to its overall antioxidant activity.[30,31]

*Antioxidant activity*

Table 2 outlines the antioxidant activity of *H. scoparia* extract and the standard compounds, evaluated using three assays: DPPH, ABTS, and FRAP. These assays are essential for assessing the antioxidant potential of substances, reflecting their ability to neutralize free radicals and counteract oxidative stress, which is crucial in health and disease.

In the DPPH assay, the extract had an $IC_{50}$ of 1.213 ± 0.290 mg/mL, reflecting its ability to neutralize 50% of DPPH free radicals. Although this value is significantly higher than that of quercetin ($IC_{50}$ = 0.005 ± 0.020 mg/mL), a reference antioxidant, it still indicates significant antioxidant activity for a crude extract. The ABTS test reveals an $IC_{50}$ of 2.671 ± 0.210 mg/mL, higher than that of ascorbic acid (0.003 ± 0.02 mg/mL), which suggests a lower efficacy compared to the standard, but remains consistent with the complex nature of the extract.

Finally, the FRAP test, which measures the reducing power of the extract, shows a value of 82.215 ± 0.970 mg AAE/g extract (ascorbic acid equivalent). This high reducing capacity confirms the presence of compounds capable of donating electrons to neutralize oxidizing species, thus reinforcing the antioxidant potential of *H. scoparia*. In summary, these results highlight that *H. scoparia* extract possesses a high content of phenolic and flavonoid compounds, as well as significant antioxidant activity, albeit less potent than pure standards such as quercetin and ascorbic acid. These properties make it a promising candidate for nutraceutical and pharmacological applications, subject to further study.

These findings highlight the potential of the extract as natural antioxidants with radical scavenging, and ferric reducing power capacities.

**Table 2:** Bioactive compounds and antioxidant activity of *H. scoparia* extract.

| | TPC (mg GAE /g E) | TFC (mg QE/g E) | Antioxidant activity | | |
|---|---|---|---|---|---|
| | | | DPPH ($IC_{50}$ in mg/mL) | ABTS ($IC_{50}$ in mg/mL) | FRAP (mg AAE/g) |
| ***H. scoparia* extract** | 55.47 ± 0.70 | 147.67 ± 1.53 | 1.213 ± 0.29 [b] | 2.671 ± 0.21 [b] | 82.215 ± 0.97 |
| **Quercetin** | - | - | 0.005 ± 0.02 [a] | - | - |
| **Ascorbic acid** | - | - | - | 0.003 ± 0.02 [a] | - |

Values are means ± standard deviation (SD), n = 3. Different lowercase letters (a, b) within the same column indicate statistically significant differences at $p < 0.05$, determined by one-way ANOVA followed by Tukey's post hoc test. GAE: Gallic Acid Equivalent. QE: Quercetin Equivalent. AAE: Ascorbic acid Equivalent. E: Extract. $IC_{50}$: The extract concentration providing 50% inhibition. DPPH: 2,2-diphenyl-1-picrylhydrazyl. ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid). FRAP: Ferric reducing antioxidant power.

*In vitro antidiabetic activity of H. Scoparia extract*

Table 3 presents the results of antidiabetic activity of *H. Scoparia* extract and acarbose as standard against both α-amylase and α-glucosidase enzymes. The values represent the concentration of the extract required to inhibit 50% of the enzyme activity.

For the α-amylase enzyme, *H. scoparia* extract exhibited an $IC_{50}$ of 175.45 ± 0.70 µg/mL, indicating the concentration required to inhibit 50% of enzyme activity. In comparison, acarbose, a reference inhibitor used in the treatment of diabetes, showed a significantly lower $IC_{50}$ of 14.000 ± 0.001 µg/mL. This significant difference ($p < 0.05$) suggests that acarbose is much more potent than crude *H. scoparia* extract in inhibiting α-amylase. However, the extract's ability to inhibit this enzyme, although less pronounced, remains interesting in the context of an unpurified natural extract.

Concerning α-glucosidase inhibition, *H. scoparia* extract displayed an $IC_{50}$ of 74.52 ± 0.18 µg/mL, which is closer to the value obtained for acarbose (18.01 ± 2.00 µg/mL). Although acarbose remains significantly more effective ($p < 0.05$), *H. scoparia* extract showed notable inhibitory activity on α-glucosidase, suggesting an interesting potential for slowing glucose absorption at the intestinal level. This activity could be attributed to the bioactive compounds present in the extract, such as the previously identified polyphenols and flavonoids, known to interfere with enzymatic activity.

In conclusion, *H. scoparia* extract demonstrated inhibitory activity against α-amylase and α-glucosidase enzymes, albeit less potent than that of acarbose. The more marked inhibition of α-glucosidase compared to α-amylase suggests that the extract may be more effective in modulating carbohydrate absorption at the intestinal level, a key mechanism in the management of type 2 diabetes. These results, obtained in an *in vitro* study, highlight the potential of *H. scoparia* as a source of bioactive compounds for therapeutic applications, but further studies, particularly *in vivo*, are needed to confirm these effects and assess their clinical relevance.

**Table 3:** α-amylase and α-glucosidase inhibitory activities of *H. Scoparia* and Acarbose

| | $IC_{50}$ (µg/mL) | |
|---|---|---|
| | **α-amylase** | **α-Glucosidase** |
| ***H. scoparia* extract** | 175.45 ± 0.7 [a] | 74.52 ± 0.18 [a] |
| **Acarbose** | 14 ± 0.001 [b] | 18.01 ± 2 [b] |

Values are means ± standard deviation (SD), n = 3. Different lowercase letters (a, b) within the same column indicate statistically significant differences at $p < 0.05$, determined by one-way ANOVA followed by Tukey's post hoc test.

*Acute toxicity of H. scoparia extract*

A study evaluating the acute toxicity of *H. scoparia* extract was conducted in mice following the OECD guidelines 423 for testing chemical substances. The results of this investigation regarding the acute toxicity of the orally administered plant extract were promising. No deaths or clinical signs of toxicity were observed following the administration of doses at 300 and 2000 mg/kg of body weight. All animals survived the 14 days observation period, indicating that the $LD_{50}$ (median lethal dose) is greater than 2000 mg/kg. In accordance with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS, 2003), the extract can be considered non-toxic when administered orally.

Moreover, monitoring the weight evolution of mice treated with the *H. scoparia* extract during the observation period revealed notable stability in body weight, particularly after 14 days. This finding confirms that *H. scoparia* extract had no toxic effect on the basic behaviour of treated mice. These results are encouraging regarding the potential use of this extract in various applications while emphasizing its safety when administered orally.

*Subacute toxicity of H. scoparia extract*

The results in Table 4 illustrate the sub-chronic toxicity evaluation of *H. scoparia* extract in a group of mice consisting of 5 males and 5 females. The extract was administered orally at a dose of 200 mg/kg over a period of 28 days. The initial body weight of the mice receiving the *H. scoparia* extract was recorded with an average value of 30.12 ± 1.41 g. After the 28$^{th}$ day, their final weight was measured, with an average value of 32.19 ± 2.15 g. Therefore, a weight gain was observed in the mice after treatment with *H. scoparia* extract. These results correlate with those obtained in the control group, where mice treated with distilled water also showed weight gain, with an average increase of 1.68 g per kg, although the magnitude of the change was lower compared to the group treated with the *H. scoparia* extract. The increase in body weight observed in both the test and control group mice over 28 days period indicates normal growth or slight weight gain during this period. However, the relatively higher difference in body weight in the group treated with *H. scoparia* compared to the control group may suggest an influence of the extract on weight-related parameters. Further investigations could explore the specific components of the *H. scoparia* extract that might contribute to this observed weight change. It is crucial to consider factors beyond weight, such as metabolic changes or any associated physiological alterations, to comprehensively evaluate the extract impact on the subject's health and well-being over a prolonged period.

Table 5 presents the relative weight of vital organs in both the control (untreated) and extract treated groups during the subacute toxicity assessment of *H. scoparia* extract. The relative organ weights depict the proportional mass of each organ concerning the total body weight. In this study, the group treated with the methanol extract of *H. scoparia* exhibited slight variations in the relative weights of certain vital organs compared to the control group. The liver, a crucial organ involved in

detoxification and metabolism, demonstrated a marginal increase in relative weight in *H. scoparia* extract treated group compared to the control group. Similarly, the kidney and lung showed slight elevations in relative weight, although these changes were not notably substantial. Conversely, the relative weights of other organs such as the pancreas and heart showed minor decreases in *H. scoparia* extract-treated group compared to the control. The variations observed in the relative weights of these organs suggest some potential influence of the extract on the physiological state of these vital organs. It is important to note that these alterations in relative organ weights, while present, fall within a range that does not imply significant adverse effects or major physiological changes. Such marginal changes, within a subacute toxicity study, may suggest a mild influence of the extract on certain organs but do not indicate adverse or concerning impacts on their functionality or health. These subtle alterations in organ weights, both increases and decreases, could hint at some level of physiological response to the extract. However, given the minimal and relatively balanced changes observed, further investigation is warranted to comprehensively understand the implications, if any, of these variations on organ function and overall health in prolonged exposure scenarios or different dosage regimens.

**Table 4:** Body weight of mice treated with the methanol extract of *H. scoparia*

| | Dose | Body weight (g) | |
|---|---|---|---|
| | | 1st day | 28th day |
| ***H. scoparia* extract** | 200 mg/kg | 30.12 ± 1.41 [b] | 32.19 ± 2.15 [b] |
| **Control group** | Distilled water | 25.75 ± 1.18 [a] | 27.43 ± 1.32 [a] |

Values are means ± standard deviation (SD), n = 5. Different lowercase letters (a, b) within the same column indicate statistically significant differences between treatments at $p < 0.05$, determined by one-way ANOVA followed by Tukey's post hoc test.

**Table 5:** Relative weight of vital organs in the sub-acute toxicity study of *H. scoparia* extract

| Organ | Weight (mg) | |
|---|---|---|
| | Control | ***H. scoparia* extract** |
| **Liver** | 1.63 ± 0.09 [a] | 1.88 ± 0.29 [b] |
| **Kidney** | 0.37 ± 0.04 [a] | 0.33 ± 0.07 [a] |
| **Pancreas** | 0.094 ± 0.06 [a] | 0.081 ± 0.01 [a] |
| **Spleen** | 0.12 ± 0.06 [a] | 0.23 ± 0.07 [b] |
| **Lung** | 0.32 ± 0.11 [a] | 0.34 ± 0.09 [a] |
| **Heart** | 0.12 ± 0.04 [a] | 0.15 ± 0.04 [b] |

Values are means ± standard deviation (SD), n = 5. Different lowercase letters (a, b) within the same line indicate statistically significant differences between treatments at $p < 0.05$, determined by one-way ANOVA followed by Tukey's post hoc test.

*Effect of the H. scoparia methanol extract on biochemical parameters in the subacute toxicity study*

Table 6 illustrates the biochemical parameters of the control group and groups treated with *H. scoparia* extract at a dose of 200 mg/kg in a subacute toxicity study. The analysis of biochemical parameters was assessed using the COBAS c 502 (ROCHE Diagnostics).

These parameters included various liver enzymes, lipid profile indicators, and markers related to kidney function. Comparing the *H. scoparia* extract-treated group to the control group revealed some variations in the biochemical parameters. Notably, the levels of liver enzymes, specifically ALT (Alanine Aminotransferase) and AST (Aspartate Aminotransferase), appear different between the groups. The extract-treated group showed a decrease in ALT levels compared to the control, while AST levels were higher in the treated group. These alterations in liver enzymes could suggest some influence on liver function due to the extract administration. Regarding lipid profiles, the treated group with *H. scoparia* extract displayed a slightly elevated HDL (High-Density Lipoprotein) cholesterol level but a decrease in LDL (Low-Density Lipoprotein) cholesterol compared to the control. This alteration in lipid levels might hint at a potential modulation of lipid metabolism by the *H. scoparia* extract.

Other parameters like alkaline phosphatase (ALP), total protein (TP), and markers related to kidney function (urea and creatinine) also exhibited marginal variations between the treated and control groups. These changes, while noticeable, were within ranges that did not suggest significant adverse effects or critical deviations from normal physiological levels.

The observed differences in these biochemical parameters between the extract-treated and control groups, although noticeable, might not necessarily indicate harmful effects. Nonetheless, they do suggest a certain level of influence on liver enzymes and lipid metabolism induced by the extract.

Further research, including more extensive studies over different time frames or varying dosages, is essential to comprehensively grasp the physiological implications of these variations. Additionally, analyzing these parameters in conjunction with histopathological studies could offer a more comprehensive understanding of the impact of the extract on organ function and overall health.

*Effect of the H. scoparia methanol extract on hematological parameters in the subacute toxicity study*

Table 7 presents the hematological parameters of the control group and groups treated with *H. scoparia* extract at a dose of 200 mg/kg in a subacute toxicity study. The results, analyzed using the DXH 800 BECKMAN COULTER apparatus, indicated variations in various hematological parameters between the control and extract-treated groups.

The *H. scoparia* extract-treated group (HSEM) demonstrated significant differences compared to the control group in several blood cell counts parameters. Notably, there was a noticeable increase in WBC (White Blood Cells) count, indicating an elevation in the immune response in the extract-treated group. Similarly, an increase in PLT (Platelets) count suggests potential modulation in the blood clotting mechanism due to the extract's administration.

Regarding specific types of white blood cells, the extract-treated group showed higher counts of lymphocytes (LYMPH), monocytes (MONO), neutrophils (NEUT), and eosinophils (EO) compared to the control group. These variations might indicate an enhanced immune response or certain immune system modulation triggered by the extract.

Interestingly, while some parameters show significant differences between the treated and control groups, other values such as hemoglobin (HB) and granulocytes (GR) exhibited marginal variations or remain relatively consistent between the two groups.

The changes observed in these hematological parameters, particularly in white blood cell counts, suggest a potential influence of *Halopteris*

*scoparia* extract on the immune system and blood clotting mechanisms. However, these alterations might not necessarily imply adverse effects, as they could also indicate an adaptive response of the body to the extract's administration.

Further research, including additional studies with different doses or time frames, and perhaps complemented by histopathological investigations, would provide a more comprehensive understanding of the extract's impact on hematological parameters and the overall immune response.

**Table 6:** Serum lipid profile and cardiovascular risk markers of mice treated with H. scoparia extract and the control in the sub-acute toxicity study

| | ALT (U/L) | AST (U/L) | TC (g/L) | TG (g/L) | HDL (g/L) | LDL (g/L) | ALP (U/L) | TP (g/L) | UREA (g/L) | CREA (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| ***H. scoparia* extract 200 mg/kg** | 60.1 ± 2.1 [b] | 285.1 ± 2.7 [b] | 1.0 ± 0.2 [a] | 1.7 ± 0.1 [a] | 0.7 ± 0.3 [a] | 0.6 ± 0.1 [a] | 97.8 ± 5.2 [a] | 62.0 ± 1.4 [a] | 0.2 ± 0.04 [a] | 0.90 ± 0.09 [a] |
| **Control** | 66.40±1.50 [a] | 248.40±1.03 [a] | 1.27±0.70 [a] | 1.85±0.22 [a] | 0.73±0.06 [a] | 0.60±1.01 [a] | 97.22±2.04 [a] | 62.88±0.22 [a] | 0.59±0.07 [a] | 1.06±0.30 [a] |

Values represented means ± standard deviation (SD), n = 5. Different lowercase letters (a, b) within the same column indicate statistically significant differences between treatments at $p < 0.05$, determined by one-way ANOVA followed by Tukey's post hoc test. ALT: Alanine Aminotransferase. AST: Aspartate Aminotransferase. TC: Total Cholesterol. TG: Triglyceride. HDL: High-Density Lipoprotein. LDL: Low-Density Lipoprotein. ALP: alkaline phosphatase. TP: total protein. CREAT: Creatinine.

**Table 7:** Haematological profile of mice treated with H. scoparia extract and the control in the sub-acute toxicity study

| | Control | *H. scoparia* extract (200 mg/kg) |
|---|---|---|
| **WBC ($10^3$/uL)** | 4.18 ± 0.34 [a] | 9.23±0.29 [b] |
| **GR ($10^6$/uL)** | 8.42 ± 1.01 [a] | 8.71 ± 0.32 [a] |
| **HB (g/L)** | 12 ± 1.55 [a] | 12.16 ± 0.67 [a] |
| **PLT ($10^3$/uL)** | 944 ± 11.33 [a] | 981.93 ± 4.74 [b] |
| **LYMPH ($10^3$/uL)** | 3.66 ± 0.39 [a] | 4.95 ± 0.15 [b] |
| **MONO ($10^3$/uL)** | 0.025 ± 0.02 [a] | 0.10 ± 0.07 [b] |
| **BASO ($10^3$/uL)** | 0.01 ± 0.00 [a] | 0.02 ± 0.005 [b] |
| **NEUT ($10^3$/uL)** | 0.48 ± 0.02 [a] | 0.97 ± 0.26 [b] |
| **EO ($10^3$/uL)** | 0.01 ± 0.00 [a] | 0.02 ± 0.004 [a] |

Values represent means ± standard deviation (SD), n - 5. Different lowercase letters (a, b) within the same line indicate statistically significant differences between treatments at $p < 0.05$, determined by one-way ANOVA followed by Tukey's post hoc test.WBC: White Blood Cells. GR: granulocytes. HB: haemoglobin. PLT: Platelets. LYMPH: lymphocytes, MONO: monocytes, NEUT: neutrophils. EO: eosinophils.

*The in-vivo effect of H. scoparia extract on blood glucose and body weight*

After breeding the mice and acclimatizing them to laboratory conditions, diabetes was induced by an injection of alloxan. Subsequently, physiological parameters such as fasting blood glucose and body weight were monitored for 3 weeks. The data in Table 8 summarized the effects of the extract on glycemia and body weight in diabetic mice. The normal mice group maintained stable fasting blood glucose levels with values between 72.50 ± 1.33 mg/dL and 86.00 ± 0.38 mg/dL, unlike the group of untreated diabetic mice, which had high blood glucose values (between 304.00 ± 0.41 mg/dL and 406.50 ± 1.27 mg/dL). Moreover, the daily oral administration of both doses of the H. scoparia extract 150 mg/kg and 250 mg/kg resulted in a significant decrease in fasting blood glucose compared to untreated diabetic mice, which maintained high blood glucose levels over the 3 weeks of the study. The same effect was observed with glibenclamide (commonly prescribed diabetes medication) which induced a significant decrease in fasting blood glucose in diabetic mice from a value of 230.60 ± 1.52 mg/dL to 68.80 ± 1.76 mg/dL over the 3 weeks study period. Regarding body weight, the normal mice group showed weight gain, unlike the group of untreated diabetic mice, which experienced weight loss. Treatment with both doses of the methanol extract of H. scoparia induced an increase in body weight toward normal weight over the 3 weeks of treatment. The results obtained show improvements in blood glucose levels and body weight throughout the 3 weeks treatment period; consequently, our extract may have beneficial effects on long-term diabetes management.

*Effect of H. scoparia extract on serum biochemical parameters in diabetic mice*

Table 9 presents the effects of *H. scoparia* extract on various biochemical parameters in diabetic mice, compared with normal control, diabetic control and reference glibenclamide treatment.

With respect to liver enzymes, the diabetic control group showed significantly elevated levels of ALT (124.750 ± 9.687 U/L) and AST (262.660 ± 10.691 U/L) compared with the normal control (41.695 ± 3.429 U/L for ALT and 162.675 ± 58.018 U/L for AST), indicating diabetes-related liver stress. Administration of *H. scoparia* extract at 500 mg/kg significantly reduced ALT (42.63 ± 5.54 U/L), reaching values close to normal control, suggesting a protective effect on the liver. However, at 250 mg/kg, ALT (62.96 ± 12.544 U/L) remained higher, and AST (309.860 ± 31.571 U/L) showed an increase compared with diabetic control, indicating a variable dose-dependent response. Glibenclamide (10 mg/kg) also reduced ALT (86.075 ± 2.001 U/L) and AST (120.365 ± 2.878 U/L), confirming its efficacy, although less marked than the 500 mg/kg extract for ALT.

In terms of lipid profile, the diabetic control group showed marked dyslipidaemia, with elevated levels of total cholesterol (TC: 3.545 ± 0.191 g/L), triglycerides (TG: 2.525 ± 0.191 g/L) and LDL (0.295 ±

0.021 g/L), as well as a decrease in HDL (0.165 ± 0.021 g/L) compared to normal control. *H. scoparia* extract at 500 mg/kg and 250 mg/kg significantly improved these parameters, with values for TC of 1.09 ± 0.24 g/L and 1.12 ± 0.296 g/L, respectively, TG of 1.38 ± 0.19 g/L and 1.23 ± 0.256 g/L, respectively, HDL of 0.76 ± 0.27 g/L and 0.80 ± 0.206 g/L, respectively and LDL of 0.16 ± 0.06 g/L and 0.12 ± 0.021 g/L, respectively. These results indicate a partial correction of dyslipidaemia, with a notable increase in HDL, particularly at 250 mg/kg, where values exceeded those of the normal control. Glibenclamide showed superior efficacy in reducing TC (0.495 ± 0.049 g/L) and TG (0.520 ± 0.156 g/L), but *H. scoparia* extract stood out for its beneficial effect on HDL.

For renal biomarkers, the diabetic control group showed elevated levels of urea (0.655 ± 0.035 g/L) and creatinine (7.800 ± 0.156 mg/L), suggesting impaired renal function. *H. scoparia* extract at 500 mg/kg significantly reduced urea (0.19 ± 0.04 g/L) and creatinine (0.93 ± 0.39 mg/L) levels close to normal control (0.54 ± 0.071 g/L for urea and 0.495 ± 0.403 mg/L for creatinine), indicating a protective effect on the kidneys. At 250 mg/kg, the effect was slightly less pronounced (urea: 0.32 ± 0.048 g/L; creatinine: 1.15 ± 0.697 mg/L), but remained significant. Glibenclamide also reduced these biomarkers (urea: 0.470 ± 0.057 g/L; creatinine: 3.615 ± 0.799 mg/L), but the 500 mg/kg extract seems more effective, particularly for creatinine.

In conclusion, *H. scoparia* extract showed beneficial effects on liver enzymes, lipid profile and kidney biomarkers, with particularly marked efficacy at the 500 mg/kg dose. These results suggest its therapeutic potential in the management of diabetic complications, notably to protect the liver and kidneys while improving the lipid profile. However, dose-dependent variations, notably for AST, call for further investigations to optimize dosage and better understand the extract's mechanisms of action.

**Table 8:** Effect of the continuous administration of *H. scoparia* methanolic extract on fasting blood glucose and body weight in alloxan-induced diabetic mice

| | Dose mg/kg | Before induction | Fasting blood glucose (mg/dL) | | | | Body weight (g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Initial day | Week 1 | Week 2 | Week 3 | Initial day | Week 1 | Week 2 | Week 3 |
| **Normal control** | - | 80.50 ± 1.30 | 72.50 ± 1.33 $^{a}$ | 86.00 ± 0.38 $^{a}$ | 79.25± 0.72 $^{b}$ | 79.0 ± 0.17 $^{ab}$ | 29.81 ± 0.95 $^{A}$ | 30.2 ± 0.38 $^{A}$ | 30.84 ± 0.30 $^{A}$ | 31.48 ± 1.72 $^{A}$ |
| **Diabetic control** | - | 80.50 ± 0.80 | 304 ± 0.41 | 373.00 ± 1.13 $^{b}$ | 406.50 ± 1.27 $^{c}$ | 324.25± 0.75 $^{d}$ | 25.05 ± 1.82 $^{A}$ | 24.28 ± 1.67 $^{A}$ | 22.0 ± 3.15 $^{B}$ | 20.03 ± 2.19 $^{B}$ |
| **Glibenclamide** | 10 | 70.00 ± 0.22 | 230.60 ± 1.52 $^{a}$ | 128.00 ± 1.67 $^{b}$ | 72.00 ± 1.37 $^{c}$ | 68.80 ± 1.76 $^{a}$ | 24.85 ± 2.7 $^{A}$ | 25.47 ± 2.57 $^{A}$ | 26.69 ± 2.79 $^{A}$ | 27.26 ± 2.93 $^{A}$ |
| ***H. scoparia extract*** | 250 | 71.20 ± 1.08 | 229.20 ± 0.18 $^{a}$ | 217.00 ± 0.97 $^{b}$ | 145.20 ± 0.92 $^{c}$ | 110.60± 0.21 $^{d}$ | 25.74 ± 3.42 $^{A}$ | 24.00 ± 3.66 $^{A}$ | 22.90 ± 3.40 $^{A}$ | 23.10 ± 3.75 $^{A}$ |
| | 500 | 67.80 ± 1.03 | 282.20 ± 0.74 $^{a}$ | 240.0± 0.83 $^{b}$ | 135.00 ± 0.05 $^{c}$ | 91.60 ± 0.07 $^{d}$ | 27.80 ± 1.51 $^{A}$ | 25.30 ± 1.49 $^{B}$ | 25.50 ± 1.85 $^{AB}$ | 27.90 ± 1.38 $^{A}$ |

Values are means ± standard deviation (SD). Different lowercase letters (a, b, c, d) and uppercase letters (A, B, C, D) within the same row indicate statistically significant differences over time ($p < 0.05$) for blood glucose and body weight, respectively, as determined by two-way ANOVA followed by Tukey's post hoc test.

**Table 9:** Effects of H. scoparia extract on hepatic enzymes (ALT, AST), lipid profile (TC, TG, HDL, LDL), and renal biomarkers (urea, creatinine) in mice.

| | Dose mg/kg | ALT (U/L) | AST (U/L) | TC (g/L) | TG (g/L) | HDL (g/L) | LDL (g/L) | UREA (g/L) | CREA (mg/L) |
|---|---|---|---|---|---|---|---|---|---|
| **Normal control** | - | 41.695±3.429 $^{a}$ | 162.675±58.018 $^{a}$ | 0.795±0.134 $^{a}$ | 0.87±0.057 $^{a}$ | 0.4±0.057 $^{a}$ | 0.06±0.014 $^{a}$ | 0.54±0.071 $^{a}$ | 0.495±0.403 $^{a}$ |
| **Diabetic control** | - | 124.75±9.687 $^{b}$ | 262.66±10.691 $^{b}$ | 3.545±0.191 $^{b}$ | 2.525±0.191 $^{b}$ | 0.165±0.021 $^{b}$ | 0.295±0.021 $^{b}$ | 0.655±0.035 $^{b}$ | 7.8±0.156 $^{b}$ |
| **Glibenclamide** | 10 | 86.075±2.001 $^{a}$ | 120.365±2.878 $^{a}$ | 0.495±0.049 $^{a}$ | 0.520±0.156 $^{a}$ | 0.270± 0.085 $^{a}$ | 0.220± 0.014 $^{a}$ | 0.470±0.057 $^{a}$ | 3.615±0.799 $^{a}$ |
| ***H. scoparia* Extract** | 500 | 62.96±12.544 $^{a}$ | 309.86±31.571 $^{b}$ | 1.12±0.296 $^{a}$ | 1.23±0.256 $^{a}$ | 0.80±0.206 $^{a}$ | 0.12±0.021 $^{a}$ | 0.32±0.048 $^{a}$ | 1.15±0.697 $^{a}$ |
| | 250 | 42.63±5.54 $^{a}$ | 255.75±68.86 $^{c}$ | 1.09±0.24 $^{a}$ | 1.38±0.19 $^{a}$ | 0.76±0.27 $^{a}$ | 0.16± 0.06 $^{a}$ | 0.19±0.04 $^{a}$ | 0.93±0.39 $^{a}$ |

Values are means ± standard deviation (SD) (n = 5). Different lowercase letters (a, b, c) within the same column indicate statistically significant differences between treatments at $p < 0.05$, determined by one-way ANOVA followed by Tukey's post hoc test. ALT: alanine aminotransferase. AST: aspartate aminotransferase. TC: total cholesterol. TG: Triglycerides. HDL: high-density lipoprotein. LDL: low-density lipoprotein. CREA: Creatinine.

*Effect of H. scoparia on malondialdehyde levels in diabetic mice*

Table 10 shows malondialdehyde (MDA) levels in various organs (pancreas, liver, and kidney) in alloxan-induced diabetic mice, comparing normal control, diabetic control, glibenclamide-treated (10 mg/kg), and *H. scoparia* extract-treated (250 mg/kg and 500 mg/kg) groups.

Levels of malondialdehyde (MDA), a marker of oxidative stress, were measured in the pancreas, liver and kidneys of mice. In the diabetic control group, MDA concentrations were significantly higher than in

the normal control group, with respective values of 0.882 ± 0.070 µmol/g in the pancreas, 0.966 ± 0.200 µmol/g in the liver and 0.760 ± 0.100 µmol/g in the kidneys, compared with 0.270 ± 0.010 µmol/g, 0.352 ± 0.010 µmol/g and 0.312 ± 0.030 µmol/g in the normal control group. These results indicate increased oxidative stress in the organs of diabetic mice, probably due to alloxan-induced hyperglycemia, which promotes free radical production.

Treatment with glibenclamide (10 mg/kg) reduced MDA levels in all organs investigated, with values of 0.299 ± 0.150 µmol/g (pancreas), 0.365 ± 0.04 µmol/g (liver) and 0.258 ± 0.030 µmol/g (kidneys). These values were close to those of the normal control group, suggesting that glibenclamide, a reference anti-diabetic drug, effectively attenuated oxidative stress. This reduction may be linked to its hypoglycemic action, which limits the oxidative damage caused by hyperglycemia.

*H. scoparia* extract at 250 mg/kg and 500 mg/kg also showed beneficial effects on reducing MDA levels. At 250 mg/kg, MDA levels were 0.32 ± 0.07 µmol/g (pancreas), 0.21 ± 0.05 µmol/g (liver) and 0.28 ± 0.02 µmol/g (kidneys), while at 500 mg/kg they were 0.25 ± 0.04 µmol/g (pancreas), 0.47 ± 0.10 µmol/g (liver) and 0.26 ± 0.08 µmol/g (kidneys). These results indicated that *H. scoparia* extract reduces oxidative stress in the liver, particularly at the 500 mg/kg dose, where the MDA value was even lower than that of the normal control group. This effectiveness could be attributed to the antioxidant compounds present in the extract, which neutralize free radicals.

The data showed that *H. scoparia* extract, particularly at 500 mg/kg, exhibited antioxidant activity comparable to, and in some cases even superior to that of glibenclamide, particularly in the liver. These results suggest that *H. scoparia* could be a promising natural alternative for alleviating the oxidative stress associated with diabetes. The significant differences ($p < 0.05$) between treatments confirm the efficacy of the interventions, although further studies are needed to elucidate the precise mechanisms and optimize the doses.

**Table 10:** Effect of *H. scoparia* extract on Malondialdehyde (MDA) levels in alloxan Induced diabetic mice.

| | Samples | Normal control | Diabetic control | Glibenclamide | *H. scoparia* extract | |
|---|---|---|---|---|---|---|
| | | | | 10 mg/kg | 500 mg/kg | 250 mg/kg |
| **Malondialdehyde** | Pancreas | 0.270±0.01 [a] | 0.882±0.7 [b] | 0.299±0.15 [c] | 0.25±0.04 [c] | 0.32±0.07 [c] |
| **(MDA)** | Liver | 0.352±0.01 [a] | 0.966±0.20 [b] | 0.365±0.04 [c] | 0.47±0.10 [c] | 0.21±0.05 [d] |
| **unit/g tissue** | Kidney | 0.312±0.03 [a] | 0.760±0.10 [b] | 0.258±0.03 [c] | 0.26±0.08 [c] | 0.28±0.02 [c] |

Values are means ± standard deviation (SD), n = 5. Different lowercase letters (a, b, c) within the same line indicate statistically significant differences between treatments at $p < 0.05$, determined by one-way ANOVA followed by Tukey's post hoc test.

*Effect of H. scoparia on antioxidant enzymes levels in in diabetic mice*

Table 11 shows the effects of *H. scoparia* extract on antioxidant enzymes (SOD, CAT, and GSH) in alloxan-induced diabetic mice.

The activity of SOD, essential for neutralizing superoxide radicals, was significantly reduced in the diabetic control group compared to the normal control in all organs. In the pancreas, for example, it decreased from 662.80 ± 0.60 U/g (normal control) to 367.55 ± 2.50 U/g (diabetic). In the liver and kidneys, similar decreases were observed, with 391.83 ± 0.94 U/g and 135.76 ± 0.93 U/g, respectively in diabetics versus 700.88 ± 1.20 U/g and 595.47 ± 0.30 U/g in normal mice. Treatment with *H. scoparia* extract at 250 mg/kg and 500 mg/kg improved SOD activity, particularly at 500 mg/kg in the liver (640.42 ± 1.32 U/g), approaching normal values. Glibenclamide (10 mg/kg) restored SOD activity to levels close to normal control, particularly in the pancreas (650.11 ± 2.03 U/g) and liver (709.16 ± 0.62 U/g), showing slightly greater efficacy than the extract.

**Table 11:** Effect of *H. scoparia* extract on antioxidant enzymes activities in alloxan-iInduced diabetic mice.

| | Sample | Normal control | Diabetic control | Glibenclamide | *H. scoparia* extract | |
|---|---|---|---|---|---|---|
| | | | | 10 mg/kg | 250 mg/kg | 500 mg/kg |
| **SOD** | Pancreas | 662.80±0.60 [a] | 367.55±2.50 [b] | 650.11±2.03 [a] | 426.41±0.44 [c] | 460.63±0.84 [c] |
| **unit/g tissue** | Liver | 700.88±1.20 [a] | 391.83±0.94 [b] | 709.16±0.62 [a] | 526.12±1.18 [c] | 640.42±1.32 [d] |
| | Kidney | 595.47±0.30 [a] | 135.76±0.93 [b] | 502.21±1.40 [c] | 502.21±1.40 [c] | 465.78±0.75 [c] |
| **CAT** | Pancreas | 0.57±0.10 [a] | 0.16±0.20 [b] | 0.58±0.20 [a] | 0.52±0.13 [a] | 0.41±0.21 [c] |
| **U/mg protein** | Liver | 0.44±0.14 [a] | 0.19±0.27 [b] | 0.43±0.13 [a] | 0.50±0.18 [a] | 0.49±0.13 [a] |
| | Kidney | 0.47±0.20 [a] | 0.23±0.07 [b] | 0.47±0.12 [a] | 0.51±0.07 [a] | 0.40±0.12 [c] |
| **GSH** | Pancreas | 85.33±4.1 [a] | 29.17±0.51 [b] | 75.18±1.54 [a] | 64.13±1.92 [c] | 72.86±1.43 [a] |
| **µmol/min/mg of** | Liver | 87.32±1.02 [a] | 49.28±2.05 [b] | 82.61±0.72 [a] | 67.75±0.51 [c] | 64.89±1.70 [c] |
| **prot** | Kidney | 79.89±2.0 [a] | 30.07±3.07 [b] | 89.49±1.02 [a] | 76.32±1.11 [c] | 75.52±2.46 [c] |

Values are means ± standard deviation (SD), n = 5. SOD: superoxide dismutase. CAT: Catalase. GSH: glutathione. Different letters in the line indicate significant differences among treatments at $p < 0.05$.

The activity of CAT, which breaks down hydrogen peroxide, was also impaired by diabetes. In the diabetic control group, it drops drastically: 0.16 ± 0.20 U/mg in the pancreas versus 0.57 ± 0.10 U/mg for the normal control, 0.19 ± 0.27 U/mg in the liver versus 0.44 ± 0.14 U/mg for the normal control, and 0.23 ± 0.07 U/mg in the kidneys versus 0.47 ± 0.20 U/mg for the normal control. *H. scoparia* extract improved these values, with notable effects at 250 mg/kg in the liver (0.50 ± 0.18 U/mg) and kidneys (0.51 ± 0.07 U/mg), sometimes exceeding normal control. At 500 mg/kg, improvement was less pronounced in the pancreas (0.41 ± 0.21 U/mg). Glibenclamide restored CAT activity to levels close to normal control in all organs, with a value of 0.58 ± 0.20 U/mg in the pancreas.

GSH, a non-enzymatic antioxidant, was severely reduced in the diabetic control group. GSH levels decreased from 85.33 ± 4.10 µmol/min/mg protein (normal control) to 29.17 ± 0.51 µmol/min/mg in the pancreas, from 87.32 ± 1.02 to 49.28 ± 2.05 µmol/min/mg in the liver, and from 79.89 ± 2.0 to 30.07 ± 3.07 µmol/min/mg in the kidneys. *H. scoparia* extract at 250 mg/kg and 500 mg/kg significantly increased GSH levels, reaching 72.86 ± 1.43 µmol/min/mg in the pancreas and 75.52 ± 2.46 µmol/min/mg in the kidneys at 500 mg/kg, values close to normal

control. Glibenclamide was slightly more effective, with GSH levels restored to 89.49 ± 1.02 µmol/min/mg in the kidneys, even exceeding normal control.

These results indicate that alloxan-induced diabetes causes marked oxidative stress, characterized by decreased SOD, CAT and GSH activities in the pancreas, liver and kidneys. *H. scoparia* extract, particularly at 500 mg/kg, attenuated this oxidative stress by enhancing antioxidant activities, although its effects were generally less marked than those of glibenclamide. These observations suggest that *H. scoparia* could be a promising natural alternative for reducing diabetes-related oxidative stress, but further studies are needed to optimize its use and understand its mechanisms of action.

In conclusion, the methanol extract of *Halopteris scoparia* (HSEM) emerged as a promising natural source of bioactive compounds, showcasing antioxidant, enzyme inhibitory, anti-inflammatory, and cytotoxic properties, alongside a reassuring safety profile. However, further investigations into its specific bioactive components, mechanisms of action, and clinical studies are necessary to fully realize its therapeutic potentials across various health domains.

## Conclusion

The multifaceted evaluation of *Halopteris scoparia* methanol extract (HSEM) revealed a robust spectrum of bioactivities, representing a significant advance in natural product-based therapeutics. The findings demonstrate strong antioxidant and antidiabetic properties. The methanolic extract of *H. scoparia* exhibited significant inhibition of α-amylase and α-glucosidase enzymes. Moreover, after three weeks of treatment, administering the methanolic extract at doses of 150 mg/kg and 250 mg/kg to alloxan-induced diabetic mice significantly reduced hyperglycemia, comparable to the effect of glibenclamide. These results indicate that the phenolic compounds in the extract possess notable antioxidant, antihyperglycemic, and antihyperlipidemic activities. Overall, the study confirms the potential of *H. scoparia* as an antidiabetic agent and indicates its promise as an alternative therapy for managing or preventing diabetes and its associated complications.

## Conflict of Interest

The author`s declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the data presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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