

## Green extraction protocols of *Mitragynaspeciosa* leaves leading to a possible large scale production

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### Objectives

*Mitragynaspeciosa* (K.) H. (*Rubiaceae*), is a tropical tree that is indigenous to Southeast Asia and Indochina. Also known as Kratom, it has been widely used, for hundreds of years, for its stimulant and opioid-like analgesic effects. The present study aims to design a green protocol for alkaloids extraction, in particular mitragynine, from the leaves using green techniques and solvents both in pretreatment and in extraction steps. For this purpose, we compared several non-conventional techniques (ultrasound, microwave, hydrodynamic cavitation) with classic methods.

### Methods

Dried *M. speciosa* leaves belonging to a red vein variety from Bali were in some cases pretreated with a phosphate buffer (pH = 7.5) and then extracted with ethanol, ethanol/water mixture or acidic water (pH = 3), using ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE). Moreover, hydrodynamic cavitation (HC) was also used for the scaling-up of the processes, using a pilot scale reactor (Rotocav<sup>®</sup>). All the samples have been analyzed using HPLC-DAD for the quantification of the principal alkaloids present based on previous literature data.

### Results

The extraction technique and solvent choice influenced both the raw product yield and the relative alkaloid content of *M. speciosa* leaves. Of the several methods tested, UAE with an immersion horn (21.4 kHz, 150 W, 30 min at 25-50°C with acidic water or 15 min at rt with ethanol/water 7:3 mixture) showed the best yield for mitragynine. HC demonstrated to be determinant both in the pretreatment and in the extraction step, increasing the final mitragynine content in the extract, paving the way to a future large scale production of *M. speciosa* extracts with different potential applications.