Optimization extraction study for the isolation of a bioactive diterpene from *Plectranthus ornatus* Codd.

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**Background**

Tuberculosis (TB) infects thousands of people every year and is a serious public health problem worldwide. The causative agent, *Mycobacterium tuberculosis*, is a bacterium that has elaborated survival mechanisms in the host.

The discovery of new antibiotics is essential for reducing TB deaths and natural products offer an excellent starting point for the discovery of these compounds due to their structural and functional diversity.

The genus *Plectranthus* belonging to the Lamiaceae family, such as mint and sage, exhibit a wide range of ethnobotanical uses. The *P. ornatus* species has diuretic, antipyretic, analgesic, antibiotic and anti-inflammatory properties and is used to relieve stomach and liver disorders.

Halimane’s backbone diterpene (11R*-acetoxy-halima-5,13E-dien-15-oic acid) was previously isolated for the first time from an acetone extract of *P. ornatus*. This compound is described due to its antimicrobial, namely antitubercular activity. Thus, in this work, for a large-scale of the compound isolation, we optimized its extraction. Moreover, an acetonitrile ultrasound extraction was performed (extraction yield 7.082% (w/w)). Chromatographic isolation of 5.3 mg of pure diterpene, identified by HPLC-DAD, was also performed by comparison with an authentic sample.

**Methodology**

1. Extract production procedure

Collection, drying and milling of the plant

The milled plant (1,261 Kg) was put in acetone (13L) and subjected to ultrasound waves for 30 minutes.

Extract solution was filtered and the solvent evaporated

![Image](https://example.com/fig1.png)

Fig 1 - *Plectranthus Ornatus* Codd.

2. Isolation and purification

- Flash column
  - Polarity gradient type elution from n-hexane to ethyl acetate
  - 14 Fractions produced

Based on TLC similarity 5 fraction were united and re-cromatographed

Dry column

Based on TLC analysis the purest fraction was recrystalized to obtained pure Hal (5.3 mg)

Recrystallization served as a purifying process of the compound Hal

![Image](https://example.com/fig2.png)

Fig 2 - Absorption spectrum of authentic sample

![Image](https://example.com/fig3.png)

Fig 3 - Isolated compound absorption spectrum

3. Characterization by HPLC-DAD

Two UV spectrums were performed from HPLC–DAD, the Hal isolated and an authentic sample from the group o made the RMN characterization of the compound

![Image](https://example.com/fig4.png)

Fig 4 - Compound Hal - 11R*-acetoxy halima-5,13E-dien-15-oic acid

**Results**

**Conclusion**

- An ultrasonic-assisted acetone extraction of *P. ornatus* was performed (extraction yield 7.082% (w/w)).
- This extract was chromatographed, and the purest fractions were recrystallized and 5.3 mg of a bioactive diterpene was obtained.
- The presence of this compound was confirmed by HPLC-DAD by comparison with the spectrum of an authentic sample.
- In future studies, it will be possible the exhaustive isolation of the diterpene of this extract allowing new biological studies with potential for the development of new tuberculostatic drugs.

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