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Research Article

Phytochemical Potential of *Heliotropium marifolium* (J. kocnig ex Retz.)- An *in vitro* Antioxidant and Antidiabetic Analysis

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ABSTRACT

This study aimed to assess the antioxidant, anti-inflammatory, and α-amylase inhibitory activities of *H. marifolium* leaf and stem extracts using *in vitro* assays to validate its pharmacological potential. Antioxidant assays including DPPH, ABTS, hydrogen peroxide, superoxide, hydroxyl, and nitric oxide radical scavenging tests were conducted at different extract concentrations (50–300 µg/mL). Anti-inflammatory activity was assessed via protein denaturation inhibition, and α-amylase inhibition was determined using starch–iodine complex assays. Ascorbic acid and acarbose were used as standards. Both extracts exhibited concentration-dependent antioxidant activity, with the leaf extract showing superior scavenging efficiency across all radicals (DPPH, 88.32 ± 1.66%; H_2O_2, 91.29 ± 1.70%) and lower IC_{50} values (130.52–181.27 µg/mL) compared with the stem extract. The anti-inflammatory assay revealed significant inhibition (79.90 ± 1.59%) for the leaf extract at 300 µg/mL, with an IC_{50} of 178.30 µg/mL versus 202.18 µg/mL for the stem. In the α-amylase inhibition assay, the leaf extract (IC_{50} = 187.30 µg/mL) again showed higher potency than the stem (IC_{50} = 209.26 µg/mL), though both were lower than acarbose (IC_{50} = 122.37 µg/mL). The findings demonstrate that *Heliotropium marifolium* leaf extract possesses strong antioxidant, anti-inflammatory, and α-amylase inhibitory properties, supporting its potential as a natural source of bioactive compounds for managing oxidative stress, inflammation, and hyperglycemia.

Keywords: Antidiabetic, *Heliotropium* sp, Antioxidant, Inflammation, DPPH.

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INTRODUCTION

Plants of the genus Heliotropium (family Boraginaceae) have long been exploited in traditional medicine for their diverse therapeutic properties, including anti-inflammatory, wound-healing, antimicrobial, and antioxidant effects. *Heliotropium marifolium* is a member of the Boraginaceae family. *H. bacciferum, H. ovalifolium,* and *H. pterocarpum* are further species of this genus. The genus is biochemically notable for its rich repertoire of secondary metabolites such as phenolic compounds, flavonoids, terpenoids, steroids, tannins, saponins, and pyrrolizidine alkaloids, which often serve as chemotaxonomic markers in the Boraginaceae family.[1,2] These bioactive constituents mediate diverse biological activities, notably free radical scavenging and modulation of inflammatory pathways.[3] *H. Indicum* is used in ethnomedicine for giving protection to human from many diseases from ancient time. In fact, studies on *Heliotropium indicum* have documented potent antioxidant activity.[4] The anti-inflammatory properties are further supported by *in vivo* models,[5] where *H. indicum* methanolic extracts manifested significant reductions in edema and granuloma formation in rats. Although *Heliotropium marifolium* has been comparatively less studied, its genus affiliation and documented presence of bioactive secondary metabolites suggest its strong potential as a natural source of antioxidant[6] and anti-inflammatory agents.

Because direct research is limited, understanding of *H. marifolium* often builds upon the broader phytochemical and medicinal knowledge of the Heliotropium genus. Plants in this genus are well known to contain pyrrolizidine alkaloids as major secondary metabolites, along with terpenoids, flavonoids, phenolic compounds, and other classes of bioactives.[7] These compounds are often responsible for the biological activities reported, but also contribute to toxicity concerns (especially liver toxicity) associated with internal use of Heliotropium species.[8] Its extracts have demonstrated anti-inflammatory effects (e.g.

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in LPS-induced uveitis in animal models) and gastroprotective activities against experimentally induced gastric ulcers.[9] Numerous scientific studies into the medicinal properties of *H. indicum* have been conducted as a result of its widespread use as conventional remedies worldwide. Some of these studies have led to the isolation of some alkaloids of pharmacological significance. Chemical constituents such as indicine, indicine-N-oxide, heliotrine, and other pyrrolizidine alkaloids have been identified from *H. indicum*, along with tannins, saponins, and flavonoids.[10]

MATERIALS AND METHODS

Extraction

The whole plant was shade-dried on clean white sheets. Once dried, the leaves and stems were separated, cut into small pieces, and ground into a coarse powder. Five grams of this powder were then macerated in 100 mL of 99% ethanol (w/v) for ten days, with intermittent stirring at 150 rpm for 12 hr at room temperature, followed by refrigeration for another 12 hr. After the maceration period, the ethanol-soluble extract was filtered, and the filtrate was concentrated under reduced pressure at a low temperature (40°C) using a rotary evaporator

Antioxidant assays

DPPH Radical Scavenging Assay

A 0.1 mM DPPH solution was prepared in methanol.[11] To 1.0 mL of this solution, 1.0 mL of various concentrations of the plant extract (50–300 µg/mL) was added. The reaction mixture was incubated in the dark at room temperature for 30 min, and the absorbance was measured at 517 nm against a methanol blank. Ascorbic acid was used as the standard. The percentage of inhibition was calculated using the formula:

$$\% \text{ Inhibition} = [Ac - As/Ac] \times 100$$

Where Ac is the absorbance of control and As is the absorbance of sample.

ABTS Radical Cation Decolorization Assay

The ABTS radical was generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark for 12–16 hr at room temperature. Before use, the ABTS solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 1.0 mL of ABTS solution was mixed with 100 µL of the extract at different concentrations and incubated for 6 min, and absorbance was recorded at 734 nm. Ascorbic acid was used as standard

Hydrogen Peroxide (H_2O_2) Scavenging Assay

Hydrogen peroxide scavenging activity was determined by using solution of H_2O_2 (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). Plant extract (1 mL) at various concentrations was added to 0.6 mL of H_2O_2 solution. After 10 min of incubation at room temperature, the absorbance was read at 230 nm against a blank solution containing phosphate buffer without H_2O_2. Ascorbic acid was used as a reference compound.

Superoxide Anion Scavenging Assay

The reaction mixture contained 1 mL of nitroblue tetrazolium (NBT, 156 µM), 1 mL of NADH (468 µM), and 1 mL of plant extract at different concentrations. The reaction was initiated by adding 100 µL of phenazine methosulfate (PMS, 60 µM). After 5 min of incubation at 25°C, the absorbance was measured at 560 nm against the corresponding blank. Decreased absorbance indicated increased superoxide scavenging activity.

Hydroxyl Radical Scavenging Assay

The reaction mixture contained 0.45 mL of phosphate buffer (50 mM, pH 7.4), 0.15 mL of 10 mM $FeSO_4$-EDTA, 0.15 mL of 10 mM deoxyribose, 0.15 mL of 10 mM H_2O_2, and 0.525 mL of distilled water. Plant extract (0.15 mL) at various concentrations was added, and the mixture was incubated at 37°C for 1 hr. After incubation, 0.75 mL of 2.8% trichloroacetic acid and 0.75 mL of 1% thiobarbituric acid were added, and the tubes were heated at 90°C for 15 min. The absorbance was measured at 532 nm.

Nitric Oxide (NO) Radical Scavenging Assay

Sodium nitroprusside (10 mM) in phosphate buffer (0.5 mL, pH 7.4) was mixed with 0.5 mL of plant extract at different concentrations and incubated at 25°C for 150 min. After incubation, 0.5 mL of the reaction mixture was added to 0.5 mL of Griess reagent (1% sulfanilamide, 0.1% naphthyl-ethylene-diamine dihydrochloride, and 2% phosphoric acid). The absorbance was read at 540 nm. Ascorbic acid served as standard

Anti-inflammatory assay

200 µL of 1% BSA was added to 800 µL of cold normal saline, and dissolved completely under gentle shaking. 1.3 mL of PBS buffer and 0.2 mL standard (Diclofenac sodium) and sample extract, at different concentration was prepared and mixed with above BSA solution. Only distilled water was combined to make a total volume of 5 mL of the control. After 30 min of incubation at 37±2°C, the reaction tubes were placed in a water bath set at 70±5°C for 15 min. An appropriate UV/vis spectrophotometer was used to determine the absorption at 280 nm following cooling down, using PBS serving as the blank.

$$\% \text{ of inhibition} = \text{OD of Control- OD of Test/ OD of Control x } 100$$

Inhibition of α–amylase by the crude extracts

working samples were prepared by combining 0.2 mL of the plant extracts with 0.1 mL of the 100 U amylase and pre incubated for 15 min. After 100 µL of 1% starch was added and incubated for 5 min, This was then stopped with the addition of 0.5 mL of 0.1

M HCl. the amount of starch hydrolysed from test and blank evaluated by the addition of 200 µL of 1 µM iodine solution. The percentage inhibition was calculated from the OD using the formula below.

OD of control -OD of Test /OD of control x 100

RESULTS AND DISCUSSION

The antioxidant potential of Heliotropium marifolium leaf and stem extracts was evaluated through multiple *in vitro* radical scavenging assays, namely DPPH, ABTS, Hydrogen Peroxide (H_2O_2), superoxide, Hydroxyl (•OH), and Nitric Oxide (NO) scavenging assays (Table 1). Antioxidant activity was found to increase in a concentration-dependent manner for both stem (SE) and Leaf (LE) extracts, indicating the presence of phytoconstituents capable of donating electrons or hydrogen atoms to neutralize reactive free radicals. At 50 µg/mL, the scavenging activities were relatively low, ranging from 12.31 ± 0.38% (DPPH) to 17.60 ± 0.44% (NO) for the stem extract and from 16.52 ± 0.55% (•OH) to 24.65 ± 0.60% (H_2O_2) for the leaf extract. However, at 300 µg/mL, both extracts exhibited significantly enhanced activity, with the leaf extract showing the highest inhibition values across all assays - 88.32 ± 1.66% (DPPH), 91.29 ± 1.70% (H_2O_2), and 85.40 ± 1.53% (NO) - closely approaching that of the standard ascorbic acid (96.09 ± 1.85% NO scavenging at 300 µg/mL).

The DPPH assay reflects the extract's ability to neutralize the stable DPPH radical by hydrogen or electron transfer.[12] The high activity of the leaf extract suggests the presence of potent hydrogen-donating antioxidants such as phenolics and flavonoids, which are well-known in Heliotropium species.[13] Similarly, the ABTS assay, which evaluates both lipophilic and hydrophilic antioxidant capacity, showed strong activity (88.40 ± 1.88%), further confirming the extract's broad radical-neutralizing potential.[14]

Hydrogen peroxide and superoxide scavenging activities also increased markedly with concentration, indicating the capacity of *H. marifolium* phytochemicals to act as reducing agents and metal chelators that prevent the formation of highly reactive hydroxyl radicals via the Fenton reaction.[15] Hydroxyl radical scavenging activity (83.72 ± 1.55% for the leaf extract) demonstrated efficient quenching of the most damaging ROS species, preventing oxidative damage to biomolecules such as DNA, proteins, and lipids.[16] Nitric oxide scavenging activity was also substantial (85.40 ± 1.53% at 300 µg/mL for both extracts), suggesting that *H. marifolium* constituents may modulate NO-related oxidative stress, which contributes to inflammation and tissue damage.[17] The leaf extract consistently outperformed the stem extract across all assays, implying that leaves are richer in antioxidant phytochemicals such as flavonoids, tannins, alkaloids, and phenolic acids. This aligns with prior reports on other Heliotropium species, such as *H. indicum* and *H. curassavicum*, which possess significant antioxidant, anti-inflammatory, and antimicrobial properties attributed to similar classes of compounds.[18,19]

The antioxidant potential of the *Heliotropium marifolium* leaf and stem extracts was quantitatively assessed by determining the IC_{50} values (the concentration required to inhibit 50% of radicals) in various *in vitro* assays, including DPPH, ABTS, hydrogen peroxide, superoxide, hydroxyl, and nitric oxide scavenging (Figures 1 and 2). The leaf extract exhibited notably lower IC_{50} values across all assays-indicating higher radical scavenging efficiency-compared with the stem extract. Among the leaf assays, the IC_{50} values ranged from 130.52 µg/mL (H_2O_2) to 181.27 µg/mL (ABTS). The lowest IC_{50} value in the DPPH assay (153.23 µg/mL) reflects strong hydrogen-donating capacity, whereas moderate values were recorded for nitric oxide (164.42 µg/mL) and hydroxyl radical (172.53 µg/mL) scavenging. In contrast, the stem extract showed relatively higher IC_{50} values, ranging from 147.49 µg/mL (hydroxyl radical) to 209.26 µg/mL (anti-lipid peroxidation), indicating comparatively weaker antioxidant potency. The higher IC_{50} values of the stem extract suggest a lower abundance or activity of free-radical-neutralizing constituents. The difference between the two extracts can be attributed to variation in phytochemical composition. Similar findings have been reported in other Heliotropium species such as *H. indicum* and *H. curassavicum*, where leaf extracts exhibited stronger

Table 1: Free radical scavenging activity of *Heliotropium marifolium*.

Concentration µG	DPPH	ABTS	H_2O_2	Superoxide	OH	Nitric oxide
Stem extract (SE) 50 µg	12.31±0.38	12.51±0.50	14.51±0.55	16.62±0.51	13.24±0.41	17.60±0.44
SE 300 µg	72.37±1.40	80.11±1.52	80.72±1.52	82.73±1.57	78.40±1.37	85.40±1.53
Leaf extract (LE) 50 µg	24.39±0.51	18.62±0.81	24.65±0.60	18.52±0.67	16.52±0.55	17.60±0.44
LE 300 µg	88.32±1.66	88.40±1.88	91.29±1.70	87.50±1.69	83.72±1.55	85.40±1.53
Ascorbic acid 50 µg	30.68±0.89	25.13±0.83	36.52±0.89	28.62±0.80	25.33±0.60	34.41±0.70
Ascorbic acid 300 µg	95.47±1.85	92.70±1.82	96.40±1.75	93.45±1.78	90.19±1.70	96.09±1.85

antioxidant and anti-inflammatory effects than other plant parts due to higher total phenolic and flavonoid contents.[20,21]

The anti-inflammatory potential of *Heliotropium marifolium* leaf and stem extracts was evaluated by measuring their inhibition percentage at different concentrations (50 and 300 µg/mL) in comparison with the standard drug (ascorbic acid or diclofenac sodium). The results (Table 2) indicate a dose-dependent increase in anti-inflammatory activity for both extracts. At 50 µg/mL, the leaf extract exhibited 17.20 ± 0.45% inhibition, while the stem extract showed 13.52 ± 0.33%, both lower than the standard (34.12 ± 0.75%). However, at 300 µg/mL, the leaf extract demonstrated a marked increase (79.90 ± 1.59%), surpassing the stem extract (72.68 ± 1.33%) and showing activity comparable to the standard (97.19 ± 1.77%). The calculated IC_{50} values further confirm this trend: the leaf extract (178.30 µg/mL) displayed stronger inhibitory potential than the stem extract (202.18 µg/mL), indicating higher anti-inflammatory efficacy. The standard exhibited an IC_{50} of 98.01 µg/mL, reflecting its potent reference activity. The higher activity of the leaf extract suggests a greater concentration of these compounds, consistent with earlier reports on *Heliotropium indicum* and *H. curassavicum*, which showed comparable *in vitro* and *in vivo* anti-inflammatory effects.[22] Ozay and Keleş (2024) reported that *H. dolosum* extracts significantly suppressed nitric oxide production and reactive oxygen species in LPS-induced macrophages, validating the genus's anti-inflammatory potential.[23]

The α-amylase inhibitory assay was performed to evaluate the antidiabetic potential of *Heliotropium marifolium* leaf and stem extracts. The results are summarized in Table 3. Both extracts showed a concentration-dependent increase in inhibitory activity, indicating that the bioactive compounds in the extracts interfere with starch hydrolysis and glucose release. At 50 µg/mL, the leaf extract exhibited moderate inhibition (17.35 ± 0.40%) compared with the stem extract (9.86 ± 0.30%) and the standard acarbose

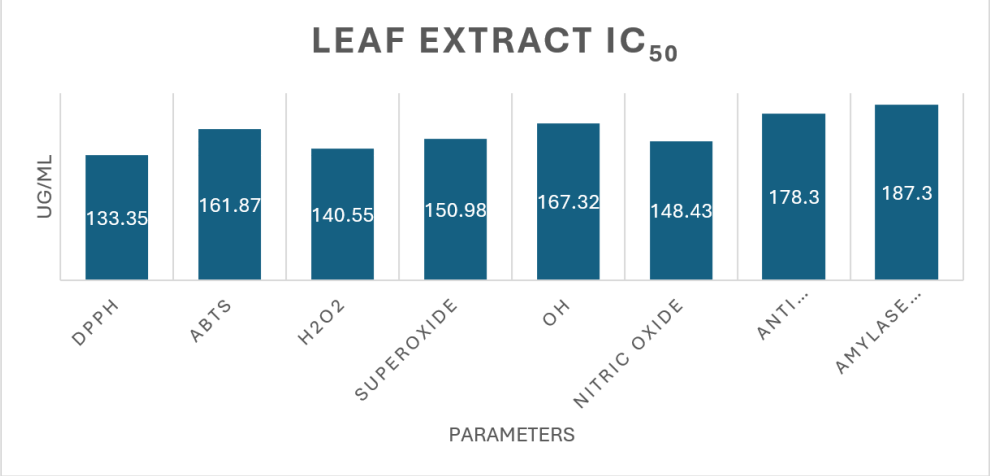

Figure 1: The bar graph presents the IC_{50} values (µg/mL) of various biological and antioxidant assays for the leaf extract of *Heliotropium marifolium*.

Table 2: Percentage of anti-inflammatory activity of *Heliotropium marifolium*.

Concentration µg	Leaf extract	Stem extract	Standard
50	17.20±0.45	13.52±0.33	34.12±0.75
300	79.90±1.59	72.68±1.33	97.19±1.77
IC_{50} Value µg/mL	178.30	202.18	98.01

Table 3: Percentage of amylase activity inhibition by *Heliotropium marifolium*.

Concentration µg	Leaf extract	Stem extract	Standard
50	17.35±0.40	9.86±0.30	25.10±0.65
100	22.73±0.61	15.50±0.45	42.40±0.65
150	41.10±0.93	32.44±0.61	56.06±1.15
200	57.15±1.15	53.18±0.90	78.11±1.15
250	69.80±1.30	60.45±1.27	84.42±1.50
300	76.25±1.15	71.18±1.44	92.27±1.74
IC_{50} µg/mL	187.30	209.26	122.37

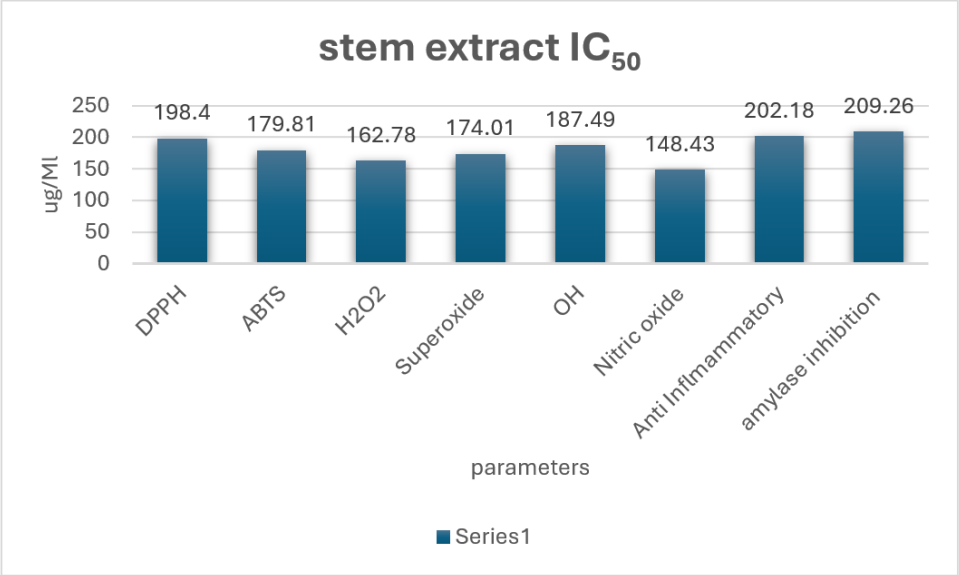

Figure 2: The bar graph presents the IC_{50} values (µg/mL) of various biological and antioxidant assays for the stem extract of *Heliotropium marifolium*.

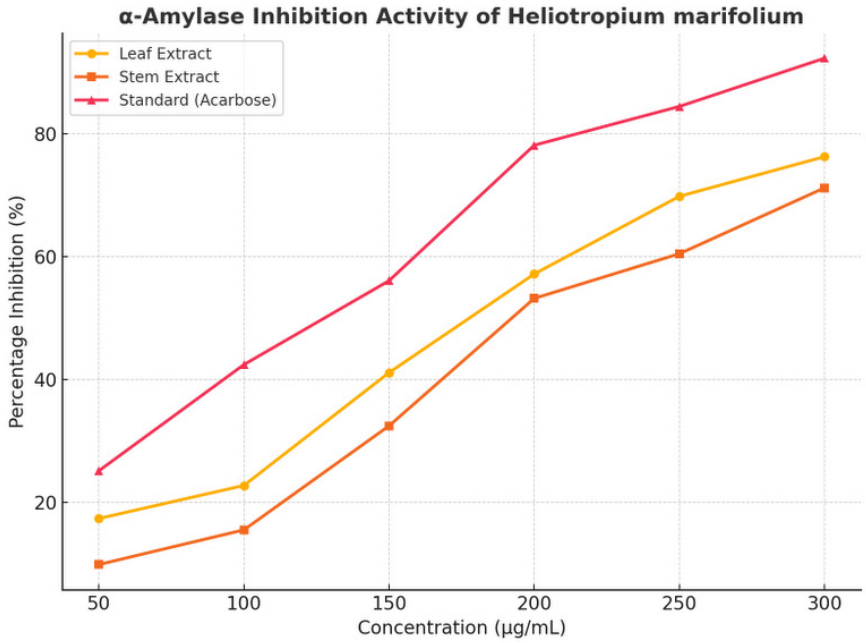

Figure 3: Dose-dependent α-amylase inhibition by *Heliotropium marifolium*.

(25.10 ± 0.65%). As the concentration increased to 300 µg/mL, the leaf extract achieved a substantial inhibition of 76.25 ± 1.15%, while the stem extract reached 71.18 ± 1.44%; both were near the activity of the standard (92.27 ± 1.74%). Comparative IC_{50} (µg/mL) values of leaf and stem extracts of *Heliotropium marifolium* showing dose-dependent α-amylase inhibition relative to standard given in Figure 3. It clearly shows the dose-dependent increase in inhibitory activity for both leaf and stem extracts, with the leaf extract demonstrating higher inhibition than the stem extract across all concentrations, and the standard (acarbose) showing the strongest inhibition. The IC_{50} values (the concentration producing 50% enzyme inhibition) further confirmed this trend, with the leaf extract showing a lower IC_{50} (187.30 µg/mL) compared to the stem extract (209.26 µg/mL), indicating higher α-amylase inhibitory potency of the leaf fraction. The reference drug acarbose demonstrated the strongest inhibition with an IC_{50} of 122.37 µg/mL. α-amylase and α-glucosidas inhibition property of Heliotropium plants previously reported by researchers.[24]

SUMMARY

This study aimed to assess the antioxidant, anti-inflammatory, and alpha-amylase inhibitory activities of *H. marifolium* leaf and stem extracts using *in vitro* assays to validate its pharmacological potential. Antioxidant assays including DPPH, ABTS, hydrogen peroxide, superoxide, hydroxyl, and nitric

oxide radical scavenging tests were conducted at different extract concentrations (50–300 ug/mL). Anti-inflammatory activity was assessed via protein denaturation inhibition, and alpha-amylase inhibition was determined using starch–iodine complex assays. Ascorbic acid and acarbose were used as standards. Both extracts exhibited concentration-dependent antioxidant activity, with the leaf extract showing superior scavenging efficiency across all radicals (DPPH, 88.32 +/- 1.66%; H2O2, 91.29 +/- 1.70%) and lower IC_{50} values (130.52–181.27 ug/mL) compared with the stem extract. The anti-inflammatory assay revealed significant inhibition (79.90 +/- 1.59%) for the leaf extract at 300 ug/mL, with an IC_{50} of 178.30 ug/mL versus 202.18 ug/mL for the stem. In the alpha-amylase inhibition assay, the leaf extract (IC_{50} = 187.30 ug/mL) again showed higher potency than the stem (IC_{50} = 209.26 ug/mL), though both were lower than acarbose (IC_{50} = 122.37 ug/mL). The findings demonstrate that *Heliotropium marifolium* leaf extract possesses strong antioxidant, anti-inflammatory, and alpha-amylase inhibitory properties, supporting its potential as a natural source of bioactive compounds for managing oxidative stress, inflammation, and hyperglycemia.

CONCLUSION

This study demonstrated the *in vitro* biopotential of *Heliotropium marifolium* leaf and stem extracts. Both extracts showed significant, concentration-dependent antioxidant, anti-inflammatory, and alpha-amylase inhibitory activities. Notably, the leaf extract consistently exhibited superior potency compared to the stem extract across all assays. The leaf extract showed strong free radical scavenging, with IC_{50} values ranging from 130.52 ug/mL to 181.27 ug/mL. Furthermore, it displayed more potent anti-inflammatory (IC_{50} 178.30 ug/mL) and alpha-amylase inhibitory (IC_{50} 187.30 ug/mL) effects than the stem extract (IC_{50} 202.18 ug/mL and 209.26 ug/mL, respectively). These findings suggest that *H. marifolium* leaf extract is a promising natural source of bioactive compounds that could be further explored for managing conditions associated with oxidative stress, inflammation, and hyperglycemia.

ABBREVIATIONS

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); **BSA:** Bovine Serum Albumin; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **H_2O_2:** Hydrogen Peroxide; **IC_{50}:** Half-maximal inhibitory concentration; **LE:** Leaf Extract; **NBT:** Nitroblue tetrazolium; **NO:** Nitric Oxide; **ROS:** Reactive Oxygen Species; **SE:** Stem Extract.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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