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Copy of Protocol for the production of crude alcoholic extracts from native plants

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ARSTRACT

This protocol describes the method to obtaining crude alcoholic extracts from plant organs, such leaves and inflorescences. The used plants were the popularly called "João Brandinho" *Piper Callosum* and "Jambu" *Spilanthes acmella*. The crude extracts were used in our laboratory to anesthetize the native silver tetra fish *Ctenobrycon sp.* and Zebrafish *Danio rerio*, by immersion method.

MATERIALS NAME CATALOG # VENDOR Distilled Water 70% alcohol 793213 Sigma Aldrich Paper towels Beaker View Fluted Qualitative Filter Paper Circles 0979014G Thermo Fisher Wyllie Micro - te 650 Knife Mill **Analytical Balance** Amber glass Tray Glass funnel crucible

Separate the leaves and inflorescences from plants with the help of scissors



2 Rinse the material under running water and then with distilled water to remove debris.



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3 Dry the material with paper towels to remove the excess of water.



Grind the leaves and flowers with a knife mill or blender and / or just cut with the help of scissors, in order to allow a better extraction of secondary compounds.



5 Weight the vegetable mass into an analytical balance and dilute in 70% alcohol.

After quantifying the vegetable mass, dilute and mash the crushed and / or cut material into 70% alcohol in the desired ratio, such as 1:1 (1 gram of vegetal material to 1 milliliter of 70% alcohol) until the thoroughly solution mix.



7 After maceration and dilution in 70% alcohol filter the obtained solution with the aid of filter paper in a beaker.



 ${\tt 8} \quad {\tt Identify the alcoholic crude \ extract \ and \ store \ in \ an \ amber \ glass \ in \ the \ refrigerator}$

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