

Extraction of Coumarins from Leaves, Petioles, Stems and Roots of *Ruta graveolens* and *Nicotiana benthamiana*

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[Abstract] This method describes the extraction of coumarins and furanocoumarins from leaves of *Ruta graveolens* (a natural furanocoumarin) producer, and *Nicotiana benthamiana*.

Materials and Reagents

1. *Ruta graveolens* or *Nicotiana benthamiana* plants
2. Liquid nitrogen
3. Ethanol
4. Methanol
5. (+)- Taxifolin (Extrasynthèse, catalog number: 1036 <http://www.extrasynthese.com>)

Equipment

1. 2 ml microtubes
2. Centrifuge for 2 ml tubes
3. Pestle and mortar
4. Bench top blender (Polytron PT2100, Kinematica, <http://www.kinematica.ch>)
5. Vacuum concentrator (RC10.10 speed vacuum, <http://www.thermoscientific.com>)

Procedure

1. Harvest 200 mg fresh material of each organ which is supposed to be analyzed.
2. Freeze it immediately in liquid nitrogen. Avoid thawing.
3. Grind the material in a cooled mortar. Keep the material frozen during the grinding process.
4. Add 2 ml of 80% ethanol and 50 µl of taxifolin 2 mg/ml to the mixture and homogenize with a bench top blender for 1 min (taxifolin will be an external control to compare the efficiency of the extraction between two samples. As the amount of taxifolin is the same in all the

- samples, final results can be compared to each other on the basis of the amount taxifolin detected).
5. Transfer the crushed material into a 2 ml microtube.
 6. Centrifuge at 16,000 x *g* for 10 min at room temperature.
 7. Harvest the supernatant and transfert the sample in a fresh tube.
 8. Evaporate the sample overnight with a vacuum concentrator.
 9. Resuspend the pellet in 200 µl methanol prior to analyses by HPLC (analyses are performed between 300 and 350 nm wavelength. Identification of furanocoumarin is done by comparison with standard molecules).

Acknowledgments

The protocol described here is adapted from one reported previously (Vialart *et al.*, 2012).

References

1. Vialart, G., Hehn, A., Olry, A., Ito, K., Krieger, C., Larbat, R., Paris, C., Shimizu, B., Sugimoto, Y., Mizutani, M. and Bourgaud, F. (2012). [A 2-oxoglutarate-dependent dioxygenase from *Ruta graveolens* L. exhibits p-coumaroyl CoA 2'-hydroxylase activity \(C2'H\): a missing step in the synthesis of umbelliferone in plants.](#) *Plant J* 70(3): 460-470.