**Reviewer #2:**  
The manuscript "Metabolic profiling reveals organ-specific flavone accumulation in Scutellaria and identifies a scutellarin isomer isoscutellarin 8-O-β-glucuronopyranoside" was submitted to Plantdirect for publication.  
  
The present study investigates three types of organs (roots, stems, and leaves) in different species of Scutellaria and draw conclusions on the distribution of the necessary enzymes, especially with regard to the occurrence of 4'-hydroxy- and 4'-deoxyflavones.  
The study is well-planned and concise and also the experimental details are satisfactory, with only a few exceptions, leaving only a few points to be improved/clarified from my side.  
  
Major points:  
One major point which needs improvement is the first part of the results section. Here it would be better to not repeat results as given in the table (e.g. with standard deviation) but give ranges or total contents (as the authors did anyway). Thank you for the comment. We have reworked much of the first section of the results to avoid repeating specific data points included in Table 1. Additionally, instead of reporting ranges of concentrations, we opted to make this section more analytical by calculating significance values relative to *S. baicalensis*. We hope that this has improved the conciseness and clarity of this section.

With regard to tables 1 and 2, the concentrations of 0.00 {plus minus} 0.00 µmol/g fresh weight should be changed to n.d. for not detected. Corrected. Furthermore, tables 1 and 2 should be combined and the concentration of isoscutellarein 8-G calculated using the calibration curve of the isomer scutellarin. The tentative quantitation should then by mentioned in the Table legend or indicated by an asterisk and mentioned at the bottom of the table. We agree with the reviewer’s suggestion to merge Tables 1 and 2, but think it is more appropriate to leave the units of isoscutellarein 8-G as peak areas. This is because the UV absorbance spectra of isoscutellarein 8-G and scutellarin don’t resemble each other closely (Fig. S2), so isoscutellarein 8-G concentrations calculated using scutellarin as a reference may be completely inaccurate. We would rather be explicit about our lack of a standard for isoscutellarein 8-G, than potentially mislead readers with a tentative quantification.

Another major point is the missing chromatograms. As the determination of flavonoid concentrations is the basis of the study, a figure showing chromatograms of a standard solution and each investigated species should be given. One option would be to expand Figure 4 making the current Figure 4 a part of the new figure. Thank you for the comment. Although we showed the profiles of 15 metabolites in three organs of seven species, there are various unidentified peaks in the chromatograms of our samples, particularly leaf and stem samples, and some of them are highly abundant in some species but not all species. This variation made the chromatograms complex, and without spectra information, it is hard to tell the identity of the peaks. To identify peaks during our analysis, we compared both retention time and absorbance spectra against our standards. Although peaks in the chromatograms may appear to coincide in retention time between samples, we could have identified them as being different metabolites based on their absorbance spectra. Therefore, we think showing all chromatograms may make readers (particularly non-experts) distracted rather than help them to understand our manuscript. Thus, we prefer not to show all chromatograms. Instead we provide spectra for each metabolite we analyzed as a supporting figure (Fig. S2). We hope the reviewer agrees with us.

The third major point is the unknown compound mentioned in tables S1 and S2. According to the manuscript one previously undescribed compound was isolated and revealed as isoscutellarein 8-glucuronide. However, the tables compare the shift values of isoscutellarein 8-glucuronide and scutellarin to another unknown compound, which was not mentioned in the main text? If so, some more information on this component should be added to the main text. Otherwise, please clarify. The unknown compound in Tables S1 and S2 refers to the isoscutellarein 8-G fraction which we isolated from our *S. barbata* leaf extracts. In the tables, data in the scutellarin and isoscutellarein 8-O-β-glucuronopyranoside columns were taken from prior publications, and are not our own data. This was indicated in the original submission with a superscript beside the compound names. However, to make this more clear, we reworded the labels of both Tables S1 and S2, and added extra notes at the bottom of the tables.  
  
Minor points:  
Line 103: According to ThePlantList Scutellaria leonardii is a synonym for Scutellaria parvula var. leonardii. Therefore, please change it to S. parvula (with or without the respective variation). *S. leonardii* was changed to *S. parvula* in the manuscript, and in all figures and tables.   
Line 144: Please add particle size of the HPLC column. Added to text.  
Line 162: Please add the column used for LC-HRMS or indicate if the same column was used. Added to text. Also specify if both acetonitrile and water contained 0.1% formic acid. It is not clear. Clarified in text - both contained 0.1% formic acid.  
Line 296 and 298: Please write NOESY and ROESY instead of NOSEY and ROSEY. Corrected.  
Line 306: Please write "at position 8" instead of "at 8 position". Corrected  
  
**Reviewer #3:**  
The manuscript entitled "Metabolite profiling reveals organ-specific flavone accumulation in Scutellaria and identifies a scutellarin isomer isoscutellarein 8-O-b-glucuronopyranoside" by Askey et al. describes the flavone biosynethesis pathway of seven Scutellaria species. In particular, these species demonstrate organ-specific flavones, with 4-deoxy flavones localized to the roots and 4-hydroxy flavones to the aerial tissue. The authors quantitatively demonstrate the distrubition of these metabolites across seven species using HPLC and identify two species that differed from other species tested, S.racemosa and S. wrightii, by containing 4-deoxy flavone in the aerial tissue and lacking the characterized scutellarin isomer isoscutellarein 8-G. Overall, the work is of high quality, the subject is interesting and appropriate for Plant Direct, and the manuscript is relatively well written.  
I have several minor suggestions for improvement prior to publication listed below.

1. The authors compare compounds across species however no statistical analysis has been reported. Some of the figures could use some statistical comparison at least to one of the species such as S.barbata or S. baicalensis. Thank you for the comment. We have added statistical analysis to our work using *S. baicalensis* as a reference. Figures 3 and 6, and Table 1 have been updated to include asterisks (\*) above significant values. We added a description of the method used for statistical testing to the “Flavone extraction and quantification” subsection of the methods section, and rewrote several parts of our results to reference these results. Additionally, we removed parts of our results section which made comparisons between species without statistical evidence.

2. It would be interesting to know how divergent S.racemosa and S. wrightii, are from the other species, this is possible by a phylogeny tree. We are also interested in this divergence, but believe this sort of analysis is outside of the scope of this work. We are currently preparing another manuscript which includes this phylogenetic analysis, and should be submitted for publishing soon.

3. Method section:  
Method of extraction of flavones: was the tissue pulverized or crushed using liquid nitrogen prior to sonication? If so, indicate in the method section The following sentence was added to clarify: “Samples which did not fit in a 1.5 mL Eppendorf tube were compressed or bent to fit, but were otherwise kept whole.”  
  
Line 149 indicated the used of a calibration mix, indicate the chemical standards and calibration mix used A description of the method used to prepare the calibration mixes is provided in the sentences following their initial mention.  
  
Was any statistical analysis done on the data? Include a section A description of the statistical analysis method was added to the “Flavone extraction and quantification” subsection of our methods section. References to the results of the statistical analysis were added to multiple parts of the results section.

4. Lines 225-227 compare metabolites across species and would benefit from statistical analysis or by plotting a separate bar graph as the colors used in Figure 2 are hard to distinguish As mentioned previously, we have added statistical analysis using *S. baicalensis* as a reference. We have highlighted oroxylin A and oroxyloside in figure 3, and hope that readers can refer to Table 1 to find more detailed information.

5. Lines 237-243 reports several differences in amount of 4-hydroxy flavones vs 4-deoxy flavone across the species. These lines would benefit from a figure showing the total hydroxy and deoxy flavones across tissues with statistical support. We have removed these sentences which compared total accumulation of 4´-hydroxyflavones and 4´-deoxyflavones.   
6. Minor corrections:  
Line 137, 139: use the term 30 mg tissue/mL solvent Corrected.  
Line 145: gradient -8 to 0 min, correct the time Corrected.  
Line 148: the unit of measurement is incorrect, I believe the authors meant "nm" Corrected.  
Line 162: suggestion to use alternate sentence "0.1% formic acid in water (A) and acetonitrile (B) were used as mobile phase" The sentence was changed to: “0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) were used as the mobile phase ...”  
Lines 164: space between 0.5 ml/min Corrected  
Lines 164-166: use "min" instead of "mins" Corrected..  
Lines 258: spelling of species Corrected.  
Line 260: Figure 3 is not about chrysin Corrected.  
Line 263-264: restructure the sentence as it appears that to indicate the amount in root is low The sentence was deleted.  
Table 1: check table alignments on page 632 Table font size has been decreased to prevent line breaks within cells.  
Figure 2: Color palette used is hard to distinguish and there are not enough lines connecting the numbers. They are either missing or not connected to the numbers We have redrawn figure 2 to include lines connecting every number. In an attempt to clarify which bar section each label corresponds to, we also slightly adjusted the width allocated to the bars versus the lines and number labels. The color palette in the figure was chosen to maintain a constant lightness (i.e. shade) across all colors, and even steps in hue within 4´-hydroxyflavone and 4´-deoxyflavone classes. We worry that changing shade between flavones may give readers the impression that some flavones are more important or significant than others. As we wanted to emphasize overall differences in 4´-hydroxyflavone and 4´-deoxyflavone accumulation, the steps in hue separating flavones between the classes are larger. As the number of total flavones analyzed makes it difficult to visualize species with diverse, yet low concentrations of flavones, we hope that readers will refer to table 1 to get more detailed information.

Figure 6: Add breaks/indents to the y-axis We believe that the reviewer might have been confused by our inclusion of error bars on data points actually equal to 0. We have removed these extra error bars from figures 3 and 6.

Figure S5: The NMR data uses black and green which is hard to distinguish, use black and red or alternate color that stands out We changed the colors in the figure to gray and red.