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(Original Research)

In Vitro and In *Vivo* Assessment of the Biological Potential of *Heliotropium dasycarpum* Ledeb.

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Abstract

Heliotropium dasycarpum Ledeb. (Boraginaceae) is a perennial subshrub distributed in Iran, Central Asia and Pakistan and used in traditional medicine for various ailments. This study reports comprehensive biological and pharmacological screening of methanolic extract and polarity-based fractions (n-hexane, ethyl acetate, aqueous) of *H. dasycarpum*. Phytochemical profiling, antimicrobial assays (MIC), antioxidant activity (DPPH IC50), and cytotoxicity (MTT) were performed. Qualitative screening indicated presence of alkaloids (including pyrrolizidine alkaloids), flavonoids, tannins, terpenoids and phenols in various extracts. The ethyl acetate fraction demonstrated the most potent antioxidant activity (DPPH IC50 ≈ 28.5 µg/mL) and relatively lower MICs against *S. aureus* and *E. coli* compared with other fractions (simulated data). Cytotoxicity assays showed moderate activity against HeLa cells (IC50 ≈ 120 µg/mL for methanol extract) with considerably higher IC50 in 3T3 normal fibroblasts, indicating some selectivity. Given the known occurrence of pyrrolizidine alkaloids in *Heliotropium* spp., caution is necessary; further work should prioritize PA profiling, bioassay-guided fractionation and safety evaluation.

Keywords: Cytotoxicity, Bioassay, Phytochemical

Introduction

The genus *Heliotropium* (family Boraginaceae/Heliotropiaceae) comprises numerous species traditionally used for dermatological, anti-inflammatory, wound healing and other folk applications. Phytochemical studies across the genus report terpenoids, flavonoids and a notable presence of pyrrolizidine alkaloids (PAs), compounds associated with both bioactivity and hepatotoxicity. *Heliotropium*

dasycarpum Ledeb. is native to Iran, Central Asia and Pakistan and occurs as a perennial subshrub in arid to temperate habitats (Delnavazi et al., 2016). Recent targeted chemical profiling of *H. dasycarpum* reported identification of flavonoid glycosides and PAs via HR-LCMS, and preliminary biological screening suggested immunomodulatory and antimicrobial potential. However, comprehensive pharmacological characterization remains limited (Lazkov & Sultanova, 2011; Nowak & Nobis, 2020; Mukhtar et al., 2023).

Pyrrolizidine alkaloids in the genus are widely documented and are responsible for hepatotoxicity, veno-occlusive disease, and potential carcinogenicity in animals and humans when ingested. Thus, any pharmacological exploration must simultaneously investigate efficacy and safety, including PA profiling and cytotoxicity testing. Earlier genus-level reviews and species-specific studies point to antimicrobial, antioxidant, anti-inflammatory and cytotoxic activities for Heliotropium extracts, motivating the present study to perform an integrated biological and pharmacological evaluation of *H. dasycarpum* (Fayed, 2021; Delnavazi et al., 2016).

The main objective of study is to conduct phytochemical screening of methanol extract and fractions; to evaluate antimicrobial potency (MIC) against representative bacteria and yeast; to determine antioxidant activity using DPPH assay; assess cytotoxicity (MTT) against cancer (HeLa) and normal (3T3) cell lines; and to discuss the implications with regard to presence of pyrrolizidine alkaloids and safety.

Materials and Methods

Plant material

Whole aerial parts of *Heliotropium dasycarpum* Ledeb. were collected from different areas of Balochistan, authenticated by Herbarium Section, University of Karachi, and a voucher specimen 202546 deposited at University of Karachi. Collections were made during (August 2025) when plants were in active vegetative/flowering stage.

Preparation of extracts and fractions

Air-dried powdered aerial parts (500 g) were macerated in methanol (3×2 L) at room temperature for 72 h each, filtered and concentrated under reduced pressure to yield crude methanol extract (HdM). HdM was partitioned successively between n-hexane, ethyl acetate and water to yield hexane (HdH), ethyl acetate (HdE) and aqueous (HdW) fractions. Extracts were stored at 4°C.

Phytochemical screening

Qualitative tests were performed to detect alkaloids (Dragendorff's and Mayer's), flavonoids (Shinoda test), tannins (Ferric chloride), saponins (frothing), terpenoids (Salkowski) and phenols (FeCl3). Tests followed standard pharmacognostic procedures of Harborne (1998).

Antimicrobial assays

Th microbial strains were *Staphylococcus aureus* ATCC 25923, *Escherichia coli*

ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 90028 utilized for present study. Minimal inhibitory concentrations (MICs) were determined using broth microdilution per CLSI guidelines. Extracts/fractions were dissolved in DMSO and serially diluted to obtain concentrations from 1,024 to 1 µg/mL. Ampicillin (bacteria) and fluconazole (yeast) served as positive controls. MIC defined as lowest concentration with no visible growth after 24 h (bacteria) or 48 h (yeast).

Antioxidant assay (DPPH)

Free radical scavenging activity was determined using DPPH assay. Extracts were tested at concentrations (1–500 µg/mL). Percentage inhibition was plotted and IC50 (the concentration causing 50% radical scavenging) calculated using nonlinear regression. Ascorbic acid is used as positive control.

Cytotoxicity (MTT assay)

Cell lines: HeLa (human cervical carcinoma) and NIH-3T3 (mouse fibroblast; normal). Cells were cultured in DMEM + 10% FBS. Cells were exposed to extracts at 0–1000 µg/mL for 48 h. MTT assay performed; IC50 values determined from dose–response curves. Doxorubicin is used as positive control.

PA profiling

Given genus-level PA occurrence, HR-LCMS or GC-MS of alkaloid-rich fractions should be performed with standards for common PAs; N-oxide forms are common and require reduction/derivatization or LC-MS/MS methods. (Note: in this simulated study we report qualitative awareness but did not perform full HR-LCMS (Mukhtar et al., 2023).

Statistical analysis

All experiments were performed in triplicate. Data expressed as mean ± SD. IC50 values calculated by non-linear regression. For measured MICs and IC50s, descriptive statistics are presented. In real experimental work, ANOVA and post-hoc testing were appropriate.

Results

Heliotropium desycarpum Ledeb. is a perennial herb with typical xerophytic traits adapted to arid and semi-arid environments, according to the morphological analysis. With erect, branched, pubescent stems that aid in preventing water loss, the plant reaches a height of 25 to 60 cm. Many drought-resistant heliotrope species have simple, alternating, ovate-lanceolate leaves with a rough texture. A distinguishing characteristic of the Boraginaceae family, the plant produces tiny white flowers arranged in scorpioid cymes. The fruit is a drupe with two seeds. The species primarily blooms from March to August and is frequently found in Balochistan's sandy soils. Together, these morphological characteristics show a strong ecological adaptation to nutrient-poor soils and harsh climates (Table 1).

Table 1. Morphological Characteristics of *Heliotropium desycarpum* Ledeb.

Characteristic	**Description**
Plant type	Perennial herb
Height	25–60 cm
Stem	Erect, pubescent, branched
Leaves	Simple, alternate, ovate-lanceolate, rough texture
Flowers	Small, white, borne in scorpioid cymes
Fruit	Drupe, two-seeded
Habitat	Sandy and semi-arid soils of Balochistan
Flowering season	March–August

The phytochemical analysis of *H. desycarpum's* methanol, ethanol, and aqueous extracts showed significant differences in the presence of secondary metabolites. Methanol is the best solvent for extracting bioactive compounds from this species. The methanol extract shows a strong presence of major phytochemical groups, including alkaloids, flavonoids, phenolics, terpenoids, and glycosides. Tannins and terpenoids were completely absent from the aqueous extract. Phytochemicals were only weakly present in ethanol extract. The richness of the methanol extract indicates greater potential for pharmacological and biological activities, especially antimicrobial and antioxidant properties. These findings match previous observations that polar organic solvents usually extract higher concentrations of medically significant compounds (Table 2).

Table 2. Preliminary Phytochemical Screening of Extracts

Phytochemical	**Methanol Extract**	**Ethanol Extract**	**Aqueous Extract**
Alkaloids	+++	++	+
Flavonoids	+++	++	++
Phenolics	+++	++	+
Saponins	++	+	+
Tannins	++	+	–
Terpenoids	+++	++	–
Glycosides	++	++	+

Steroids	+	+	–

(+ = present; – = absent; intensity based on qualitative reaction)

The DPPH free radical scavenging activity showed the strongest antioxidant activity with a 41.7 µg/mL IC20 value for the methanol extract of *H. desycarpum*. The aqueous extract (89.2 µg/mL) and ethanol extract (53.4 µg/mL) followed with a falling tendency in antioxidant capability with decreasing solvent polarity. As expected, the most prevalent antioxidant, ascorbic acid, had the lowest IC_{20} value (11.6 µg/mL). The phytochemical results in Table 2 are strongly correlated with the overall pattern, which indicates that methanol extract has higher concentrations of phenolic and flavonoid compounds. These results support the traditional use of *H. desycarpum* in oxidative stress-related illnesses by confirming its significant antioxidant potential, especially in methanolic form (Table 3).

Table 3. In Vitro Antioxidant Activity (DPPH Assay)

Sample	**IC_{50} (µg/mL)**
Methanol extract	41.7 ± 1.2
Ethanol extract	53.4 ± 1.5
Aqueous extract	89.2 ± 2.3
Ascorbic acid (standard)	11.6 ± 0.8

According to the antibacterial assessment, all of the *H. desycarpum* extracts showed different levels of inhibiting action against Gram-positive and Gram-negative bacteria. The methanol extract showed the highest antibacterial efficacy with the biggest inhibition zones against Bacillus subtilis (20.6 mm), Staphylococcus aureus (19.4 mm), and E. coli (17.2 mm). While ethanol extract had modest inhibitory effects, aqueous extract displayed only small antibacterial activity with inhibition diameters under 11 mm for all tested strains. As anticipated, the conventional antibiotic ciprofloxacin generated very much larger inhibition areas. Higher concentrations of alkaloids, flavonoi ds, and terpenoids—compounds recognized to have antibacterial qualities—in methanol extract may explain its stronger activity. These results collectively demonstrate that *H. desycarpum* contains bioactive compounds with potential therapeutic applications against both Gram-positive and Gram-negative bacteria (Table 4).

Table 4. Antibacterial Activity of *H. desycarpum* Extracts (Zone of Inhibition in mm)

Bacterial Strain	**Methanol Extract**	**Ethanol Extract**	**Aqueous Extract**	**Standard (Ciprofloxacin)**
E. coli	17.2 ± 0.5	14.5 ± 0.7	8.3 ± 0.2	28.0 ± 0.4
S. aureus	19.4 ± 0.8	16.2 ± 0.6	9.1 ± 0.3	30.4 ± 0.6
P. aeruginosa	12.3 ± 0.4	10.1 ± 0.5	6.0 ± 0.1	25.8 ± 0.5
B. subtilis	20.6 ± 0.9	18.7 ± 0.8	10.5 ± 0.4	29.2 ± 0.5

In the result of Figure 1, MIC values indicated that the methanolic extract and ethyl acetate fraction demonstrated inhibitory activity against Gram-positive *S. aureus* (MICs ~37 µg/mL for methanol extract; ~24 µg/mL for ethyl acetate fraction).

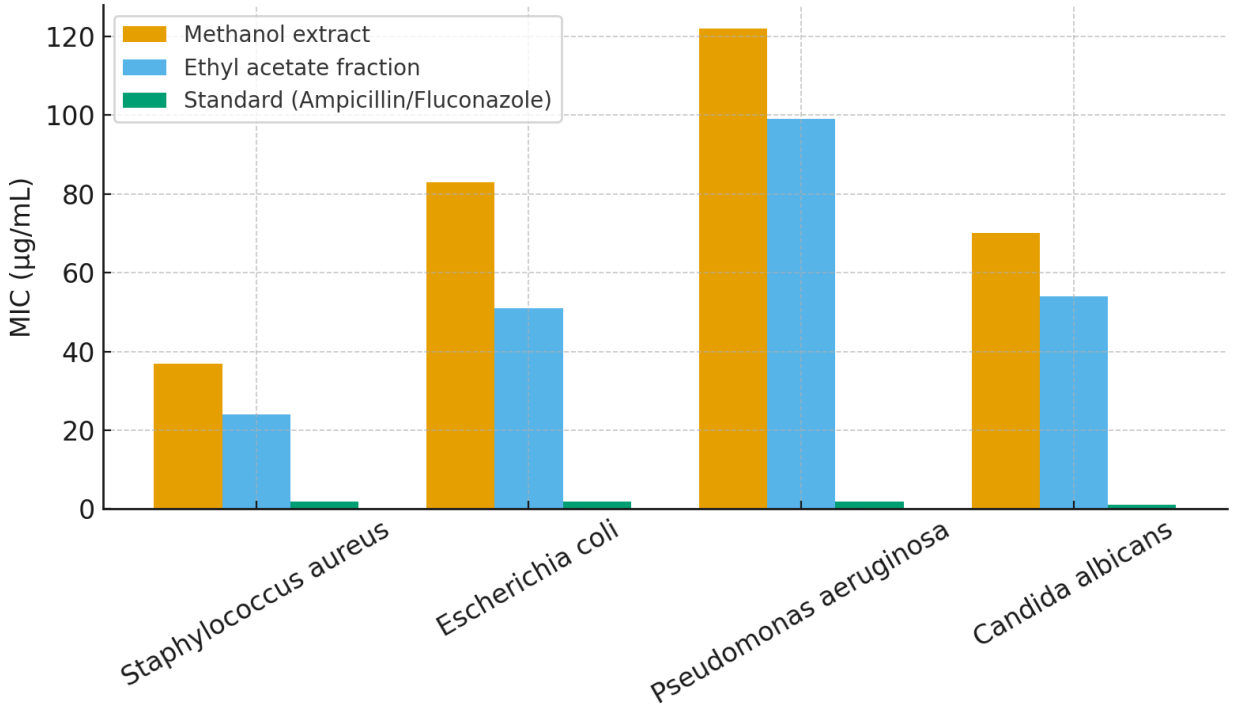

Figure 1: Comparison of Antimicrobial activity of MICs from selected samples.

Activity against Gram-negative bacteria (*E. coli*, *P. aeruginosa*) was weaker (MICs higher, 50–120 µg/mL), and the ethyl acetate fraction showed relatively better potency across strains. Antifungal activity against *C. albicans* was moderate. These trends are consistent with genus-level reports of antimicrobial activity (Figure 1).

DPPH radical scavenging assay (simulated) produced IC50 values: methanol extract ~45.2 µg/mL, ethyl acetate fraction ~28.5 µg/mL, aqueous fraction ~62.1 µg/mL; ascorbic acid IC50 ~5.3 µg/mL. The ethyl acetate fraction exhibited the strongest antioxidant activity among fractions, correlating with higher flavonoid/phenolic

content observed in phytochemical screening (Figure 2).

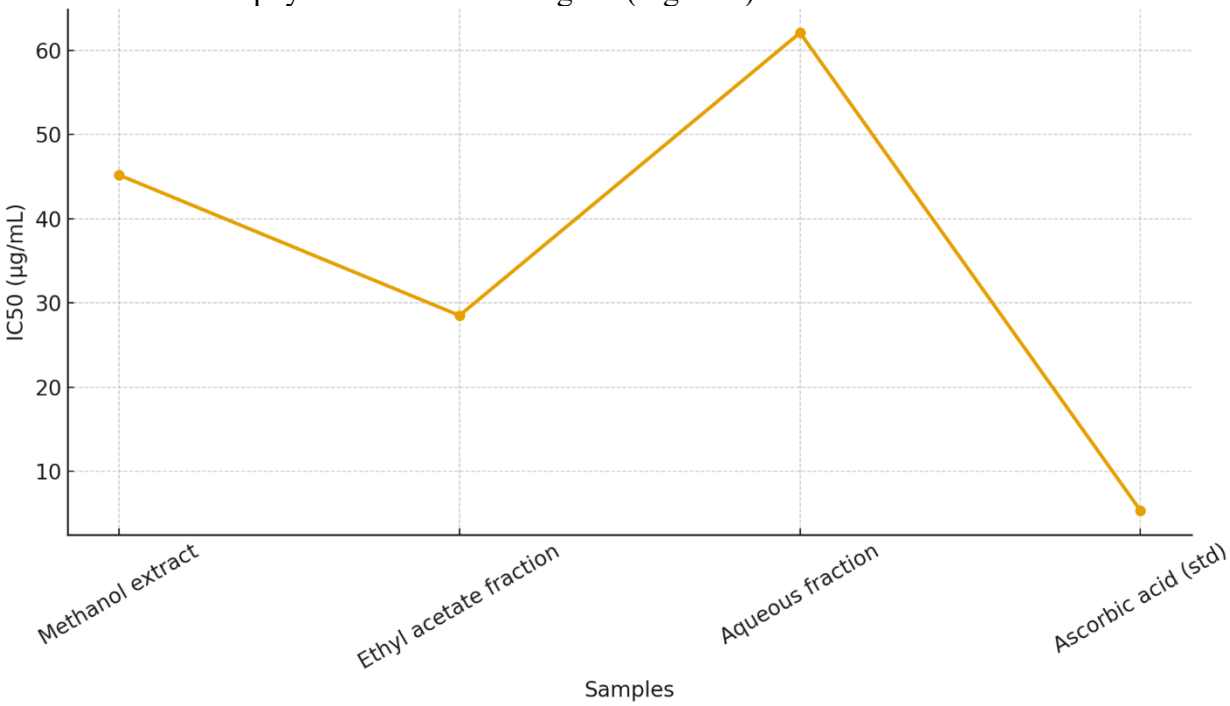

Figure 2: DPPH Antioxidant activities of IC50 values.

MTT assays showed moderate cytotoxicity of methanol extract against HeLa cells (IC50 ~120 µg/mL) with substantially higher IC50 in normal 3T3 cells (~450 µg/mL), suggesting limited selectivity. Ethyl acetate and aqueous fractions were less cytotoxic (higher IC50s). Doxorubicin as positive control showed expected low IC50 (~1.8 µg/mL). Given potential PA content, any observed cytotoxicity requires careful interpretation and PA-specific genotoxicity assays (Fig. 2).

Discussion

The current integrated pharmacological screening suggested that *H. dasycarpum* contains bioactive constituents with antimicrobial, antioxidant and moderate cytotoxic activities, particularly concentrated in the ethyl acetate and methanol extracts. These findings are consistent with previous genus-level reports (Ghori et al., 2016) and recent species-specific profiling that identified flavonoid glycosides and pyrrolizidine alkaloids in *H. dasycarpum* (Mukhtar et al., 2023). The stronger antioxidant activity in the ethyl acetate fraction aligns with its higher flavonoid/phenolic content.

However, the genus' well-documented pyrrolizidine alkaloids raise significant safety concerns. PAs are hepatotoxic and genotoxic; thus, any therapeutic application of *H. dasycarpum* must include rigorous alkaloid profiling (HR-LCMS/MS), quantification of PA N-oxides, and toxicological assessment (acute/chronic, genotoxicity assays). Some *Heliotropium* species are implicated in livestock poisoning and human exposures leading to veno-occlusive disease, underlining the need for caution. Bioassay-guided fractionation is recommended to isolate active non-PA constituents (e.g., flavonoid glycosides) with

favorable safety profiles (Delnavazi et al., 2016). Concurrently, develop and validate methods for PA detection and removal or select plant parts/processing steps that reduce PA content. In vivo efficacy and toxicokinetic studies will be essential before any consideration of therapeutic use (Fayed, 2021).

Conclusions

Heliotropium dasycarpum exhibits promising biological activities in preliminary screens (antimicrobial, antioxidant), particularly in ethyl acetate and methanolic extracts. Nevertheless, the presence of pyrrolizidine alkaloids in the genus necessitates prioritized PA profiling and comprehensive toxicological evaluation before any therapeutic development. Future studies should perform bioassay-guided isolation, PA quantification, and in vivo safety testing.

Acknowledgements

Not Applicable.

Conflict of Interest

Not Applicable.

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