











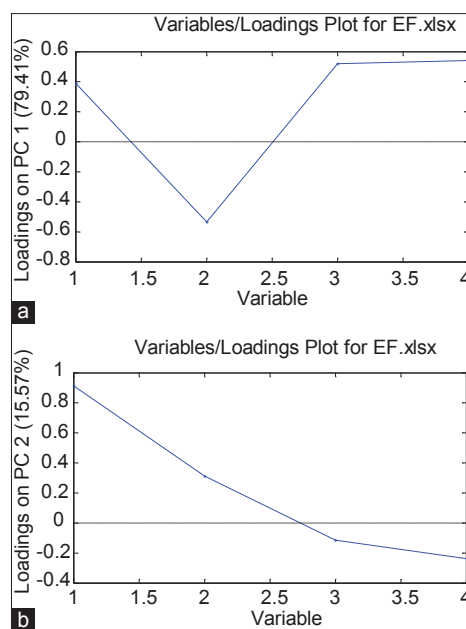




that contents of dehydroevodiamine, evodiamine, and rutaecarpine contribute to positive positions of EF samples in PC1 in the scores plot. What it means is that samples with higher contents of the three analytes are placed to the right in the PCA scores plot. Meanwhile, higher content of limonin makes samples get lower PC1 scores, placing them on the left in the scores plot. The combined action of the contents ultimately decides the position of each sample in the scores plot.

EF-9 from Dejiang, Tongren, Guizhou, gets the second highest content of evodiamine at 0.0877%, the third highest content of rutaecarpine at 0.2483%, and the second lowest content of limonin at 0.0158%, making it be placed to the most right in the scores plot. In a similar way, EF-7, EF-8, and the samples from group B (EF-6, EF-14, and EF-17) have relatively higher contents of dehydroevodiamine, evodiamine, and rutaecarpine and relatively lower contents of limonin, resulting in their higher PC1 scores ( $>1.5$ ). In contrast, EF-4 from Jiangkou, Tongren, Guizhou, has the lowest content of evodiamine at 0.0004% and the lowest content of rutaecarpine at 0.0176%. EF-2 from Shiqian, Tongren, Guizhou, has the lowest content of dehydroevodiamine at just 0.0020%. EF-3 from Jiangkou, Tongren, Guizhou, contains the second lowest content of dehydroevodiamine. EF-5 from Yuping, Tongren, Guizhou, contains the second lowest content of evodiamine at 0.0036% and rutaecarpine at 0.0250% in all the samples. These content characteristics make them locate in the left of other samples. Other samples from group "A" also have lower contents of dehydroevodiamine, evodiamine, and rutaecarpine as well as higher content of limonin. Therefore, all the samples from group "A" get PC1 scores  $<0$ . It is interesting to mention that although EF-18 from Pan'an, Jianghua, Zhejiang, and EF-19 from Taizhou, Zhejiang, have the highest and the second highest contents of dehydroevodiamine at 0.2943% and 0.2927, respectively, they also have the second highest and the highest contents of limonin at 1.5908% and 1.9340%, respectively, at the same time. The combined action of the contents ultimately makes the two samples lie in the left.

According to PC2 loadings plot, the contents of dehydroevodiamine and limonin mainly contribute to positive positions of EF samples in PC2, conversely, the contents of evodiamine and rutaecarpine contribute to negative positions of EF samples in PC2. However, due to nearer distances to zero line, contents of evodiamine and rutaecarpine play weaker roles in samples' positions in PC2 compared with those of dehydroevodiamine and limonin. EF-8 has the third lowest content of dehydroevodiamine. EF-9 has the second highest content of evodiamine and the third highest content of rutaecarpine. They are both located at the bottom of scores plot and are both from Dejiang of



**Figure 5:** PC1 (a) and PC2 (b) loadings plot of the four variables

Guizhou. It is worth noting that the contents of the four analytes of EF-16, regarded as the outlier in all the EF samples, are not the most or the least. The concentrations of the four markers are in the middle. That's why it is positioned near the elliptic line. The sample is from the Guiyang city.

Through the PCA scores plot, the similarities of the analyzed EF samples can be obtained clearly and intuitively. Then, PCA loading plots tell us how the variables (contents of the four analytes in this study) contribute to the positions of the samples in the scores plot. The method can help us to assess on the qualities of EF samples from different sources quickly and visually.

## CONCLUSIONS

EF is regarded as one of the geoauthentic herbs of Guizhou province and is one of the important herbs in many compound preparations. Evodiamine and rutaecarpine were required to be assayed in CP (edition 2005) for quality control of EF,<sup>[24]</sup> whereas limonin was designated as another characteristic chemical marker in CP (edition 2010).<sup>[1]</sup> As one of the chemical compounds with high yield from EF samples through the phytochemistry study in our lab, dehydroevodiamine is recommended as another chemical marker for quality assessment on this herb. Therefore, in this study, a sensitive, accurate, and precise UPLC-ESI-MS/MS method for assay of dehydroevodiamine, limonin, evodiamine, and rutaecarpine was established, with which 19 batches of EF samples from different regions were analyzed. Each analysis can be finished in 10 min. With



the population of MS instrument, the method can be accepted and applied widely for assessment on EF and EF-derived products.

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