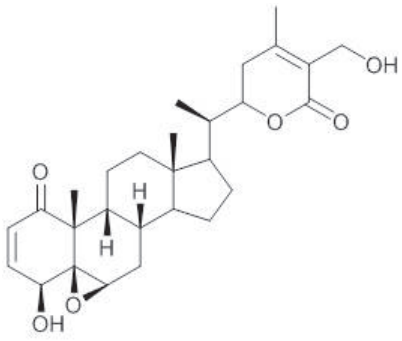
Hot Topics in Pharmacognosy:
What do you do when you need a ready supply of a phytochemical?

By Dr. David Newman

Most people who work in natural products have faced the problem of isolating a small amount of a potent agent in your biological assay of choice and then realizing that you were going to need multi-kilogram quantities of the plant and a process chemistry laboratory, to say nothing of a regular supply of funding for collection, isolation and purification. In today's funding world, or even that of a few years ago, this type of project was rapidly becoming unfundable unless you could prove that you now had the immediate cure for Alzheimer's Disease (sarcasm intended!).



Withanolide A

How did Dr. Leslie Gunatilaka, Professor at the University of Arizona Cancer Center, Tucson, Arizona, solve this problem when he wished to produce some novel withanolides?

Although the story started earlier, in 2006, Gunatilaka et al reported that withaferin A (1) induced the aggregation of actin microfilaments and was mediated by the adapter protein annexin II[1]. The producing plant *Withaferin somnifera* (L.) Dunai has an over 3,000 year history in Ayurvedic medicine as a source of materials to treat a variety of human diseases, including anti-inflammatory, immunomodulation, and antitumor activity, with some relatively recent reviews covering these topics (references 7-9 in Xu et al).[2]

Although the plant was well known and available in the United States of America, the task of obtaining enough to continue work and to investigate the potential of other withanolides led Gunatilaka to investigate other methods of obtaining reproducible quantities of this plant, grown under reproducible conditions. All readers are well aware of the variation in secondary metabolites in wild collections.

The antitumor activity of withaferin A was first reported by Kupchan in 1965 (references 12 in Xu et al)[2] with significant in vivo activity reported by Shohat et al in 1967 (ref 13 in Xu et al)[2]. In order to generate "active biomass" rather than attempt to fund large scale collections and work-up, the Gunatilaka group utilized an aeroponic process to produce withanolide-containing biomass under reproducible conditions, Xu et al[2]. Two years later, the group reported on other previously unknown withanolides[3] using this technique, which was first pioneered for an entirely different reason by Hubick et al in 1982.[4]

In the images on the following page, which were provided to the author by Dr. Gunatilaka, the results are shown together with the differences in growth that can be seen when the same plant is grown under regular condifions versus the aeroponic method. In addition, the second image shows the production of another steroidal-type secondary metabolite from *Physalis crassifolia* that is not available commercially. The success of this process for generation of novel agents under conditions evading the "usual" natural products chemist can be seen in two further publications from the Gunatilaka group, the first in 2012 by Santgata et al[5] and the second by Wijeranatne[6] in 2014, showing the different withanolides isolatable from this process.

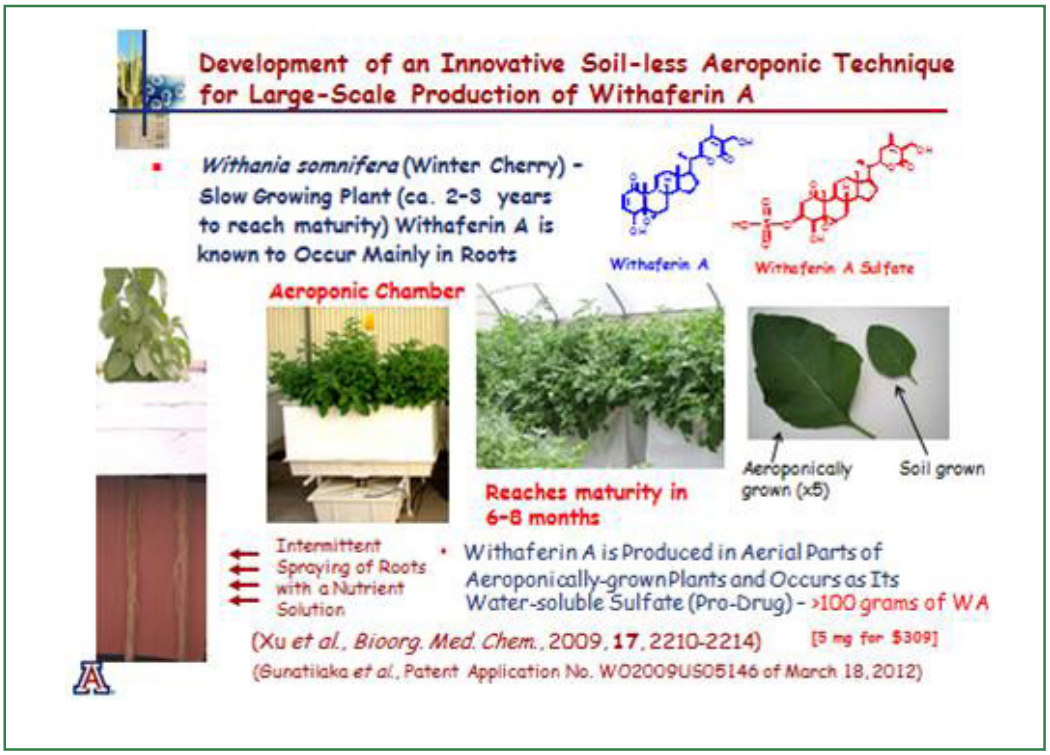
Adding to these published works is a patent for the process and the activity, applied for in 2010. This shows the processes used in greater detail, and the potential for this method of production of plant secondary metabolites is clear. ■

continued on page 19

REFERENCES

1. Falsey, R. R., Marron, M. T., Gunaherath, G. M. K. B., Shirahatti, N., Mahadevan, D., Gunatilaka, A. A. L. and Whitesell, L. Actin microfilament aggregation induced by withaferin A is mediated by annexin II. *Nat Chem Biol.,* **2006,** 2, 33-38
2. Xu, Y-M, Marron, M. T., Seddon, E., McLaughlin, S. P., Ray, D. T., Whitesell, L. and Gunatilaka, A. A. L., 2-3-Dihydrowithaferin A-3β-O-sulfate, a new potential prodrug of withaferin A from aeroponically grown *Withania somnifera. Bioorg Med Chem.,* **2009,** 17, 2210-2214
3. Xu, Y-M, Gao, S., Bunting, D. P. and Gunatilaka, A. A. L., Unusual withanolides from aeroponically grown *Withania somnifera, Phytochem.*, **2011,** 72, 518-522
4. Hubick, K. T/, Drakeford, D. R. and Reid, D. M., A comparison of two techniques for growing minimally water-stressed plants. *Can J Bot.,* **1982,** 60, 219-223
5. Santagata, S., Xu, Y-M., Wijeratne, E. M. K., Kontnik, R., Rooney, C., Perley. C. C., Kwon, H., Clady, J., Kesari, S., Whitesell, L., Lindquist, S. and Gunatilaka, A. A. L. Using the heat shock response to discover anticancer compounds that target protein homeostasis. *ACS Chem Biol.,* **2012,** 7, 340-349
6. Wijeranatne, E.M. K., Xu, Y-M., Scherz-Shouval, R., Marron, M. T., Rocha, D. D., Liu, M. X., Costa-Lotufo, L. V;, Santagata, S., Lindquist, S., Whitesell, L. and Gunatilaka, A. A. L., Structure-activity relationships for withanolides as inducers of the cellular heat-shock response. *J Med Chem.,* **2014,** 57, 2851-2863.
7. Gunatilaka, A. A. L., Wijeratne, E. M. K., Xu, Y-M., Whitesell, L. J. and Lindquiat, S. L., withaferin a analogs and uses thereof, WO 2010/030395 A2, 15SEP2009.

continued from page 18



...the differences in growth that can be seen when the same plant is grown under regular condifions versus the aeroponic method.