**Title**:

**Authors:**

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**Abstract**

The *Scutellaria* genus contains multiple plant species used extensively in traditional medicines due to their various anti-inflammatory, sedative, and neuroprotective effects. *S. baicalensis* is one of the most well-known of these species, and previous works have identified its accumulation of bioactive flavones as a primary source of these effects. Specifically, the biosynthetic pathway of *S. baicalensis* … root-specific accumulation of 4´-deoxyflavones

**OUTLINE**

1. INTRODUCTION
   1. Background of medicinal plants
      1. Importance in traditional medicines
      2. Limitations – endangerment of native populations, production efficiency
      3. Necessity of biotechnology
   2. Introduction to *Scutellaria* and well-known *Scutellaria* species
      1. *S. barbata*, *S. lateriflora*, *S. baicalensis*
   3. 4´-hydroxyflavones and 4´-deoxyflavones
      1. Medicinal activities
      2. Organ-specific pattern in *S. baicalensis*
      3. Current state of knowledge of flavone pathway in *Scutellaria*
   4. Gaps in knowledge
      1. 470+ species in genus, only several studied
      2. Focus in *S. baicalensis* has been on 4´-deoxyflavones, not hydroxyflavones
      3. F8H for deoxyflavones has been identified in *S. baicalensis*, but unknown if analogous pathway exists for hydroxyflavones
   5. Goal of present study
      1. Identify medicinally valuable species that were previously unrecognized
      2. Assess how well organ-specific accumulation pattern is conserved
      3. Unexpectedly, we have identified a novel 4´-hydroxyflavone in *Scutellaria* which shows organ- and species-specific accumulation patterns. We also identify the enzyme responsible, and quantify it in various species
2. RESULTS
   1. Organ-specific flavone profiling
      1. 4´-deoxyflavone pathway is well conserved
      2. Relative abundance of glycone vs aglycone forms
      3. 4´-hydroxyflavone pathway is less well conserved, but is largely specific to aerial parts
      4. Root specificity of 4´-deoxyflavones as observed in *S. baicalensis* is not well conserved
      5. Accumulation of baicalein + baicalein by *S. wrightii*
      6. Accumulation of oroxylin A + oroxyloside by *S. racemosa*
   2. Identification of unknown peak as isoscutellarin
      1. HPLC chromatogram comparison – peak is present in aerial parts (aerial part specificity of 4´-hydroxyflavones) but only in some species (not *S. racemosa* or *S. wrightii*)
      2. Fractionation of unknown peak
      3. LC-MS and MS/MS results (compare with scutellarin) – identical fragmentation pattern, but different retention time
      4. NMR results to elucidate structure
   3. Establish apigenin as a precursor to isoscutellarin
      1. Apigenin feeding in *S. barbata*
   4. Identification of enzyme responsible for 8-hydroxylation
      1. Discuss previous studies with RTO (Zhao et al., 2018; Berim et al., 2014)
      2. Yeast activity test + *N. benthamiana* and *A. thaliana* infiltration
      3. Detection of new peak in yeast / infiltration data – isoscutellarein (aglycone) OR reduce isoscutellarin to isoscutellarein
      4. Confirm aglycone structure with LC-MS, MS/MS, and NMR (figures go in appendix?)
   5. Organ-specific quantification of isoscutellarein and isoscutellarin
3. DISCUSSION
   1. Overall difference in conservation of 4´-hydroxyflavone and 4´-deoxyflavone pathways across species
      1. Physiological roles – herbivory defense?
      2. 4´-deoxyflavones more common – *Scutellaria* has evolved to use 4´-deoxyflavones instead of 4´-hydroxyflavones
   2. Flavone accumulation is organ-specific in majority of species analyzed
      1. Substrate specificity of enzymes in flavone pathway is conserved and/or gene expression is organ-specific
      2. Pattern of organ-specificity differs in multiple species from that of *S. baicalensis*
   3. Patterns of 4´-hydroxyflavone and 4´-deoxyflavone accumulation with respect to native environment
      1. *S. baicalensis + S. barbata* – cool climate, little 4´-deoxyflavones in aerial parts (isoscutellarin is present)
      2. *S. racemosa + S. wrightii* – warm climate, significant 4´-deoxyflavones in aerial parts (isoscutellarin is not present)
   4. Significance of isoscutellarin discovery
      1. Potential medicinal effects
      2. Isolated in other plant species? – biosynthesis route similar or different?
   5. Species as targets for further medicinal development
      1. *S. racemosa* – oroxylin A + oroxyloside (and overview of previous work with 6-OMTs)
      2. *S*. *wrightii* – baicalein + baicalin
4. MATERIALS AND METHODS
   1. Plant growing conditions
   2. Flavone extraction and quantification
   3. HPLC fractionation to isolate isoscutellarin
   4. LC-MS and MS/MS
   5. NMR
   6. Apigenin feeding
   7. Yeast activity
   8. *N. benthamiana* and *A. thaliana* infiltration

**INTRODUCTION**

**RESULTS**

**DISCUSSION**

**MATERIALS AND METHODS**

**REFERENCES**