Distribution of naphthoquinones, plumbagin, droserone, and 5-O-methyl droserone in chitin-induced and uninduced *Nepenthes khasiana*: molecular events in prey capture

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**Abstract**

Prey capture and digestion in *Nepenthes* spp. through their leaf-evolved biological traps involve a sequence of exciting events. Sugar-rich nectar, aroma chemicals, narcotic alkaloid secretions, slippery wax crystals, and other biochemicals take part in attracting, capturing, and digesting preys in *Nepenthes* pitchers. Here we report the distribution of three potent naphthoquinones in *N. khasiana* and their roles in prey capture. Plumbagin was first detected in *N. khasiana*, and its content (root: $1.33 \pm 0.02\%$, dry wt.) was the highest found in any natural source. Chitin induction enhanced plumbagin levels in *N. khasiana* (root: $2.17 \pm 0.02\%$, dry wt.). Potted *N. khasiana* plants with limited growth of roots and aerial parts, showed higher levels of plumbagin accumulation (root: $1.92 \pm 0.02\%$; root, chitin induction: $3.30 \pm 0.21\%$, dry wt.) compared with field plants. Plumbagin, a known toxin, insect ecdysis inhibitor, and antimicrobial, was also found embedded in the waxy layers at the top prey capture region of *N. khasiana* pitchers. 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 as molecular triggers in prey capture and digestion in the carnivorous plant, *N. khasiana*.

**Key words:** *Nepenthes khasiana*, plumbagin, chitin induction, droserone, 5-O-methyl droserone, molecular prey capture.

**Introduction**

*Nepenthes*, a genus of carnivorous plants, has unique ways of attracting their preys into leaf-extended biological traps known as pitchers. Prey capture and digestion through pitchers in these plants are primarily an adaptation to live in low nutrient soils (Bohn and Federle, 2004; Hatano and Hamada, 2008; Bauer et al., 2011). Insects, spiders, ants, termites, snails, and other small organisms are attracted or drawn by chance encounters into these biological prey traps (Merbach et al., 2002; Ellison and Gotelli, 2009). Pitcher colour, nectar, biochemicals, and aroma are the primary attractants in pitchers (Moran, 1996; Moran et al., 1999; Moran and Clarke, 2010). *Nepenthes* pitcher resembles an elongated jar with an open lid, which is partially filled with fluid that contains proteases, chitinases, and other enzymes in a watery medium (Owen and Lennon, 1999; Eilenberg et al., 2006; Hatano and Hamada, 2008). This enzyme mix effects the digestion of the trapped prey. These enzymes also play a role in inhibiting microbial growth within the pitcher fluid (Owen and Lennon, 1999; Eilenberg et al., 2006; Hatano and Hamada, 2008).

*Nepenthes khasiana* Hook f. (Nepenthaceae) is native to India and is largely endemic to the Khasi Hills in North East
India (Venugopal and Devi, 2003). Recent studies reported the isolation of chinatine genes and the secretion of droserone and 5-O-methyl droserone in N. khasiana pitcher fluid on chitin injection (Eilenberg et al., 2006, 2010). Chitin, a long chain polymer of N-acetylglucosamine, is one of the most abundant macromolecules in nature. It forms the major component of the exoskeletons of insects, arthropods, crustaceans, and a variety of other organisms. Chitin synthesis and its enzymatic degradation are continuous processes in nature. Colloidal chinatinjection into N. khasiana pitcher fluid triggers up chinatine activity. This, in turn, initiates control mechanisms through the formation of low molecular weight GlcNAc oligomers (Kubo et al., 1983; Eilenberg et al., 2010).

Recent studies reported the involvement of narcotic alkaloidal secretions, sugar rich nectar, slippery wax crystals, volatile metabolites, and other biochemicals in various events in prey capture (Moran, 1996; Owen and Lennon, 1999; Riedel et al., 2003; Bohn and Federle, 2004; Bauer et al., 2008; Di Giusto et al., 2010). The composition of the epicuticular wax in Nepenthes pitchers has been reported by various workers recently (Riedel et al., 2003; Gorb et al., 2005). On the other hand, the hydrophilic, low molecular size metabolites in pitcher secretions, nectars, and fragrance emissions in Nepenthes remain mostly uncharacterized. Moreover, various aspects of their biological roles in prey capture are not well understood. This paper reports (i) the distribution of naphthoquinones, plumbagin, droserone, and 5-O-methyl droserone in N. khasiana and (ii) their role in prey capture events. In addition, (iii) the synthesis and distribution of these metabolites on chitin induction in N. khasiana are also probed.

Materials and methods

Plants, collection of plant parts, pitchers

Nepenthes khasiana was introduced in the Tropical Botanic Garden and Research Institute from the Khasi Hills in North East India in 1988. Over the years, it became well established in the Institute garden. For this study, root, stem, leaves, and pitchers of N. khasiana were repeatedly collected from our Institute garden sites (field plant). Plant parts of potted N. khasiana (potted plant) grown in the garden sites were also collected.

Detection of plumbagin in pitcher washes, GC-EIMS analyses

Just opened pitchers were cut above the level of the pitcher fluid and then the fluid in the lower portion of the pitcher was emptied into a vial. The cut pitcher top was repeatedly washed with pentane (3), then the fluid in the lower portion of the pitcher was emptied into a vial. The cut pitcher top was repeatedly washed with pentane (3) at room temperature for 6 h. The dichloromethane soluble fraction was concentrated in a rotary evaporator (Buchi, Switzerland). Preparative TLC of this extract in 1:1 (v:v) petroleum ether–dichloromethane yielded a reddish yellow crystalline solid, which was subjected to 1H-NMR, 13C-NMR, 2D-NMR (JEOL GSX 500 MHz FT-NMR spectrometer, Japan), FT-IR (ABB FTLA 2000 FT-IR spectrometer, Canada), and EIMS analyses (Agilent Technologies, USA). NMR data: 1H NMR (6, CDCl3, 400 MHz): 6.8 (1H, s, H-3), 7.25 (1H, m, H-9), 7.63 (2H, m, H-7 and H-8), 2.19 (3H, s, -CH3), 11.95 (1H, s, -OH); 13C-NMR (6, CDCl3, 125 MHz): 184.75 (C-1), 149.54 (C-2), 136.07 (C-3), 124.14 (C-6), 135.44 (C-7), 119.26 (C-5); mass spectra: electron impact (EI+) mode, 70 eV; ion source temp. 240 °C. Detected compounds were identified by NIST05a.L and Adams database matches (Adams, 2007). Their relative contents were obtained from the area-% charts.

Extraction, HPTLC-based estimation of plumbagin, validation

Detection of plumbagin in the pitcher wash by GC-EIMS prompted us to look into its content in the roots, stem, leaves, and pitchers of N. khasiana. These N. khasiana samples were collected from both field and potted plants. Plant parts were shade dried, powdered in a laboratory grinder, and repeatedly extracted with methanol (4×20, dry wt./vol.). Extracts were concentrated under vacuum on a rotary evaporator (Buchi, Switzerland) and subjected to plumbagin estimation by HPTLC-densitometry (CAMAG, Switzerland). An HPTLC system with Linomat V sample applicator, CAMAG twin-trough plate development chamber, CAMAG TLC Scanner 3, and WinCATS Software 4.03 was used for the estimation of plumbagin in the N. khasiana samples. Known weights of extracts uniformly dissolved in methanol were filtered through a nylon 0.45 μ membrane filter (PALL Gelman Laboratory, India). Each solution (10 μL) was repeatedly applied to silica gel HPTLC plates (60F-254, E. Merck, Germany, 10×10 cm, 0.2 mm thickness) as 6 mm wide bands with the automatic Linomat V sample applicator, fitted with a Camag microsyringe in N2 flow (application rate: 150 nl s−1; space between two bands: 13 mm; slit dimension: 6×0.45 mm; scanning speed: 20 mm s−1). Plumbagin standard (Sigma-Aldrich, India) was also applied as control along with N. khasiana samples.

Plants were developed up to 80 mm, under saturation conditions, with 10 ml of toluene:glacial acetic acid (9.5:0.5, v:v) as the mobile phase in the CAMAG twin-trough glass chamber. This mobile phase composition was chosen after testing different solvent systems of varying polarities. Plumbagin peaks at Rf 0.49 in N. khasiana extracts were well resolved from other peaks in 9.5:0.5 (v:v) toluene:glacial acetic acid (Fig. 1). These plates were scanned densitometrically at 265 nm (deuterium lamp) using a TLC Scanner 3 equipped with WinCATS software.

The HPTLC method was validated in terms of accuracy, precision, repeatability, and linearity. A calibration curve of the amounts of analyte (plumbagin) versus average response (peak area) was developed with a linear relationship (y = 11380+3.721, R2 = 0.997) in the range 0.05–0.90 μg. Specificity of the assay was tested by repeated application of the standard, plumbagin. Rf values of plumbagin were reproducible, and they were same as the values found in N. khasiana extracts. Robustness of the method, limit of detection (LOD), limit of quantification (LOQ), % recovery, and % residual standard deviations (RSD) determined were within the acceptable limits. Plumbagin content in each N. khasiana sample was calculated from its average peak area against its response in the standard curve. Again, plumbagin percentage in each sample was calculated with respect to its dry weight (Fig. 2). Each data point in Fig. 2 is an average of the data obtained for 4–6 samples and the % data distribution is expressed as standard deviation.

Isolation and spectral identification of plumbagin

Dried leaf powder of N. khasiana was repeatedly extracted with dichloromethane (100 ml × 3) at room temperature for 6 h. The dichloromethane soluble fraction was concentrated in a rotary evaporator (Buchi, Switzerland). Preparative TLC of this extract in 1:1 (v:v) petroleum ether–dichloromethane yielded a reddish yellow crystalline solid, which was subjected to 1H-NMR, 13C-NMR, 2D-NMR (JEOL GSX 500 MHz FT-NMR spectrometer, Japan), FT-IR (ABB FTLA 2000 FT-IR spectrometer, Canada), and EIMS analyses (Agilent Technologies, USA). NMR data: 1H NMR (6, CDCl3, 400 MHz): 6.8 (1H, s, H-3), 7.25 (1H, m, H-9), 7.63 (2H, m, H-7 and H-8), 2.19 (3H, s, -CH3), 11.95 (1H, s, -OH); 13C-NMR (6, CDCl3, 125 MHz): 184.75 (C-1), 149.54 (C-2), 136.07 (C-3), 124.14 (C-6), 135.44 (C-7), 119.26 (C-5); mass spectra: electron impact (EL+) mode, 70 eV; ion source temp. 240 °C; scanning speed: 20 mm s−1; space between two bands: 13 mm; slit dimension: 6×0.45 mm; scanning speed: 20 mm s−1. Plumbagin standard (Sigma-Aldrich, India) was also applied as control along with N. khasiana samples. Plates were developed up to 80 mm, under saturation conditions, with 10 ml of toluene:glacial acetic acid (9.5:0.5, v:v) as the mobile phase in the CAMAG twin-trough glass chamber. This mobile phase composition was chosen after testing different solvent systems of varying polarities. Plumbagin peaks at Rf 0.49 in N. khasiana extracts were well resolved from other peaks in 9.5:0.5 (v:v) toluene:glacial acetic acid (Fig. 1). These plates were scanned densitometrically at 265 nm (deuterium lamp) using a TLC Scanner 3 equipped with WinCATS software.

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Chitin induction on pitchers; HPTLC profiling of pitcher tissues, pitcher fluid on chitin induction and prey capture

Colloidal chitin was prepared by boiling 1 g crustacean chitin powder (HiMedia, RM 1356) with distilled water for 1 h, centrifuged, and the solid portion was separated by filtration (Eilenberg et al., 2010). This pellet was stirred in an ethanol–ether–HCl mix (38:38:0.8, v:v:v) for 15 min. This mixture was filtered through a metallic gauze. The solid phase was solubilized in 0.2 M NaClO for 1 h at 75 °C. After filtration, the solid phase was again solubilized by stirring in acetone–32% HCl (1:150, v:v) at 4 °C. After centrifugation, the supernatant was collected and chitin was precipitated by adding ice-cold water and incubating at 4 °C overnight. The acid was removed by subsequent washes with cold water until the pH of the washings reached 2.5. This chitin was allowed to dry on a filter paper and then it (80 mg) was homogenized with 10 ml distilled water and the pH was adjusted to 5.0 with 1 N NaOH. This colloidal chitin was added drop by drop into the fluid at the bottom of a near mature N. khasiana pitcher using a syringe inserted half-way through the pitcher top under sterile conditions. After 5 days, pitcher fluid was collected from the unopened pitcher. On chitin induction (colloidal chitin injection), the colour of the pitcher fluid changed to orange red. This N. khasiana pitcher fluid was lyophilized (DPG 001, India) and subjected to Direct Analysis in Real Time-Mass Spectrometry (DART-MS) with uninduced pitcher fluid (not injected with colloidal chitin) as control (Fig. 3).

HPTLC-based plumbagin estimation was carried out on root, stem, leaves, and pitchers of both field and potted N. khasiana after chitin induction (Fig. 2). Prey-captured pitcher specimens were also subjected to plumbagin estimation. Further, the chemical profiles of methanol extracts of chitin-induced and uninduced pitcher tissues, and the respective pitcher fluids of both field and potted plants, were analysed on HPTLC using plumbagin as standard (Fig. 1, 2).

DART-MS and DART-HRMS analyses of pitcher and pitcher fluid on chitin induction

Methanol extracts of chitin-induced and uninduced pitchers, and the respective pitcher fluids after lyophilization, were subjected to DART-MS profiling on an AccuTOF JMS-T100LC Mass Spectrometer having a DART (JEOL, USA). Samples were analysed directly in front of the DART source. Dry helium was used at a flow rate of 4 LPM for ionization at 350 °C. Orifice 1 was set at 28 V, spectra were collected, and the data from 6–8 scans were averaged. DART-HRMS spectra of signals corresponding to molecular masses 204.0971 and 219.0740 in DART-MS profile (Fig. 3) of chitin-induced pitcher fluid were also recorded.
Solubility of plumbagin in pitcher fluid, physical parameters, and pH measurements of pitcher fluid

Solubility of plumbagin in *N. khasiana* pitcher fluid was tested. Optical rotation (Autopol IV Polarimeter, Rudolph Research Analytical, USA), refractive index (J257 Refractometer, Rudolph Research Analytical, USA), and specific gravity of the pitcher fluid were measured by standard procedures. pH of *N. khasiana* pitcher fluids collected from unopened pitchers and pitchers with captured prey were measured using a calibrated pH meter (Cyberscan Ion 510 Bench Meter, Cole-Parmer, India).

Results and discussion

GC-EIMS profile of the dichloromethane wash of *N. khasiana* pitcher top showed a high content of plumbagin (92.15, area %) in it. The major peak in the gas chromatogram at retention time 28.58 min gave 97% match with 5-hydroxy-2-methyl-1,4-naphthalenedione ([plumbagin, EIMS, m/z]: 188 [M⁺] (100), 173 (26), 160 (20), 131 (36), 120 (21), 92 (20), 63 (14)) in the NIST05a.L database search. This study was the first to detect the biologically active, naphthoquinone plumbagin in *N. khasiana*. Plumbagin, a yellow dye, is considered to be a biological toxin (Kubo et al., 1983). It is also known for insect antifeedant, antitumour, cardiotonic, antimarial, and antimicrobial activities (Sandur et al., 2006; Shin et al., 2007; Aziz et al., 2008; Sreelatha et al., 2009). The pentane wash of the pitcher top showed only waxy compounds on GC profiling, with dioctyl phthalate (76.51, area %) and dibutyl phthalate (8.86, area %) as its major constituents. These results showed that the pentane washing eluted out only the outer waxy layers on the top inside portion of *N. khasiana* pitcher. On subsequent dichloromethane washing plumbagin embedded in the inner layers of the *N. khasiana* pitcher top, was eluted to the solvent along with traces of wax compounds. This observation proved the presence of plumbagin as a component associated with the wax crystals, water, and nectar at the top prey-capturing region of the *N. khasiana* pitcher. Kubo et al. (1983) reported plumbagin as an inhibitor of insect ecdysis. At the pitcher top, plumbagin could be playing a multiple role of an anesthetic to visiting prey, growth inhibitor of trapped visitors, and also as an antimicrobial constituent protecting the pitcher tissues from infections (Kubo et al., 1983; Shin et al., 2007).

Plumbagin detection in the pitcher wash prompted us to look into its content throughout *N. khasiana* including the pitcher fluid. Plumbagin was detected in its root, stem, leaves, and pitchers, but it was not found in *N. khasiana* pitcher fluid at any stage. The content of plumbagin in the roots and aerial parts of *N. khasiana* field and potted plants were estimated by HPTLC-densitometry. Plumbagin content was also estimated in chitin-induced plant parts of both field and potted *N. khasiana* and in prey-captured pitchers (Fig. 1, 2). Plumbagin contents in field and potted *N. khasiana* root samples were 1.33±0.02% and 1.92±0.02% (dry wt.), respectively. On chitin induction, these figures in the roots of field and potted *N. khasiana* plants were enhanced to 2.17±0.02% and 3.30±0.21% (dry wt.), respectively. Again, plumbagin contents in stem samples of both field (0.21 ± 0.00% to 0.33 ± 0.01%, dry wt.) and potted (0.51 ± 0.01% to 0.89 ± 0.02%, dry wt.) plants rose on chitin induction (Fig. 2). But, chitin induction did not result in a noticeable rise in the plumbagin contents in leaf samples of both field and
Fig. 3. 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 important parts of the DART profiles.
potted plants (Fig. 2). Plumbagin content in *N. khasiana* pitchers of uninduced field and potted plants were as low as 
0.03±0.00% and 0.11±0.00% (dry wt.), respectively. On chitin induction, these values were enhanced to 0.22±0.00%
and 0.38±0.00% (dry wt.), respectively, in field and potted samples. On prey capture, plumbagin contents in pitchers 
were 0.26±0.00% and 0.34±0.00% (dry wt.) in field and potted plants, respectively (Fig. 2). Figure 1A, B, D, and E indicating that chitin induction enhanced the metabolic activity in *N. khasiana*, particularly resulting in higher levels of plumbagin. In Fig. 1A and B, it is evident that peaks corresponding to other metabolites in *N. khasiana* tissues also gained prominence on chitin induction. Figure 1D and E showed the same pattern, enhanced peaks of all metabolites in addition to the formation of droserone and 5-O-methyl droserone on chitin injection into the pitcher fluid. Moreover, potted *N. khasiana* plants with restricted growth of root structures and aerial parts, showed high levels of plumbagin accumulation compared with the plants grown in the field (Fig. 2). This plumbagin accumulation is due to physical stress in potted plants, and also triggered by chemical signalling on chitin induction (Bringmann and Feineis, 2001).

Until now, the best known source of plumbagin is the roots of *Plumbago* species, namely, *P. rosea, P. europea,* and *P. zeylanica.* The highest content reported from the roots of wild *Plumbago* spp. is 0.49±0.13% (dry wt.) (Hsieh et al., 2005; Wang and Huang, 2005; Shin et al., 2007). Various tissue culture protocols attempted to enhance the plumbagin content in these plant systems. A recent study assessed the variation of energy resources by *N. khasiana* as an isotropic peristome surface and aquaplaning involving the present study, droserone and 5-O-methyl droserone were detected in chitin-induced pitcher fluids by DART analyses. 
DART-HRMS (ES+): droserone: *m/z* [M+1]⁺, calc. mass for C₁₇H₂₀O₂ 205.0501; found 205.0647; 5-O-methyl droserone: *m/z* [M+1]⁺, calc. mass for C₁₇H₂₁O₂ 219.0657; found 219.0671 (Fig. 3). On chitin induction, plumbagin levels showed an increase in most parts of *N. khasiana* (Fig. 2) but, surprisingly, it was not detected in the pitcher fluid even in traces (Fig. 1, 3). On testing, plumbagin was found to be soluble in the enzymatic pitcher fluid of *N. khasiana.* It appears as if an ‘unknown barrier’ exists for plumbagin between the pitcher surface and the fluid inside. By contrast, based on DART-MS and DART-HRMS data, chitin injection generated droserone and 5-O-methyl droserone only in the pitcher fluid (Fig. 3A, B) and not in the pitcher tissues (Fig. 3C, D). On the HPTLC profiles, plumbagin was found at Rf 0.49 in uninduced and induced pitchers of both field and potted *N. khasiana* plants (Fig. 1A-C). However, plumbagin was not found in the pitcher fluids of uninduced and induced pitchers (Fig. 1D, E). Furthermore, induced pitcher fluids of both field and potted plants showed two additional overlapped signals at Rf 0.07–0.15 (Fig. 1E). These signals correspond to droserone and 5-
O-methyl droserone based on a comparison with the DART-MS and DART-HRMS profiles of pitchers and pitcher fluids in chitin-induced and uninduced conditions (Fig. 1, 3). Thus, both DART-MS and HPTLC-densitometry data confirmed a ‘two-way barrier’ between plumbagin, found only in tissues, and its more polar derivatives, droserone and 5-O-methyl droserone released only into the pitcher fluid on chitin induction. The biosynthesis of these two polar derivatives triggered by chitin injection could be through diversion of the molecular resources in the plumbagin synthesis pathway (Bringmann and Feineis, 2001). This suggests an optimization of energy resources by *N. khasiana* in synthesizing these two antimicrobial plumbagin derivatives in its pitcher fluid.

Specific gravity, refractive index, specific rotation and pH of the pitcher fluid from unopened pitchers were 0.9740,

1.3344, [α]D²⁰ -0.667 (c = 0.97%, H₂O) and 3.62, respectively. Similar values for prey-captured pitcher fluid were 0.9901, [α]D²⁰ 1.3379, [α]D²⁰ -0.606 (c = 0.97%, H₂O) and 2.72, respectively. *N. khasiana* pitcher fluid became more acidic on prey capture and digestion. In the field, a number of ants were found occasionally on the top prey capture region of the open *N. khasiana* pitchers. A variety of insects, spiders, ants and other small organisms were observed by us in the pitcher fluids of open pitchers of *N. khasiana.* These small organisms were trapped by the plant and they were in various stages of digestion. The futile escape efforts of insects and other organisms trapped within *N. khasiana* pitcher fluid were also witnessed by us. As in most other *Nepenthes* spp., no specificity was found in the nature of captured preys in *N. khasiana* pitcher liquid.

The present study leads to a new hypothesis of molecular prey capture in *N. khasiana.* Visiting insects, ants etc. guided by a fatal attraction or a chance encounter into the pitchers of *N. khasiana* are trapped at the pitcher top by a sequence of events. Once the visiting prey ‘lands’ at the pitcher rim, the anisotropic peristome surface and aquaplaning involving the
water, slippery wax, and nectar lead to its first stage ‘trapping’. Subsequently, waxy constituents, plumbagin, and other metabolites in situ exert a toxic or anaesthetic influence on the prey, so that it loses its normal ‘escape’ and ‘defence’ capabilities. Plumbagin is also known for causing ecdysis and developmental abnormalities in insects. Plumbagin found embedded in the waxy layer at the N. khasiana pitcher top and distributed throughout its tissues could also be providing protection against fungal infections from visiting preys and other ecological factors. Once trapped, the ‘visitor’ loses its grip at the pitcher top, slips into the pitcher fluid which, in fact, is an ‘enzymatic-watery-grave’. Chitinases, proteases, and other enzymes in the pitcher fluid initiate the digestion of the ‘prey’. Chitin induction into the pitcher fluid mimics the prey capture scenario in N. khasiana. Sensing the rise in chitin levels, N. khasiana produces higher contents of plumbagin in its tissues. It mostly gets accumulated in its roots. Once chitin levels are high in the fluid, N. khasiana converts a portion of plumbagin into its derivatives drosorone and 5-O-methyl drosorone and releases them into the pitcher fluid. Both these derivatives are potent antifungal metabolites. They keep the pitcher fluid free of possible infections from the ‘prey’ falling into it. Briefly, these three naphthoquinones, plumbagin, drosorone, and 5-O-methyl drosorone, act like molecular triggers at various stages of prey capture in N. khasiana. Plumbagin exerts a toxic influence on the prey at the pitcher top, provides antifungal protection to pitcher tissues and the other two derivatives provide an infection-free environment for the enzymatic digestion of captured preys in the pitcher fluid. Our findings show that the carnivorous plant N. khasiana has evolved an energy-efficient strategy, involving molecular triggers, towards its prey capture.

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