



Scutellaria as a medicinal crop: Cryopreservation, hairy root culture, organic farming and anticancer activity

JOSHEE, Nirmal (PD)

Agricultural Research Station, Fort Valley State University, Fort Valley, Georgia 31030

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Objectives:

Objective 1: Germplasm collection, micropropagation and conservation: Expand genetic base by adding new species, refine and scale up plant micropropagation protocols for Liquid Lab Rockers, develop protocols for conservation of threatened *Scutellaria montana* and *S. ocmulgee*, and study white flowered morph of *S. ocmulgee* initiating scientific documentation.

Objective 2: Devising agritechology for organically growing skullcaps as medicinal crops: Organic production of *Scutellaria lateriflora* and *S. baicalensis*.

Objective 3: Developing hairy root cultures for selecting flavonoid over expressing clones: Genetic transformation of four *Scutellaria* spp. using *A. rhizogenes* and selection of higher flavonoids / diterpene producing clones. Elicitation as a tool to maximize biosynthesis and flavonoids profile.

Objective 4: Phytochemical screening of bioactive flavonoids: Analysis will be carried out to screen flavonoids and diterpenes in the plants grown in LLR, organically grown plants and hairy root leachates to understand biosynthesis in relation to culture system and parameters used.

Objective 5: Anti-tumor activity of select Scutellaria extracts either individually or in combination with Temodar: To investigate therapeutic efficacy of select *Scutellaria* extracts through laboratory as well as animal experiments in order to demonstrate the supplementary influence of the skullcap flavonoids on chemotherapy of malignant human tumors (in vitro as well as in rat xenograft models of human gliomas).

Team Members:

Anand K Yadav Agricultural Research Station, Fort Valley State University, Fort Valley, GA

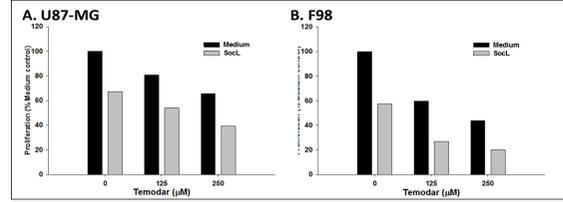
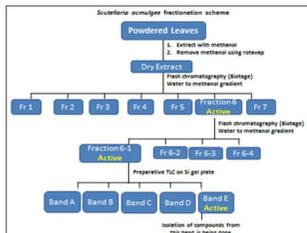
Prahlad Parajuli Department of Neurosurgery, Wayne State University & Karmanos Cancer Institute, Detroit, MI

Fabricio Medina-Bolivar Department of Biological Sciences and Arkansas Biosciences Institute, Arkansas State University, AR

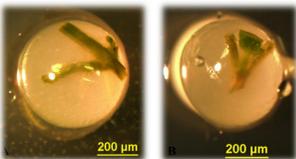
USDA Cooperators: a). Agnes M Rimando USDA-ARS, Natural Products Utilization Research Unit, University, MS; and **b). David D Ellis** National Ctr. for Genetic Resources Preservation, Fort Collins, CO

Proposed research is a **high priority area** being related to **human health (I.C.2.a.1)** and addresses **need area pharmaceutical qualities of medicinal plants (I.3.b.i).**

CSREES Award # 2008-38814-04737
2008 – 2011



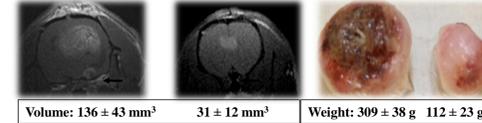
Positive interaction between temodar and SocL extract on inhibition of tumor proliferation in vitro. Human (U87-MG) and rat (F98) glioma cells were cultured with SocL extract (100 mg/ml) in the presence of indicated doses of Temodar. After 72 hours, cell proliferation was determined by MTT assay. Temodar inhibited the proliferation of both tumor cells in a dose-dependent manner. Proliferation was further inhibited when cells were treated with Temodar in the presence of SocL extract, indicating a positive interaction (additive effect) between temodar and SocL extract.



Sodium alginate beads for conservation studies



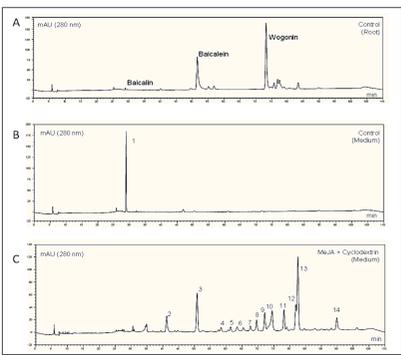
USDA-ARS
Natural Products Utilization Res Unit
Agnes M Rimando



Volume: 136 ± 43 mm³ 31 ± 12 mm³ Weight: 309 ± 38 g 112 ± 23 g



Scutellaria drying facility constructed at Auburn University



HPLC analysis of hairy root cultures of *Scutellaria lateriflora* line 4. Compounds were extracted with ethyl acetate from the tissue and media. A; Root tissue (control). B; Medium (control) and C; Medium (100 mM MeJA + 7.5 mM cyclodextrin). At least 13 compounds (peaks 2-14, panel C) were induced after 24 hours of treatment with MeJA and cyclodextrin.



USDA
National Ctr. for Genetic Resources Preservation
Dave D Ellis

Fort Valley State University
(Lead Institution)
Nirmal Joshee
Anand K. Yadav



Wayne State University
P. Parajuli



Effect of shade



Harvesting roots for phytochemical analysis



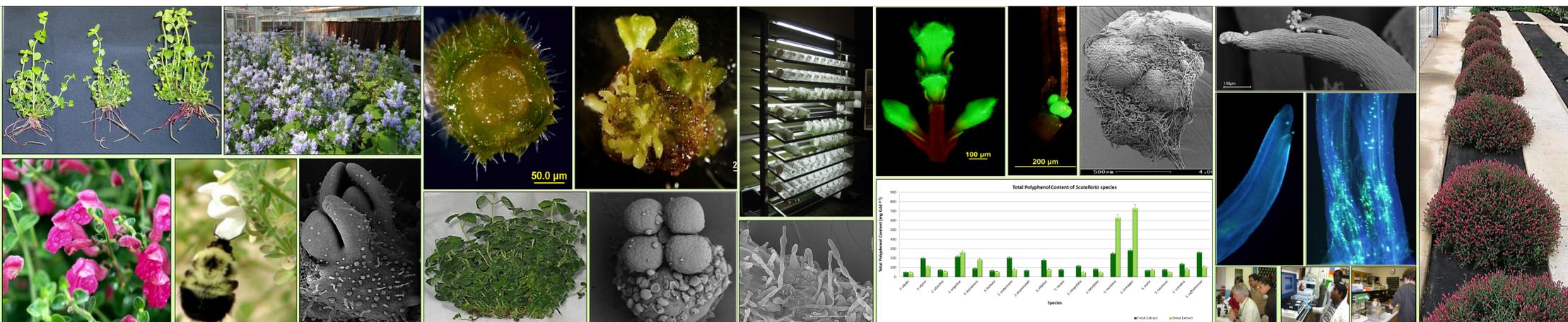
Arkansas State University
F Medina-Bolivar



Auburn University
Dennis A Shannon



Field trials to study the potential of *S. baicalensis*, *S. barbata*, and *S. lateriflora*. This study is a collaborative research program between FVSU and Auburn University (PI- N. Joshee.)



- Activities: Publications:**
1. N. Joshee, A. Tascan, Fabricio Medina- Bolivar, P. Parajuli, A. M. Rimando, Dennis A Shannon, J. W. Adelberg. **2012.** *Scutellaria*: Biotechnology, Phytochemistry and its Potential as a Commercial Medicinal Crop. Biotechnology for Medicinal Plants: Micropropagation and Improvement, Eds. S Chandra, H Lata and A Varma, Springer-Verlag, Heidelberg Germany pp. 69-99.
 2. Dandawate S, L. Williams, N. Joshee, A. M. Rimando, S. Mittal, A. Thakur, L. Lum and P. Parajuli. **2012.** *Scutellaria* extract and wogonin inhibit tumor-mediated induction of T_{reg} cells via inhibition of TGF-β1 activity. *Cancer Immunol Immunother* 61:701–711.
 3. P. Parajuli, N. Joshee, S. R. Chinni, A. M. Rimando, S. Mittal, S. Sethi and A. K. Yadav. **2011.** Delayed growth of glioma by *Scutellaria* flavonoids involve inhibition of Akt, GSK-3 and NF-κB signaling. *J Neurooncol.* 101(1):15-24.
 4. Joshee N, P. Parajuli, F. Medina-Bolivar, A. M. Rimando and A. K. Yadav. **2010.** *Scutellaria* Biotechnology: Achievements and Future Prospects. *Bulletin IASVM Horticulture*, 67(1):24-32.
 5. Tascan, A, J. W. Adelberg, A. M. Rimando, M. Tascan, N. Joshee, and A. K. Yadav. **2010.** Hyperhydricity and flavonoid content of *Scutellaria* species *in vitro* on polyester-supported liquid culture systems. *HortScience* 45(10):1723-1728.
 6. Parajuli, P., N. Joshee, A. Rimando, S. Mittal and A. K. Yadav. **2009.** *In vitro* anti-tumor mechanisms of various *Scutellaria* extracts and constituent flavonoids. *Planta Medica* 75:41-48.
 7. Joshee, N., A. M. Rimando, P. Parajuli, G. S. Rawat, and A. K. Yadav. **2009.** Investigating two medicinal *Scutellaria* species of Himalayan origin. In: *Advances in Agriculture, Environment, and Health; Fruits, Vegetables, Animals and Biomedical Sciences* (Eds. Singh SB, Chaurasia, OP, Yadav, A, Rimando, AM, and Terrill, TH). Pp. 347-356. SS Publishing House, Delhi, India.
 8. Tascan, A, J. W. Adelberg, M. Tascan, N. Joshee, and A. K. Yadav. **2009.** Polyester fiber controlled hyperhydricity for three species of *Scutellaria*: Medicinal plant. *Acta Hort.* (ISHS) 826:141-146.

M.S. Research Thesis: As major professor: Bianca L Richardson. Fort Valley State University. Thesis title: Developing *Ex Situ* Conservation Protocols for *Scutellaria ocmulgee* Small, a Threatened Plant Species Containing Anti-tumor Properties. **First MS Biotech graduate, May 2011.**

As a committee member: Arsine Similien: Auburn University, AL. Thesis title: Effect of Shade, Irrigation and Nutrient on Dry matter yield and Flavonoid Content in American Skullcap, *Scutellaria lateriflora*. 2007- 2008.

Santosh Sivakoti: Auburn University. Thesis title: Effect of Timing and Frequency of Harvest on Dry Matter Yield and Flavonoid Content of American skullcap, *Scutellaria lateriflora*. 2008-2011. Graduated in Fall 2011.

Sagar Dandwate: Studies on modulation of anti-glioma immune responses by *Scutellaria*. Wayne State University, Detroit, MI. Graduated in Fall 2011.

Beneficiaries: 1. Students and scientists, 2. Farmers and growers., 3. Herbal industry, 4. Pharmaceutical companies, 5. Ornamental plants industry/nurseries, 6. Tissue culture companies

Expected Impact: 1. In vitro conservation protocols for endangered *Scutellaria* spp., 2. Cryopreservation protocols, 3. Agronomic package for growers, 4. Development of transformation protocols for metabolic engineering, 5. Promoting ornamental *Scutellaria* spp., 6. Developing *Scutellaria* germplasm bank, 7. Scientific peer-reviewed publications.

Evaluation: Most of the objectives outlined in the project have been accomplished. Organic production of the *Scutellaria* herb at Auburn University did not succeed due to high fungal infection. We are continuing with this aspect and hope to finish it by the end of this year.

Acknowledgement: We are thankful to USDA-NIFA, 1890 Capacity Building Grant Program, for providing this research grant to carry out *Scutellaria* research.