HPTLC-based estimation of plumbagin in the carnivorous plant *Nepenthes khasiana*

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Introduction

- *Nepenthes* is a genus of carnivorous plants.
- *Nepenthes* spp. have unique ways of attracting their preys into leaf-extended biological traps known as pitchers.
- Prey capture and digestion in carnivorous plants are primarily an adaptation to live in low nutrient soils.
- Insects, spiders, ants, termites, snails and other small organisms are attracted or drawn by chance encounters into the biological prey traps.
- Nectar, biochemicals, aroma and pitcher colour are the primary attractants in *Nepenthes* plants.

Objectives

I. Study the distribution of naphthoquinones, plumbagin, droserone and 5-O-methyl droserone, in *N. khasiana.*
ii. Distribution of naphthoquinones on chitin induction in *N. khasiana.*
iii. Role of plumbagin, droserone and 5-O-methyl droserone in prey capture.

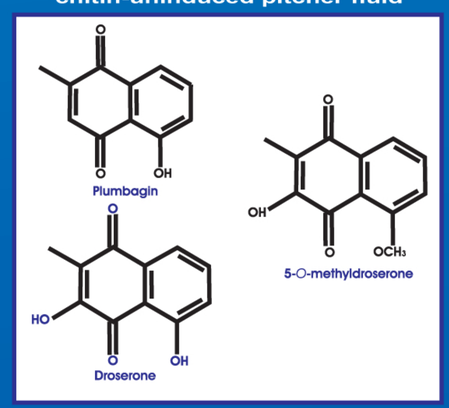
Nepenthes khasiana Hook f. is native to India and is largely endemic to the Khasi Hills in North East India.
Low molecular size metabolites in pitcher secretions, nectars and fragrance emissions in *Nepenthes* and their biological roles in prey capture are least understood.

N. khasina pitchers and semi-digested preys captured inside the pitchers.

Chitin-induced pitcher fluid and chitin-uninduced pitcher fluid

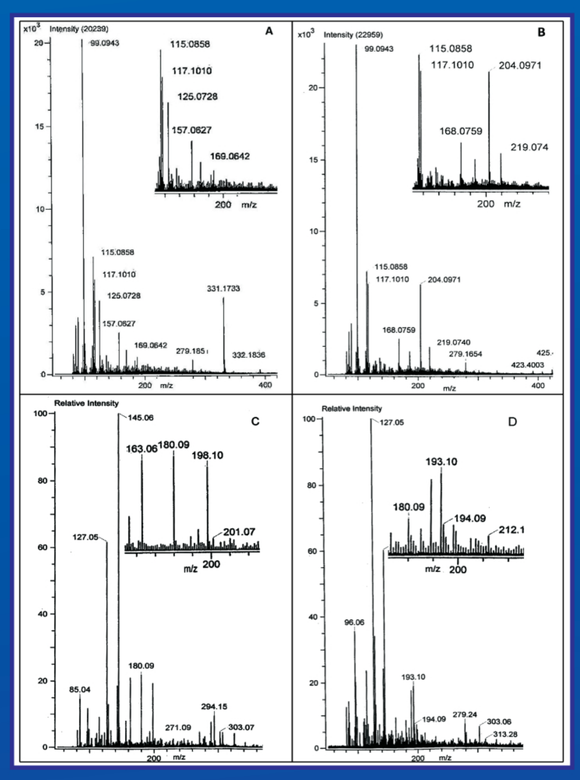
Experimental

- Collection of root, stem, leaves, pitchers and pitcher fluid from field and potted *N. khasiana* plants
- Detection of plumbagin in pitcher washes, GC-EIMS analyses.
- Extraction, HPTLC-based estimation of plumbagin in various plant parts, validation.
- Isolation of plumbagin, spectral identification.
- Chitin induction on pitchers, HPTLC profiling of pitcher tissues, pitcher fluid on chitin induction and prey capture.
- HPTLC-based estimation of plumbagin in root, stem, leaves and pitchers of *N. khasiana* after chitin induction.
- DART-MS, DART-HRMS analyses of pitcher and pitcher fluid on chitin induction.
- Solubility of plumbagin in pitcher fluid, physical parameters and pH measurements of pitcher fluid.

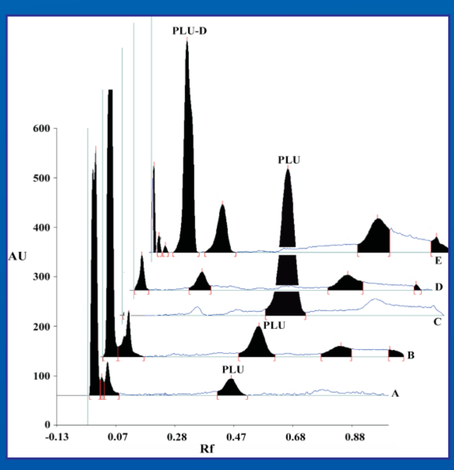


Results

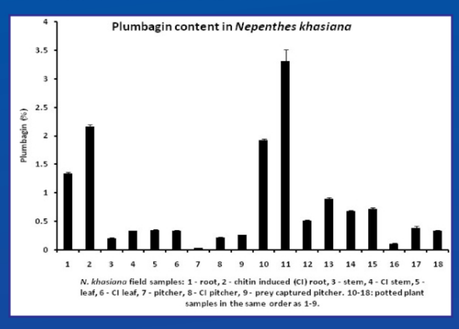
- First detection of the biologically active naphthoquinone, plumbagin, in *N. khasiana.*
- GC-EIMS analysis of the dichloromethane wash of *N. khasiana* pitcher top found 92.2 % (area %) of plumbagin in it.
- Plumbagin was detected and estimated in *N. khasiana* root, stem, leaves and pitchers. On HPTLC analysis, plumbagin peaks in *N. khasiana* methanol extracts were observed at Rf 0.49 in 9.5:0.5 toluene-glacial acetic acid at 265 nm. A linear calibration curve of plumbagin was generated in the range 0.05 to 0.90 µg ($y = 11380 + 3.721$, $R^2 = 0.997$). The HPTLC method was validated in terms of accuracy, precision, repeatability and linearity.
- Plumbagin content in both field and potted *N. khasiana* plant parts were also estimated on chitin-induction and prey-capture.
- Plumbagin content in field and potted *N. khasiana* root samples were 1.33 ± 0.02% and 1.92 ± 0.02% (dr. wt.), respectively. On chitin induction, plumbagin content in the roots of field and potted *N. khasiana* plants were enhanced to 2.17 ± 0.02% and 3.30 ± 0.21% (dr. wt.), respectively.
- Plumbagin contents in *N. khasiana* pitchers of uninduced field and potted plants were as low as 0.03 ± 0.00 % and 0.11 ± 0.00 % (dr. wt.), respectively. On chitin induction, plumbagin contents were enhanced to 0.22 ± 0.00 % and 0.38 ± 0.00 % (dr. wt.) in field and potted plant samples, respectively.
- Chitin induction and prey capture produced droserone and 5-O-methyl droserone in the enzymatic pitcher fluid of *N. khasiana*. The formation of droserone and 5-O-methyl droserone in the pitcher fluid on chitin induction was confirmed by DART-MS, DART-HRMS and HPTLC.



DART-MS profiles of (A) uninduced pitcher fluid, (B) chitin induced pitcher fluid with signals at 204 (droserone) and 219 (5-O-methyl droserone), (C) MeOH extract of uninduced pitcher with a short signal at 189 (plumbagin), (D) MeOH extract of chitin induced pitcher with an intense signal at 189 (plumbagin). The smaller profiles in A-D are expansions of parts of the DART profiles.



HPTLC profiles of (A) MeOH extract of uninduced field pitcher, (B) MeOH extract of chitin induced field pitcher, (C) standard, plumbagin, (D) uninduced field pitcher fluid and (E) chitin induced field pitcher fluid. TLC profiles A-E have similar x- and y-axes scales. PLU in A, B, C: plumbagin; PLU-D in E: plumbagin derivatives, droserone and 5-O-methyl droserone overlapped with a shoulder peak.



HPTLC-based estimation of plumbagin contents in the root, stem, leaf and pitcher samples of uninduced, chitin induced and prey-captured *N. khasiana*. Each % value is an average of data ± standard deviation (n = 4-6).

Conclusions

- HPTLC-based estimation studies proved *N. khasiana* as the highest plumbagin yielding natural source.
- Plumbagin was found throughout the plant (root, stem, leaves and pitcher), except in its pitcher fluid.
- Plumbagin, a known toxin, insect ecdysis inhibitor and antimicrobial, was found embedded in the waxy layers in the top prey capture region of *N. khasiana* pitchers.
- At the pitcher top, plumbagin could be playing a multiple role of an anesthetic to visiting preys, growth inhibitor of trapped visitors and also as an antimicrobia constituent protecting the pitcher tissues from infections.
- Chitin induction, mimicking prey capture, produced droserone and 5-O-methyl droserone in *N. khasiana* pitcher fluid. Both these naphthoquinone derivatives provide antimicrobial protection to the pitcher fluid from visiting preys.
- A two-way barrier was found between plumbagin and its two derivatives. Plumbagin was never detected in the pitcher fluid whereas both its derivatives were only found in the pitcher fluid on chitin induction or prey capture.
- The three naphthoquinones, plumbagin, droserone and 5-O-methyl droserone, act like molecular triggers in prey capture and digestion in the carnivorous plant, *N. khasiana.*

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