Nepenthes khasiana's chitin-induced pitcher liquid: a potential treatment for opportunistic fungal infection

Jessica Brown • Menlo Park • California 94025 • USA • publications.jrb@gmail.com

Keywords: *Nepenthes khasiana*, fungal infection, antimycotics, napthoquinone, secondary metabolite

The endangered tropical pitcher plant *Nepenthes khasiana* has evolved mechanisms to lure, capture, and digest prey animals (USDA 2003; Eilenberg *et al.* 2010). Native to areas with nutrient-poor soil, consuming insects enables *Nepenthes* to gain additional nourishment (Mithofer 2011). Located at the ends of leaf tendrils, their pitchers' bright colors and nectar secretions are a powerful attractant for bugs (Mithofer 2011). Venturing insects slip on the pitcher's waxy mouth and fall inside, ultimately drowning in the liquid (Rischer *et al.* 2002). Glands located at the base of the pitcher secrete hydrolyzing enzymes (Eilenberg *et al.* 2006). This serves to break the arthropod's body apart so it can be absorbed by the plant as sustenance (Eilenberg *et al.* 2006). Under special circumstances, *Nepenthes* pitcher liquid will also contain potent antifungal compounds called naphthoquinones (Eilenberg *et al.* 2010). Their purpose, it is believed, is to prevent captured prey from being consumed by fungi, a competing organism (Eilenberg *et al.* 2010). For hundreds of years, naphthoquinones have been utilized in Asia and South America for medicinal purposes (Babula *et al.* 2009). In the United States, emerging studies are investigating how these compounds can be produced, as well as their efficacy against opportunistic human fungal pathogens (Eilenberg *et al.* 2010). The importance of this research is underscored by the limited variety of antifungal drugs that are commercially available, as well as their susceptibility to pathogenic resistance and cross-tolerance (Kayser *et al.* 2003).

Naphthoquinones are secondary metabolites produced by *Nepenthes*' pitcher liquid (Rischer *et al.* 2002). Secondary metabolites are not essential for a plant's regular growth, but aid in lesser functions like defense against herbivory (Bio-Medicine 2012). For centuries, many cultures have been utilizing this type of organic compound for medicinal applications (Karuppusamy 2009). Antibiotics, widely used to treat bacterial infections, are secondary metabolites of mold (ePlant-science.com 2009). One major drawback of antibiotic medication is that its use can suppress the immune system by killing microbes which ward off infection (Merck 2008). Women taking antibiotics are at risk for developing candidiasis, which is named after its causal fungal strain *Candida albicans* (NIH 2010). Also known as vaginal yeast infection, candidiasis is responsible for more than 10 million doctor visits in the United States each year (Merck 2008). People with compromised immune systems, like the elderly and those with HIV/AIDs, are at risk for developing a more serious form of candidiasis (Merck 2008). This potentially fatal infection occurs when *C. albican* spores infiltrate the bloodstream (Merck 2008). There is a limited variety of commercial antimycotics (antifungal drugs) that are currently available (Eilenberg *et al.* 2010). Over-usage of these antimycotics, which tend to operate by the same mechanism of action, is creating drug-resistant fungal strains (Kayser *et al.* 2003). Moreover, concomitant use of antifungal drugs is causing cross-resistance in pathogens (Eilenberg *et al.* 2006). Developing antimycotics which function differently than current ones is needed to ensure our continuing ability to treat fungal infections.

Eilenberg *et al.* (2006) demonstrated a method of inducing the production of antifungal secondary metabolites known as naphthoquinones in *Nepenthes khasiana*. Published by the Journal of Experimental Botany, the article entitled "Isolation and characterization of chitinase genes from

pitchers of the carnivorous plant *Nepenthes khasiana*" showed that naphthoquinone production can be induced via colloidal chitin injections into closed *N. khasiana* pitchers. Chitin is the main component of insect exoskeleton, and is also an integral part of fungal cell walls (Collinge *et al.* 1993). Chitinase, an enzyme which degrades chitin, was found to be an implicit component of *N. khasiana* pitcher liquid. Chitinase appears to function in both carnivory, by breaking-down insects, and defense, by degrading fungi. The liquid of closed *N. khasiana* pitchers (referred to as "prey-challenged") did not contain naphthoquinones, but two types of chitinases were present. When immature *Nepenthes* pitchers were injected with chitin, napthiquinones and a third type of chitinase were detected. Chitinase was also found in open pitchers containing prey, but naphthoquinones were not. Though the pitcher liquid does not appear to contain the potent antifungal naphthoquinones under normal circumstances, it may still exhibit antifungal activities due to the endogenous presence of chitinase. This might explain why there is "anthropological evidence of [people] using the liquid from unopened traps as a curing means" (Eilenberg *et al.* 2010).

In 2010, the Journal of Experimental Botany published a compelling article entitled "Induced production of antifungal naphthoquinones in the pitchers of the carnivorous plant *Nepenthes khasiana*". In this study, Eilenberg *et al.* (2010) examined the antifungal effects of naphthoquinones on various strains of human pathogenic fungi. Similar to previous experiments, the team induced naphthoquinone production by injecting closed *Nepenthes khasiana* pitchers with colloidal chitin. The *N. khasiana* pitcher liquid of chitin-induced, prey-challenged, and open pitchers containing prey was then screened for the presence of naphthoquinones. Only the chitin-induced pitcher liquid contained these secondary metabolites. The absence of naphthoquinones from open pitchers containing prey implies that chitin alone is not responsible for its production. However, there is evidence that the components of chitin are needed to form the naphthoquinone compound (Rischer *et al.* 2002). Eilenberg *et al.* postulate that injecting the pitchers with a syringe simulates a predatory presence. As previously discussed, the major natural role of secondary metabolites is to aid in defense (Biomedicine 2012). It is possible that naphthoquinones are synthesized "to avoid the fast consumption of organic compounds by competitors before being absorbed by the pitcher cells" (Eilenberg *et al.* 2010). That is, *Nepenthes* may produce naphthoquinones as a way of preventing insects from decomposing due to fungal activity before the plant is able to digest them.

The Eilenberg *et al.* study in 2010 demonstrated that chitin-induced pitcher liquid exerts antifungal and fungicidal effects on the yeast strain *Candida albicans.* The article cited an *in vitro* experiment in which naphthoquinone derivatives inhibited *C. albicans* as effectively as Diflucan, the leading clinical yeast infection treatment (Tandon *et al.* 2009). Plumbagin is a naphthoquinone derivative that has been isolated and studied extensively due to its proven ability to inhibit and kill human pathogenic fungi (Eilenberg *et al.* 2010). The major problem with plumbagin is its high cytotoxicy and low therapeutic selectivity (Eilenberg *et al.* 2010). Droserone, an oxygenated derivative of plumbagin, exerted an antifungal effect on opportunistic fungal pathogens with a much lower level of toxicity. In addition, droserone demonstrated a different mechanism of action from that of mainstream antimycotics, like Diflucan. The majority of mainstream antimycotics prevent fungal growth by inhibiting fungal enzymes (Kayser *et al.* 2003). Droserone instead catabolizes, or breaks-down, fungal spores. This is advantageous considering the growing prevalence of antimycotic resistance (Kayser *et al.* 2003). In addition to inhibiting *C. albicans*, naphthoquinones demonstrated lethality to *Aspergillu*s, a common household fungus which causes the life-threatening disease aspergillosis (Merck 2008).

Extracting secondary metabolites from plant cell cultures can often be a difficult and inefficient process (Eilenberg *et al.* 2010). When injected with chitin, *Nepenthes* pitchers produce moderate

amounts of medicinal compounds which require no additional purification (Eilenberg *et al.* 2010). In addition to serving as an antifungal agent, naphthoquinones derived from *Nepenthes* pitchers have the potential for a wide array of applications. These compounds are so versatile, they demonstrate antifungal, anti-cancer (Eilenberg *et al.* 2010; Sandur *et al.* 2006), anti-inflammatory (Lien *et al.* 1996), antimalarial (Biot *et al.* 2004), antiviral (Sacau *et al*. 2003), antiallergic (Lien *et al.* 1996), and antibacterial (Tandon *et al.* 2009) activities. Napthoquinones may also serve as a totally "green" insect control because of their ability to inhibit insect ecdysis, or moulting, which is essential for insect survival (Eilenberg *et al.* 2010). Considering all of the potential applications in which *Nepenthes'* chitin-induced pitcher liquid can be used, it is lucky that this tropical plant is able to produce exceptional yields of pure compounds, relatively simply.

References

Babula, P., Adam,V., Havel, L., and Kizek, R. 2009. Noteworthy secondary metabolites naphthoquinones - their occurrence, pharmacological properties and analysis. Curr. Pharm. Anal. 5(1): 47-68.

Bio-Medicine. 2012. Secondary metabolites. http://www.bio-medicine.org/biology-definition/Secondary_metabolite/, accessed 8 March 2013.

Biot, C., Bauer, H., Schirmer, R.H., and Davioud-Charvet, E. 2004. 5-substituted tetrazoles as bioisosteres of carboxylic acids. Bioisosterism and mechanistic studies on glutathione reductase inhibitors as antimalarials. J. Med. Chem. 47(24): 5972-5983.

Collinge, D.B., Kragh, K.M., Mikkelsen, J.D., Nielsen, K.K., Rasmussen, U., and Vad, K. 1993. Plant chitinases. Plant J. 3(1): 31-40.

Eilenberg, H., Pnini-Cohen, S., Rahamim, Y., Sionov, E., Segal, E., Carmeli, S., and Zilberstein, A. 2010. Induced production of antifungal naphthoquinones in the pitchers of the carnivorous plant *Nepenthes khasiana*. J. Exp. Bot. 61(3): 911-922.

Eilenberg, H., Pnini-Cohen, S., Shuster, S., Movtchan, A., and Zilberstein, A. 2006. Isolation and characterization of chitinase genes from pitchers of the carnivorous plant *Nepenthes khasiana*. J. Exp. Bot. 57(11): 2775-2784.

ePlantscience.com. 2009. An Online Botanical Encyclopedia. http://www.eplantscience.com/index_files/biotechnology/Microbial%20biotechnology/Secondary%20Metabolites/biotech_antibiotics.php, accessed 8 March 2013.

Karuppusamy, S. 2009. A review on trends in production of secondary metabolites from higher plants by *in vitro* tissue, organ and cell cultures. J. Med. Pl. Res. 3(13): 1222-1239.

Kayser, O., Kiderlen, A., and Croft, S. 2003. Natural products as antiparasitic drugs. Parasitol. Res. 90 Suppl 2: S55-62.

Lien, J.C., Huang, L.J., Wang, J.P., Teng, C.M., Lee, K.H., and Kuo, S.C. 1996. Synthesis and antiplatelet, antiinflammatory and antiallergic activities of 2,3-disubstituted 1,4-naphthoquinones. Chem. Pharm. Bull. (Tokyo) 44(6): 1181-1187.

Merck. 2008. The Manual Home Health Handbook. http://www.merckmanuals.com/home/skin_disorders/fungal_skin_infections/candidiasis.html
http://www.merckmanuals.com/home/infections/fungal_infections/aspergillosis.html, accessed 8 March 2013.

Mithofer, A. 2011. Carnivorous pitcher plants: insights in an old topic. Phytochem. 72(13): 1678-1682.

NIH. 2010. Medline Plus. http://www.niaid.nih.gov/topics/vaginalyeast/Pages/Default.aspx, accessed 8 March 2013.

Sacau, E.P., Esteves-Braun, A., Ravelo, A.G., Ferro, E.A., Toduka, H., Mukainaka, T., and Nishino, H. 2003. Inhibitory effects of lapachol derivatives on epstein-barr virus activation. Bioorg. Med. Chem. 11(4): 483-488.

Rischer, H., Hamm, A., and Brigmann, G. 2002. *Nepenthes insignis* uses a C_2-portion of the carbon skeleton of L-alanine acquired via its carnivorous organs, to build up the allelochemical plumbagin. Phytochem. 59(6): 603-609.

Sandur, S.K., Ichikawa, H., Sethi, G., Ahn, K.S., and Aggarwal, B.B. 2006. Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) suppresses NF-kappaB activation and NF-kappaB-regulated gene products through modulation of p65 and IkappaBalpha kinase activation, leading to potentiation of apoptosis induced by cytokine and chemotherapeutic agents. J. Biol. Chem. 281(25): 17023-17033.

Tandon, V.K., Maurya, H.K., Tripathi, A., ShivaKeshava, G.B., Shukla, P.K., Srivastava, P., and Panda, D. 2009. 2,3-Disubstituted-1,4-naphthoquinones, 12H-benzo[b]phenothiazine-6,11-diones and related compounds: synthesis and biological evaluation as potential antiproliferative and antifungal agents. Eur. J. Med. Chem. 44(3): 1086-1092.

USDA. 2003. Germplasm Resources Information Network. http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?25155, accessed 8 March 2013.