



XXII Encontro Luso-Galego  
**Química**

**9 a 11 novembro 2016**

Instituto Politécnico de Bragança | BRAGANÇA - PORTUGAL



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Helder T. Gomes, Maria Olga A. S. Ferreira, João Barreira, Joana Amaral

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## **XXII ENCONTRO LUSO-GALEGO DE QUÍMICA**

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## ***In silico* and experimental analysis of DNA markers for *Citrus aurantium* identification in herbal medicines**

**Inês Sousa<sup>1</sup>, Joana Costa<sup>1</sup>, Joana S. Amaral<sup>1,2,\*</sup>, M. Beatriz P. P. Oliveira<sup>1</sup>,  
Isabel Mafra<sup>1,\*</sup>**

<sup>1</sup>REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

<sup>2</sup>ESTiG, Instituto Politécnico de Bragança, Bragança, Portugal

\*[jamaral@ipb.pt](mailto:jamaral@ipb.pt), [isabel.mafra@ff.up.pt](mailto:isabel.mafra@ff.up.pt)

*Citrus aurantium* L. belongs to the Rutaceae family and it is popularly known as bitter orange, Seville orange, sour orange, bigarade orange, or marmalade orange. In traditional Chinese medicine, the fruits are known as "zhi shi", being used as a stimulant of gastrointestinal function. In addition to the fruit itself, the leaves are used in herbal infusions as aids in digestion, the flowers are used for their sedative properties in cases of flu and the peel of the fruit is used as regulator of digestion. The fruit is traditionally used as an antiemetic, antitussive, diaphoretic, digestive and expectorant. Adrenergic amines (octopamine, synephrine, tyramine, methyltyramine and hordenine) are considered to be the most important active compounds of *C. aurantium*. Synephrine, tyramine and hordenine act in fat metabolism, activating lipolysis through adrenergic action and stimulation of beta-3 receptor [1]. Synephrine is a chiral amine present in nature as (R)-(-)-*p*-synephrine, which is chemically similar to other sympathomimetic amines, such as ephedrine (from *Ephedra sinica*). Therefore, bitter orange has been used in weight-loss formulations with increased popularity [2]. Since plants in herbal preparations can be substituted by others due to adulteration or to misidentification/accidental swap, to ensure consumer's safety and to verify labelling compliance, the development of adequate analytical methods is mandatory. So far, DNA-based methods have been reported as highly appropriate tools for plant authentication [3].

Therefore, the aim of this study was to develop a methodology for the specific identification of *C. aurantium* using DNA-based methods. For this purpose, voucher leaves of *C. aurantium*, as well as other 23 *Citrus* species (including *C. sinensis* – sweet orange) were gently provided by Germplasm Banks. Commercial samples of herbal infusions having bitter orange as ingredient ( $n=6$ ) were acquired in supermarkets. DNA was extracted from all samples using the commercial Nucleospin Plant II kit. An *in silico* study using 263 sequences of *C. aurantium*, available at NCBI database, was performed to eliminate high homology with other *Citrus* spp. Eight genomic sequences (NCBI accession no. HM172529, KM201256, KM201257, HM172529, HM172533, M172528, JN681151.1 and JN681151.1) were selected and used to design new primers. Qualitative PCR results showed the identification of *Citrus* genus targeting a resistance-like protein RGAs4-8-like encoding gene (NCBI accession no. HM172533) and RGAs4-1-like encoding gene for the identification of *C. aurantium* (NCBI accession no. M172528). Real-time PCR coupled to high resolution melting analysis using EvaGreen dye was also performed with *C. aurantium* specific primers. The developed methodologies were further applied to the analysis of the 6 commercial infusions labelled as containing bitter orange. PCR results showed incompliances for 5 of these samples due to the absence of bitter orange DNA. This result can possibly be related to a misidentification/swap of bitter orange (*C. aurantium*) with sweet orange (*C. sinensis*). Future work will comprise the development of a methodology for the specific identification of *C. sinensis* to confirm this hypothesis.

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