Research Article

Advances in Applied Biological Research
Year 2025, Volume-2, Issue-1 (January - June)

Study on Biomedical activity of stem and leaf extraction of *Heliotropium marifolium* (J. Kocnig ex Retz.)

S.Nithya[1], E.G.Wesely[2], Helan Soundra Rani Michael[3] and M. Shabana Begum[4]

[1]Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamilnadu, India. nithyabotany25@gmail.com
[2]Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamilnadu, India. [3]Department of Biotechnology, Manonmaniam Sundaranar University, Tirunelveli, TamilNadu, India.helanmichael@gmail.com
[4]Sri Shanmugha Institute of Medical Science and Research, Sankari, Salem.

ARTICLE INFO

Key words: Amylase, Hyperglycemia, Herbal medicine, Inflammation, DPPH

DOI: 10.48165/aabr.2025.2.1.06

ABSTRACT

New anti-diabetic compounds can be obtained from Indian herbal remedies, which are employed in the ancient Ayurvedic approach to manage hyperglycemia. A common medicinal plant, *H. marifolium* is a significant reservoir of compounds with potent antioxidant action. The main objective of the study was to find out the anti-inflammatory activities, antioxidant and antidiabetic effect of selected medicinal plant. Conventional parameters such as DPPH, ABTS, hydroxyl, superoxide radical, nitric oxide, and hydrogenperoxide assay were performed. The chromogenic iodine technique was used to quantitatively determine the activity of extracts in relation to amylase inactivation. At 250µg, the leaves extraction had a much stronger scavenge action (>75%) than the stem extraction (<75%). Both samples exhibited dose-related enhancements in antioxidant activity. When compared to its standard a low scavenging activity at 50µg concentration and comparable scavenging activity at 300µg was recorded among extract. One of the well-established roots of inflammation, protein denaturation, was successfully controlled by the use of the extract. Both extracts were discovered to have a non-steroidal anti-inflammatory activity. Additionally antidiabetic study shows that the standard inhibitor, acarbose, has an least IC50 value of 122.37 µgml^{-1}, following that the leaf and stem extracts (187.30 *µg* ml^{-1} and 209.26 *µg* ml^{-1}, respectively). The data confirms that the primary compounds found in stem and leaf extracts may promote the effectiveness of antidiabetic, anti-inflammatory, and antioxidant nature of the plant.

Introduction

Insufficient insulin function and/or production characterize diabetes mellitus, a kind of chronic metabolic disease. Numerous anti-diabetic medications are presently on the market to treat hyperglycemia, but they come with a number of negative impacts, including gaining weight, liver damage, and lactic acidosis and diarrhea (from metformin). Since historical times, diabetic medications derived from plants have been widely used since they are less expensive and safer

*Corresponding author. E.G.Wesely
*E-mail address:*egwesely@yahoo.com
Received: 10 May 2025 ; Accepted: 05 Jun 2025

than synthetic medications[1].Natural products have a large part in the development of new therapeutic agents because of their immense availability in nature, lead to the identification of bioactive compounds which enable the formation of new pharmacological drugs. Many disorders are treated using complementary or alternative medicines, which are primarily derived from natural ingredients due to the growing interest in using pharmacology in the past few years[2]. Within the Boraginaceae family, *Heliotropium* is one of the broadest and finest genera. "Don't forget me" is the common name for this blooming herb. According to[3], there are 250–300 species of "heliotropes" in the genus *Heliotropium*. The genus Heliotropium may be easily identified from related genera in the category by its highly evolved stigma caps and scorpioid cymes, which set it apart from the other species in the family[4]. According to numerous research, *Heliotropium L.* species contain a variety of secondary compounds, including phenols, terponoids, flavanoids, steroids, tannins, pyrrolizidine alkaloids, and saponins[5]. Pyrrolizidine alkaloids (PAs), the genus Heliotropium's principal ingredient and the chemotaxonomic marker of the plant family Boraginaceae, are primarily responsible for the genus' ecological relevance[6]. H. marifolium, sometimes referred to as "choti santari" in India, is frequently used to treat eye, dental, and ear pain, as well as ulcers, sores, and inflammatory condition[7]. This plant's root preparation is utilized to treat wounds[8].

Materials and method

Extraction

The entire plant was kept in fresh white sheets to dry in the shade. The leaves and stems of the shade-dried plant were separated, broken up into tiny bits, and ground into a coarse powder. After ten days, the 5 grams of coarse powder were macerated in 99% 100 mL ethanol (w/v) and stirred at 150 rpm at room temperature for 12 h and refrigerated for twelve hours. After 10 days maceration, the ethanol soluble fraction was filtered out. With the aid of a rotary evaporator, the filtrate was concentrated under vacuum at a low temperature (40°C).

Antioxidant assays

To determine the antioxidant effects of plant extracts, six different antioxidant assays for the selected plant extracts were performed according to standard procedures with minor modifications. The antioxidant activity of crude extract were determined at 50 to 300µg by using the standard test procedures of DPPH, ABTS, H_2O_2, Superoxide, hydroxyl scavenging and Nitric oxide scavenging. Ascorbic acid used as standard for all test. all the test performed in triplicate manner and the IC50 values are recorded Percentage of DPPH inhibition calculated by using 1-(OD of Test/ OD of Control) x 100 and others were calculated with (OD of Test/ OD of Control) x 100

Anti-inflammatory assay

One milliliter of the egg's white was added to one hundred milliliters of cold distilled water, and dissolved completely under gentle shaking. 2.8 mL of PBS buffer and 0.2 mL of 1% egg albumin solution were combined. Two milliliters of either the standard (Diclofenac sodium) or sample extract, in different quantities, were added. Only distilled water was combined to make a total volume of 5 mL of the control. After 30 minutes of incubation at 37±2°C, the reaction tubes were placed in a water bath set at 70±5°C for 15 minutes. An appropriate UV/Vis spectrophotometer was used to determine the absorption at 280 nm following cooling down, using PBS serving as the blank.

% of inhibition = OD of Control- OD of Test/ OD of Control x 100

Inhibition of α –amylase by the crude extracts

To assess the extract capacity to block the α-amylase enzyme, an iodine-starch test was performed. The process relies on the reaction of iron with the starch acarbose, which was employed as a standard, to generate a blue or violet product. Before being incubated at 37 °C, working samples were prepared by combining 0.2 mL of the plant extracts with 0.4 mL of the 1% starch. After adding 10U of α-amylase, the mixture was allowed to stand for an additional 15 minutes. This was then stopped with the addition of 0.8 mL of 0.1 M HCl. the amount of starch hydrolysed from test and balnk evalated by the adition 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 activity of leaves extract (LE) was given in (Table 1). The results showed that the LE had the highest activity confirmed by low IC50 when compared to the stem extracts (SE). The minimum DPPH scavenging of leave extract was 28.39± 0.51 and reached a maximum percentage 89.72±1.66% at 300µg. The extract had least ABTS 17.62±0.81% and maximum was 89.40±1.88% among 50 and 300µg.similarly the lowest hydrogen peroxide scavenging was 22.65±0.60 and reached maximal of 90.29±1.70 at 300µg. The super oxide scavenging inhibition was ranged between

19.82±0.to 88.40±1.69percent. Nitric oxide scavenging percentage was ranges between 18.60±0.44 to 86.40±1.53. More than 50% radical inhibition was recorded at 150 µg among DPPH, H_2O_2, OH, Nitric oxide scavenging. The IC 50 values of leave extract were 133.35, 61.87, 140.55, 150.98, and 167. 32, 148.43 µG respectively on DPPH, ABTS, H_2O_2, Superoxide, OH and nitric oxide comparatively higher than standard but lower than stem extract (Figure 1). The stem extract activity showed concentration dependent activity and the percentage of antioxidant is given in (Table 2). At 50 µg the percentage of antioxidant among were 14.31±0.38, 14.51±0.50, 17.51±0.55, 15.62±0.51 and 12.44±0.41. The concentration at 300µg it was 75.37±1.40, 81.11±1.52, 85.72±1.52, 81.73±1.57, 79.40±1.37 and 86.40±1.53 percentage respectively among the DPPH > ABTS > H_2O_2 > superoxide > OH > nitric oxide. More than 50% of radical inhibition by stem extract was recorded at 200 µg. Among the test, except nitric oxide all test shown 50 % scavenging at 200µg. more than 70% antioxidant potential was confirmed in ABTS, H_2O_2 and superoxide respectively at 250µg. The calculated IC_{50} values are 198.40, 179.81, 162.78, 174.01, and 187.49, 148.43µg/mL comparatively higher than standard and leaf extract. The data of standard (Table 3) shows again concentration dependent activity in standard ascorbic acid. The maximum DPPH two-tailed P value between leaf and stem equals 0.0003 and equals 0.0093 among LE and standard and this difference is considered to be very statistically significant. P value is less than 0.0001 between SE and standard considered to be extremely statistically significant. The two-tailed P value between LE and SE equals 0.0040 found statistically significant. The p value between LE and standard was significant at 0.0140 and SE and standard was equals 0.0004 found to be extremely statistically significant. Superoxide scavenging P value equals 0.0074, 0.0110 and 0.0007 respectively between SE and LE, LE vs standard and SE vs standard denotes the data is statistically significant. The NO P value equals 0.9994 between LE and SE found to be not statistically significant. P value equals 0.0015 found statistically significant between samples and standard. The H_2O_2 two-tailed P value of between samples equals 0.0256 where as LE and SE towards standard statistically significant with P value of 0.0072 and 0.0009. The hydroxyl scavenging between sample and standard have significant p value 0.0099, 0.0054 and 0.0005 Parameters like DPPH, H_2O_2, Superoxide and nitric oxide test reveals that the standard have more than 50 % antioxidant potential at 150µg with IC50 of 94.70, 121.66, 89.56, 121.23, 128.66 and 82.63µg/mL. Highest activity of antioxidants was shown by acetone was reported by[9]. Previously ethanolic extract exhibited the most potent inhibitory activity on nitric oxide production anti-inflammatory reported by[10]. The highest antioxidant activity (DPPH and ABTS) was detected in *Heliotropium elongatum* reported by[11].

Table 1 Free radical scavenging activity of leaves of *Heliotropium marifolium*

Concentration µG	DPPH	ABTS	H_2O_2	Superoxide	OH	Nitric oxide
50	28.39±0.51	17.62±0.81	22.65±0.60	19.82±0.67	15.62±0.55	18.60±0.44
100	40.15±0.88	30.48±1.02	38.41±0.77	34.66±0.91	28.44±0.64	33.72±0.51
150	55.60±1.12	46.55±1.22	53.12±1.05	50.21±1.22	45.83±0.95	57.22±0.63
200	68.53±1.30	60.38±1.40	70.63±1.44	67.55±1.40	63.27±1.22	69.77±0.90
250	80.59±1.58	78.82±1.62	82.52±1.66	80.31±1.61	75.19±1.30	78.60±1.22
300	89.72±1.66	89.40±1.88	90.29±1.70	88.40±1.69	84.92±1.55	86.40±1.53
IC50 µG	**133.35**	**161.87**	**140.55**	**150.98**	**167.32**	**148.43**

Table 2 Free radical scavenging activity of stem of *Heliotropium marifolium*

Concentration µG	DPPH	ABTS	H2O2	Superoxide	OH	Nitric oxide
50	14.31±0.38	14.51±0.50	17.51±0.55	15.62±0.51	12.44±0.41	18.60±0.44
100	21.75±0.55	25.63±0.74	31.80±0.68	28.13±0.68	23.18±0.55	33.72±0.51
150	39.51±0.70	40.81±0.95	47.62±0.81	41.53±0.87	39.55±0.68	57.22±0.63
200	50.80±0.94	57.22±1.12	61.33±1.03	60.37±1.19	55.61±0.80	69.77±0.90
250	62.92±1.14	72.60±1.33	76.45±1.31	74.22±1.40	68.92±1.04	78.60±1.22
300	75.37±1.40	81.11±1.52	85.72±1.52	81.73±1.57	79.40±1.37	86.40±1.53
IC50 µG	**198.40**	**179.81**	**162.78**	**174.01**	**187.49**	**148.43**

Table 3 Free radical scavenging activity of Standard

Concentration µG	DPPH	ABTS	H_2O_2	Superoxide	OH	**Nitric oxide**
50	33.68±0.89	26.13±0.83	34.52±0.89	28.72±0.80	26.33±0.60	35.41±0.70
100	51.82±1.21	43.62±1.12	50.41±1.15	40.31±1.02	41.12±0.72	56.33±1.15
150	68.13±1.44	60.19±1.30	73.15±1.42	61.92±1.44	59.54±1.12	72.20±1.44
200	81.41±1.60	78.35±1.51	84.69±1.65	77.60±1.53	70.28±1.35	81.62±1.65
250	90.55±1.73	86.41±1.65	90.55±1.69	85.41±1.70	85.92±1.51	90.12±1.80
300	96.47±1.85	95.70±1.82	97.40±1.75	94.75±1.78	92.19±1.70	97.09±1.85
IC50 µG	**94.70**	121.66	89.56	121.23	128.66	82.63

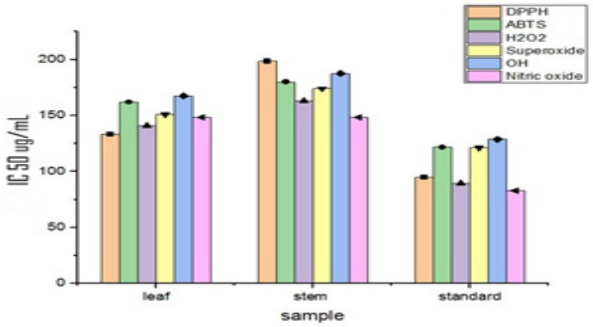

Figure 1. IC_{50} of antioxidants assay of sample and standard

Denaturation of protein has also served as an in vitro pharmacological method to screen anti-inflammatory activity of plant extract using BSA and the data is given in (Table 4). The anti-inflammatory effect of *Heliotropium marifolium* leaf was found to possess significant concentration dependent manner than stem extract. The leaf extract shown 17.20±0.45 to 79.90±1.59% anti-inflammatory activity between 50 to 300µg and 13.52±0.33 to 72.68±1.33 prcentage by stem extract. At similar concentration dichlofenac have had 34.12±0.75 to 97.19±1.77%. Similar *in vitro* anti-inflammatory activity of *Heliotropium indicum* was earlier reported by[12] and in vivo study by[13]. According to[14], the extracts which exhibit anti-denaturation property at a very low concentration (ng/ml) should be selected for further drug development processes. The IC_{50} of leaf extract was greater than standard but lower than stem extract and recorded as 178.30µg where as the stem has comparatively greater value (202.18µg) than leaf and standard (98.01µg). The crude extracts of *Heliotropium marifolium leaves* significantly reduced the amylase activity up to 77.45±1.51 with an ic50 187.30µg followed by standard acarbose (96.77±1.74) ic50 of 122.37µg. the stem extract had 209.26µg of half inhibitory concentration and 72. 28±1.44% maximal enzyme inhibition (Figure 2). Anti-diabetic agent from the crude extract of *H. curassavicum* mediated alpha-amylase inhibition was documented by[15]. At a concentration of 50µg 13.55, 9.88 and 27.12% amylase inhibition was recorded among LE, SE and standard. Both extract showed no significant enzyme inhibition up to 150µg and moderate (50 to 68%) at 200 to 250µg compared to standard. Low inhibitory potential was found at 50µg and high inhibition rate at 300µg was noted (Table 5). Amylase inhibitory activity>50% was noted at 200µg for extract and 150µg for standard.

Table 4 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
100	29.44±0.60	24.38±0.50	50.33±0.98
150	43.58±0.91	37.91±0.66	67.50±1.22
200	58.70±1.25	52.18±0.91	78.22±1.52
250	66.19±1.38	60.41±1.12	90.64±1.73
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 5.percentage of amylase activity inhibition by *Heliotropium marifolium*

Concentration µG	**Leaf extract**	**Stem extract**	**Standard**
50	13.55±0.40	9.88±0.30	27.12±0.69
100	24.63±0.61	17.62±0.45	42.60±0.81
150	40.12±0.93	31.48±0.61	59.08±1.15
200	55.73±1.15	50.13±0.90	78.41±1.39
250	68.80±1.30	63.55±1.27	85.62±1.50
300	77.45±1.51	72.28±1.44	96.77±1.74
IC_{50} µg/ml	**187.30**	**209.26**	**122.37**

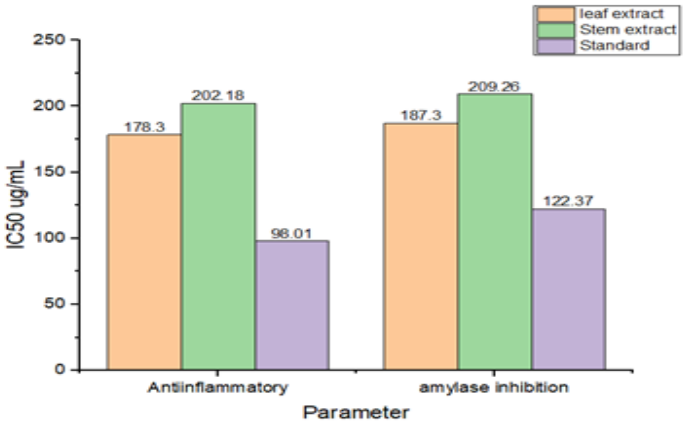

Figure 2. IC50 of anti-inflammatory and amylase inhibition

Conclusion

Stem and leaf ethanol preparations demonstrated potent inhibitory effects on α-amylase, protein denaturation, and free radical inhibition. Denaturing of proteins was used to assess the anti-inflammatory reaction, while DPPH, ABTS, NO, and H2O2 scavenging assays were used to determine the strong antioxidant capacity. At 300 ug, the highest levels of antioxidant, anti-inflammatory, and amylase inhibition were seen. According to the findings of this investigation, *Heliotropium marifolium* exhibited notable anti-diabetic and anti-inflammatory properties.

References

Aboelmagd M, Elokely K, Zaki MA, Said A, Haggag EG, Ros SA (2018). "Anti-inflammatory of pyrrolizidine alkaloids from Heliotropium digynum", Medicinal Chemistry Research. 27: 1066- 1073.

Betanabhatla KS, Jasmin Sajni R, Raamamurthy J, Christina AJ, Sasikumar S (2007). Anti-inflammatory and anyinociceptive activities of *Heliotropium indicum* Linn. In experimental animal models. Pharmacology. 3:438.

Chunthorng-Orn J, Dechayont B, Phuaklee P, Prajuabjinda O, Juckmeta T, Itharat A (2016). Cytotoxic, Anti-inflammatory and Antioxidant Activities of *Heliotropium indicum* extracts. J Med Assoc Thai. 4:S102-9.

Dash KG, Abdullah SM (2013). A Review on Heliotropium Indicum L. (Boraginaceae). International Journal of Pharmaceutical Sciences and Research.; 4(4):1253- 1258.

Kandemir N, *Çelik* A, Shah SN, Razzaq A (2020). Comparative Micro-anatomical Investigation of Genus Heliotropium (Boraginaceae) Found in Turkey. Flora. 262:151495.

Khan H, Khan MA, Gul F, Hussain S, Ashraf N (2013). "In-vivo antipyretic activity of extracts of *Heliotropium strigosum*", Medicinal Chemistry & Drug Discovery. 4(1): 55-61.

Li GQ, Kam A, Wong KH, Zhou X, Omar EA, Alqahtani A, et al (2013). Herbal Medicines for the Management of Diabetes. In: SI Ahmad, editor. *Diabetes: An Old Disease, a New Insight, Advances in Experimental Medicine and Biology*. New York, NY: Springer. p. 396–413.

Parvaze Ahmad Wani, Arowolo Mojisola Tolu, Shazia Wahid (2018). Antioxidant, antimicrobial and antibiotic resistance modifying effect of Heliotropium indicum. Biocatalysis and Agricultural Biotechnology. 15: 113-118.

Paula Sales P, Das Dores Alves de Oliveira M, Figueiredo Watanabe JM, da Silva Barbosa AP, da Mata BCA, de Jesus e Silva Viana M, de Almeida PM (2023). Phytochemical characterization, isolation, antioxidant and cytogenotoxic activity of leaves of Heliotropium elongatum (Lehm) I.M. Johnst. Journal of Toxicology and Environmental Health, Part A. 86(23): 871–897.

Roy A (2015). Pharmacological Activities of Indian Heliotrope (Heliotropium indicum L.: A Review. Journal of Pharmacognosy and Phytochemistry. 4(3):101-104.

Shah SM, Hussain S, Khan AU, Shah AH, Khan H, Barkatullah UF (2012). "Cytotoxic and phytotoxic actions of *Heliotropium strigosum*", Toxicology and Industrial Health.31:1-4.

Srinivas K, Rao ME, Rao SS (2000). Anti-inflammatory activity of *Heliotropium indicum* Linn. And *Leucas aspera Spreng*. In albino rats. Indian Journal of Pharmacology. 32:37-38.

Wasim Akram Syed Akbar, Mary Shamya Arokiarajan J, John Christopher N, Zaheer Ahmed, Rampratap Meena (2023). Evaluation of bioactive compounds as antimicrobial and antidiabetic agent from the crude extract of Heliotropium curassavicum L., Biocatalysis and Agricultural Biotechnology. 50: 102745.

Watanabe T, Rajbhandari K.R, Malla K.J, Yahara S (2005). A Hand Book of Medicinal Plants of Nepal. Volume 15 Kobfai Publishing Project; Bangkok, Thailand.

Williams L A, O'Connar A, Latore L, Dennis O, Ringer S, Whittaker JA (2008). The *In Vitro* Anti-denaturation Effects Induced by Natural Products and Non-steroidal Compounds in Heat Treated (Immunogenic) Bovine Serum Albumin Is Proposed as a Screening Assay for the Detection of Anti-inflammatory Compounds, without the Use of Animals, in the Early Stages of the Drug Discovery Process. West. Indian Med. J. 57 (4): 327–331.