Introduction

Plants have been engaging in biological warfare against other plants and competing species for millennia. At the same time, humans have used plant materials as medicine and historically, we have relied on tradition to guide their use.[1] In this presentation, we will connect the need for these evolutionary biological weapons with our ability to use them as medicine. Our studies have been able to identify and validate traditional botanical extractions with medicinal properties by using evolutionary data and laboratory proof of their efficacy. In this expanded abstract, we will discuss the distinct evolutionary adaptations that have made plants resilient, useful and even dangerous. We will also extrapolate our methods to expand these botanicals beyond their traditional use, and use their properties to fight viruses, bacteria and even cancer. Additionally, we will discuss future health obstacles that could be circumvented by the development of plant derived medicine.[2] Finally, this paper provides with guidelines to assure the safe implementation of botanicals in our evidence-based world and precautions for their proper applications.

Methods

We used taxonomy data to investigate the evolution of carnivorous plants and traced close relatives that did not develop carnivorous traits within the angiosperm clad in order to compare their antiviral activity in an *in vitro* viral reduction plaque assay.

Botanical Extract Preparation

Dried plant material was obtained from reputable sources with documentation of authenticity. All plant material was subsequently verified by qualified botanical specialists using herbal pharmacopoeia monographs and reference keys. A voucher specimen of all plant material was deposited in a repository. For tincture preparation, the dried botanicals were ground to a fine powder, resuspended in extraction solution and incubated for 3 days at room temperature. The extract was centrifuged at 3000 x g for 10 min to remove cell debris and the extraction solution filtered through a 0.2 micrometer filter. For standardization and comparison, all botanical extracts had an average non-volatile constituent concentration averaging 38.4 mg/ml (ranging from 28.4–42.6 mg/ml). This concentration (mg/ml) is based on the weight of non-volatile constituents present in the extract per ml of aqueous liquid.

Plaque Reduction Assay

HSV1 KOS (a kind gift from David Bloom, Univ. of Florida College of Medicine). Vero cells (ATCC) were maintained with Minimal Essential Media (Cellgro) supplemented with 100 IU penicillin/ml, 100 microgram streptomycin/ml, 2.5 microgram amphotericin B/ml, and 10% heat-inactivated fetal bovine serum (Hyclone). Cells were incubated at 37°C, 5% CO2 in a humidified chamber. Plaque reduction assays were performed by diluting virus stocks and preincubating 100–200 plaque-forming units (pfu) with increasing concentrations of botanical extract for 20 minutes. Monolayers were infected for 1 hour at 37°C followed by incubation in media containing the botanical for 3 days at 37°C. Plaques were visualized by staining with 0.1% crystal violet in 20% ethanol.[3,4]

Results

Our results consistently demonstrate that non-carnivorous plants possess lower evidence of antiviral activity, while carnivorous plants show an effect against the HSV virus. The carnivorous plants consistently have antiviral activity with greater antiviral potency in comparison to the non-carnivorous plants.

Conclusions

The results presented suggest that even though carnivorous plants have evolved multiple and separate times among the angiosperms, they all have developed similar antiviral capabilities.[5] Evolutionarily related non-carnivorous species do not appear to express this antiviral activity suggesting the antiviral capability may be necessary for carnivorous plant sustainability. A model is proposed where specific plants develop adaptations to deal with potential pathogens or pressures present in their environment. This model can be extrapolated to other species of plants, where we can use evolutionary data and traditional data to form a more specific use of each individual plant by exploiting their evolutionary adaptations.[6,7]

This paper does not seek to suggest that botanical medicine is the panacea for future disease processes. It seeks to elucidate that as a solution, botanical medicine should continue to be studied. Always with the knowledge that antivirals and antibacterial should be used judiciously since viruses and bacteria have been shown to be able to develop resistance to both botanical and pharmaceutical medicine.[4] We should also consider that proper extraction and application of natural medicine should be contemplated in order to ensure efficacy of the treatment. [8]

1. Hudson JB. Applications of the phytomedicine Echinacea purpurea (Purple Coneflower) in infectious diseases. *J Biomed Biotechnol*. 2012;2012:769896. doi:10.1155/2012/769896.
2. Patel D, Turner T, Nelson E, Kozin A, Ruiz G, Langland J. TREATMENT OF HERPES VIRUS-ASSOCIATED LESIONS USING A SYNERGISTIC BOTANICAL BLEND. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2016;122(5):e163. doi:10.1016/j.oooo.2016.06.046.
3. Arndt W, Mitnik C, Denzler KL, et al. In vitro characterization of a nineteenth-century therapy for smallpox. *PLoS One*. 2012;7(3):e32610. doi:10.1371/journal.pone.0032610.
4. Ruiz G, Turner T, Nelson E, Sparks L. Bacterial development of resistance to botanical antimicrobials. 2017;2(2).
5. Chen C, Chang T, Farr KM, Fujita T, Hiwatashi Y. genetic changes associated with carnivory. 2017;1(February):1-9. doi:10.1038/s41559-016-0059.
6. Jiang Y-C, Feng H, Lin Y-C, Guo X-R. New strategies against drug resistance to herpes simplex virus. *Int J Oral Sci*. 2016;8(1):1-6. doi:10.1038/ijos.2016.3.
7. Astani A, Navid MH, Schnitzler P. Attachment and penetration of acyclovir-resistant herpes simplex virus are inhibited by Melissa officinalis extract. *Phytother Res*. 2014;28(10):1547-1552. doi:10.1002/ptr.5166.
8. Nelson E, Kozin A, Ruiz G, Turner T, Jo L. Quality Control of Botanical Tinctures : Endogenous Bacterial Flora Present in Botanical Extractions. 2016:1-5.