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# Spilanthol Extraction Using Microwave: Calibration Curve for Gas Chromatography

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Jambu (*Spilanthes oleracea*) is a very popular plant in the Northern region of Brazil. It is an herbaceous plant belonging to Compositae family. The green leaves and stems are used in the Brazilian cuisine and the peculiar unique flavour is its main characteristics. Nowadays, many researchers and Brazilian companies are very interested in all parts of this plant due to its therapeutic potential and the use of its essential oil in cosmetic products. Instead of all of the components of the plant, the methodology in which one would obtain the amide spilanthol will be of interest to industry, since it is the principal compound in the essential oil. The main objective of this work was to obtain the proper calibration curve for the spilanthol from jambu flowers (area x concentration) using microwave extraction with ethanol and hexane (3:7) as solvent. The essential oil was extracted at 50 °C during 30 min and analysed in gas chromatography which confirmed the presence of spilanthol. The calibration curve was determined using concentrations of 0.05, 0.1, 0.25, 0.5 and 1.0 mg/mL in triplicates. The results showed a fast extraction method to remove efficiently the spilanthol from the flowers and a linear math model was arrived at with an excellent coefficient of determination of 99.8 %. The calibration model was used in later investigations of microwave extraction methods by the team.

## 1. Introduction

Brazil is known by its huge biodiversity, which reaches more than 50,000 different species, earning the title of world biodiversity superpower together with other six countries in Latin America and Caribbean. Among all those biomass, jambu (*Spilanthes oleracea*) is a plant that is opening researches and Brazilian companies interest due to its amide, spilanthol (N-isobutyl-2(E),6(Z),8(E)-decatrienamide), which is responsible for the peculiar flavour and properties of the plant (Garcia et al., 1996; UNDP, 2010).
As the botanical characteristics, Jambu (Figure 1) is a short plant with yellow flowers and small seeds placed inside the buds. The leaves and stems are very much used in the state of Para (Northern of Brazil) in specific local dishes, such as *tacacá* and *pato no tucupi* or in the usual form with rice and pizzas. The plant could be found in other places around the world with synonyms as *Acmella ciliata* Kunth, *Cotula pyretharia* L., *Spilanthes fusca* MART and *Bidens fervida* Lan. Torres and Chávez (2001) reported that the big distribution of *Spilanthes* gender in tropical and sub-tropical regions had influence in species as *Acmella* gender to be considered for *Spilanthes* gender, which turned out to be difficult and confusing associated with the literature about those species. Therefore, nowadays, some denominations: *Spilanthes oleracea* or *Spilanthes acmella* var *oleracea* and *Acmella oleracea* refer to the same species (Hind and Biggs, 2003; Costa, 2010).
Besides the cuisine, another interesting use of the plant is the folk medicine in treating anaemia, dyspepsia, stomatitis and colds. Recently, some researches are discovering some properties of the extract which has potential for use in industry, Among them are ovicidal, larvicidal and pupicidal activity against *Aedes aegypti*, *Anopheles culicifacies* and *Culex* quifaciatus mosquitoes, diuretic activity, antioxidant and anti-inflammatory activities beyond patents in the cosmetic field (Ratnasooriya et al., 2004; Nascimento et al., 2013; Dias et al., 2012; Simas et al., 2013).

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All those properties of the plant are attributed to its many different chemicals, but principally to the N-isobutyl-2(E),6(Z),8(E)-decatrienamide. Spilanthol is a multifaceted compound that is not available commercially. This is the reason that makes researchers interested in developing methodologies to extract it from the plant and use reference standards, as dodeca-2(E),4(E)-dienoic acid isobutylamide for example, or in the comparison for identifying the peak of the compound in chromatographic analysis. However, the literature shows extraction taking 8-12 h using processes with methanol, ethanol, etc. (Dias et al., 2012; Singh and Chaturvedi, 2012).

Processes assisted by microwaves can offer good advantages due to the volumetric heating of the material, reduced processing time and energy savings, with consequent high product quality. Microwaves are electromagnetic energy, which has frequency and wavelength in the ranges of 300 MHz to 300 GHz, and 0.001 m to 1 m, respectively. However, it is important to note that there is a rule to use this kind of energy, since some sectors as radio communication, mobile phones and radar transmissions also needs electromagnetic energy. Therefore, a range for microwave frequency (915 MHz to 2.45 GHz) use to industrial, scientific and medical (ISM) purposes was adopted and determined by Federal Communications Commission in 1959 (Copson, 1975; Venkatesh and Raghavan, 2004).

Microwave extraction (MAE) is a technique that allows significant reduction in organic solvent consumption and extraction time, working at elevated temperatures and pressures and increases the output of the sample. The radiation can penetrate in a specific material (based on its dielectric properties) and it interacts with the polar components to generate heat. The heat transfer occurs due to the oscillation of molecule dipoles interacting with the high frequency alternate electric field or by ionic conduction (Sanga et al., 2000; Chan et al., 2011).

Microwave extraction systems are classified in multi-mode or focused-mode (mono-mode). The difference is, basically, that the first one allows random dispersion of radiation inside the cavity by a mode stirrer, and the latter, focuses the microwaves in a restricted area in the cavity. The extraction in a closed system (multi-mode) is a fast and efficient process with less solvent consumption, but it is susceptible to losses of volatile compounds. On the other hand, the open system (focused-mode) is safer, suitable for extracting thermolabile compounds, increases sample yield and the solvent can be added to the system in real time during the process. This type of extraction is commonly used to remove active compounds (Chan et al., 2011).

The main intent of this work was to quantify spilanthol in the extract obtained by microwave extraction with ethanol and hexane (3:7) as solvent. The calibration curve for the compound will be used in other works of the team. It will be an excellent addition to the literature.

## 2. Materials and methodology

### 2.1 Materials

Jambu seeds (Spilanthes oleracea) were purchased from a Canadian supplier, planted and in a commercial greenhouse in Sainte-Anne-de-Bellevue, Canada. The plant (Figure 1) was cleaned with distilled water and dried using a freeze-dryer (Christ, Gamma 1-16 LSC, Canada) at -55 $^{0}$C, 0.537 mbar vacuum pressure and 21.7 $^{0}$C shelf temperature media conditions. After drying, the buds were separated from the rest of the plant, milled using a processor (Homeland Housewares, MB1001/Magic Bullet®, USA) and stored inside the *mason jars* at room temperature. The moisture content and the size of the particles were determined before the extraction process.

(a) (b)

*Figure 1: (a) Jambu (Spilanthes oleracea); (b) Buds*

The equipment used for the extraction process was a microwave oven (Figure 2) type focused-mode system (CEM Corporation, Star System 2$^{TM}$, USA, 1997). A glass vessel (250 mL) is placed inside the cavity and holds the samples during the extraction. A condenser is coupled to direct reagents, which flow

down the sides of the vessel, and its temperature is monitored by an infra-red (IR) temperature sensor. However, the real temperature of the mixture was measured with a fiber optic thermometer (NoEMI-TS Series, Nortech Fibronic Inc., Canada) that provided feedback control for the measurement during the process.


*Figure 2: Extraction process set up*

**2.2 Methodology**

*Extraction*

The dried flower samples in powder form were weighed and stored in a glass vessel. The plant material was submitted to sieve analysis with meshes 18, 20, 35, 60 and 100 and the Sauter mean diameter was evaluated (Costa et al., 2011). The inlet moisture content was determined by the oven method (Jacobs, 1973) before the extraction process. The microwave extraction was carried out using 2 g of sample with 60 mL of solvent in the proportion of 3:7 for ethanol and hexane, respectively. After the extraction, the extract was filtered and stored in a cold chamber at -5 $^0$C.The ratio sample-to-solvent and experimental conditions of microwave (50 $^0$C and 30 min) were based on previous research (Dai et al., 2010; Costa et al., 2012) and preliminary tests.

*Thin Layer Chromatography*

The spilanthol separation step was carried out using Thin Layer Chromatography (Dias et al., 2012). First, the extract was concentrated using a rotavapor (Büchi, R-205, USA) connected to a heating bath (Büchi, B-490, USA) at 313 K and 55 rpm under reduced pressure. Later, the sample was taken in disposable micro pipets (Corning, Pyrex® 100 microliters – Cat n. 7099S-100, USA) and dropped down on clean silica gel TLC plates (Analtech, Uniplate™ - Cat n. 02015, USA) measuring 20 x 20 cm. The crude extract was placed carefully on the plate in a line at 2 cm of height from the bottom and stored in a glass tank with 200 mL of hexane and ethyl acetate in proportion 2:1 (v/v). The plate was placed into the beaker in such a way that the solvent level was below the sample line. The entire set up was closed air tight to avoid solvent evaporation and was not disturbed for 3 h. Afterwards, the plate was taken out and dried at room temperature. A UV light of 265 nm was used to observe the bands.

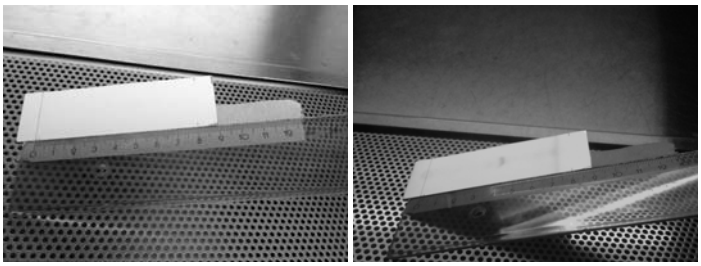
The spilanthol was identified by Rf = 0.5 (ratio between distance travelled by the sample and distance travelled by the solvent). Silica with the compound was mixed in a magnetic stirrer with 95 % ethanol and filtrated twice to remove completely the solid. The clean and green solution was subjected to an evaporative step using a rotavapor. The crude solid was weighed and solubilized in ethanol and hexane (3:7) for known concentration solutions.

*Gas Chromatographic Analysis*

Spilanthol solutions of different concentrations (0.05, 0.1, 0.25, 0.5, 1.0 mg/mL) were prepared and analyzed by gas chromatography coupled with mass spectrometer (Agilent Technologies, HP 6890N, USA). The set up was equipped with a HP-5MS column (Agilent Technologies, 190915-433, 350 °C maximum) with capillary dimensions: 30 m x 250 μm x 0,25 μm nominal. It was programmed to start at 70 $^0$C to evaporate all the solvents, increasing the ramp in 10 $^0$C/min stopping at 270 $^0$C. Helium gas was used as the carrier gas in split mode, 50:1 through the column and the spilanthol was identified by MS spectrum and NIST library. The results were analyzed by Statistica® 7.0 software using estimation method of Gauss-Newton.

## 3. Results and Discussion

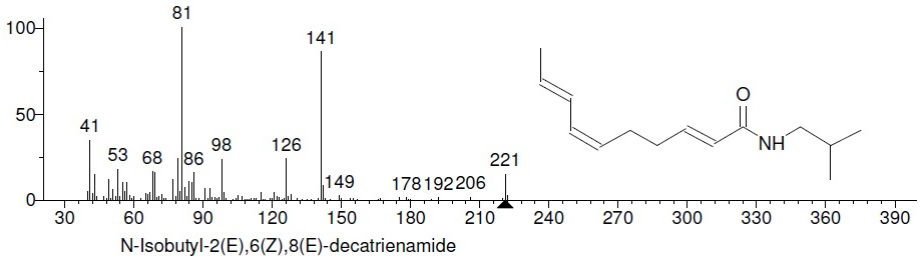
The Sauter mean diameter and the inlet moisture content of the dried flowers (powder) were $6.0013 \times 10^{-4}$ m and 15.08 % w.b., respectively. Figure 3 shows a silica plate after the spilanthol separation in thin layer chromatography. Some spots could be seen visually, but the spilanthol band could not. The UV light revealed perfectly the band in Rf = 0.5.



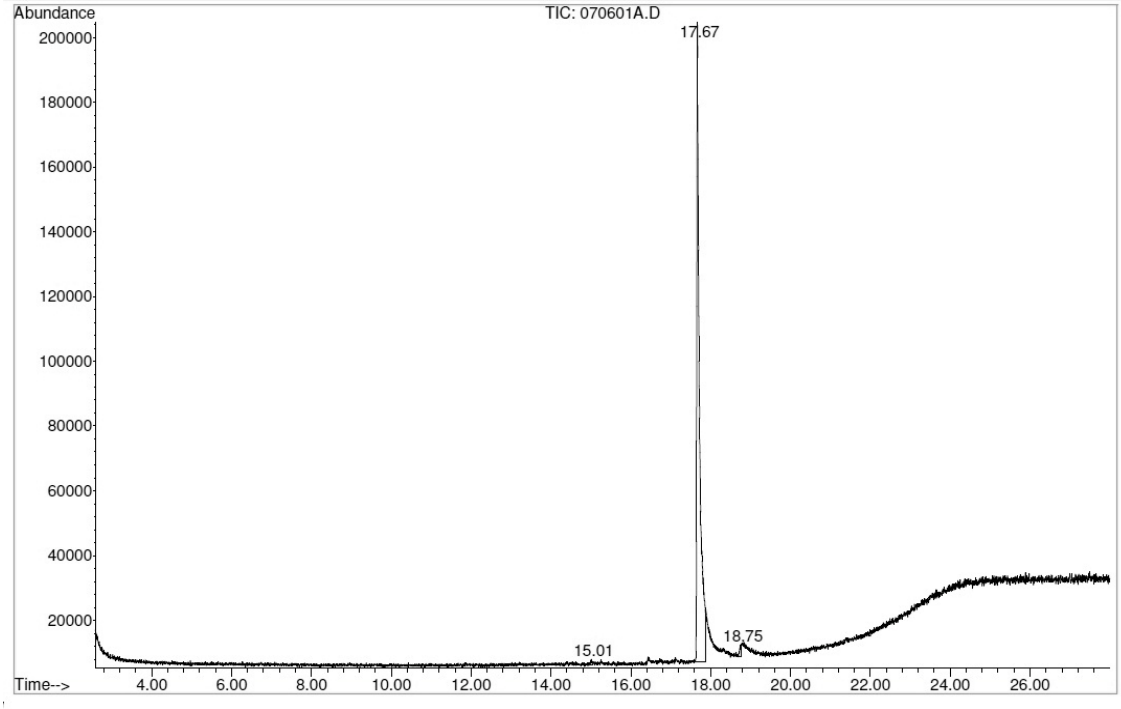
(a) without UV light (b) with UV light

*Figure 3: (a) Spilanthol separation*

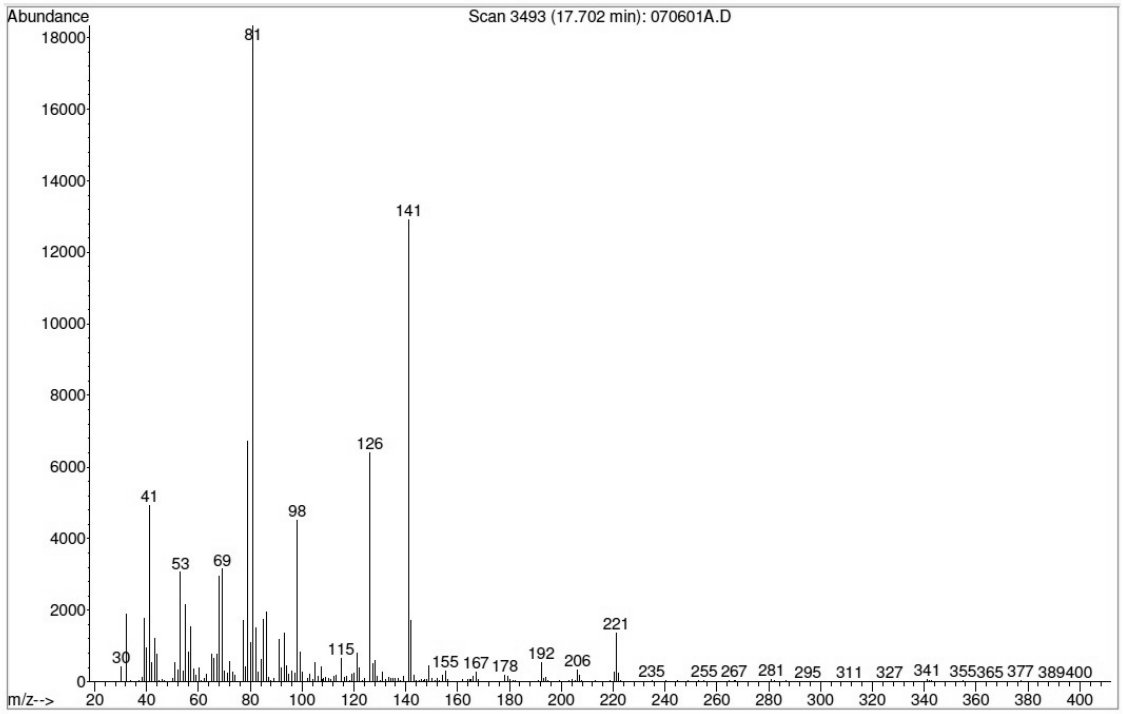
The spilanthol was identified by mass spectrometry and NIST library at integration parameters of minimum area of 0.5 % of the largest peak. It was observed that all the peaks at 17.7 min in GC/MS had the characteristics of ion spectrum pattern of spilanthol (Figure 4a).




(a)




(b)




(c)

*Figure 4: (a) GC/MS spectrum: (a) Solution of 0.1 mg/mL, (b) Mass spectroscopy of R.T. = 17.67, (c) Mass spectroscopy of spilanthol in NIST library*

Figure 5 shows the calibration curve for spilanthol and Table 1 shows the regression analysis for 95 % confidence. The standard showed high linearity with coefficient of determination ($R^2$) of 0.998 at the tested concentrations (0.05 – 1.00 mg/mL). The linear function Area = k * Concentration (mg/mL) + b was fitted and the coefficients for the standard were $1.31x10^7$ and $-6.74x10^5$, respectively. The equation was used to calculate the amount of the amide inserted in samples using microwave extraction.
Analysis of variance (ANOVA) was carried out to determine if the area of the peak was significantly influenced by the concentration changes. The ANOVA (Table 1) showed high significance confirmed by the coefficient of determination 99.8 % and p-level ($p < 0.05$) for the model regression.

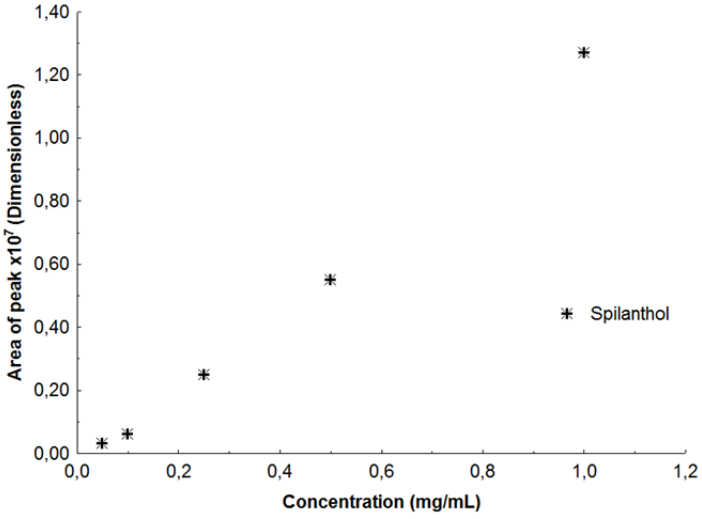



*Figure 5: Spilanthol calibration curve*

*Table 1: ANOVA*

| Effect | Sum of squares | DF | Mean Squares | F-value | p-value |
|---|---|---|---|---|---|
| Regression | $1.98x10^{14}$ | 2 | $9.88x10^{13}$ | 876.99 | 0.000071 |
| Residual | $3.38x10^{11}$ | 3 | $1.13 x10^{11}$ | | |
| Total | $1.98x10^{14}$ | 5 | | | |
| Corrected total | $1.05x10^{14}$ | 4 | | | |
| Regression vs corrected total | $1.98 x10^{14}$ | 2 | $9.88x10^{13}$ | 3.78 | 0.1197 |

## 4. Conclusion

The microwave extraction was satisfactory to remove spilanthol from the plant material. The process was faster than other methods available in the literature and preserved the compound in the extract. GC/MS was able to identify the peak and the NIST library showed a high match for the compound, confirmed by the spilanthol mass spectrum. The linear equation Area = 1.31x107 x (Concentration) - $6.74x10^5$ fitted very well the results ($R^2$ = 99.8 %, $p < 0.05$) and it has been used in other research work of the team.

### References


Chan C.; Yusoff R.; Ngoh G.; Kung F.W., 2011, Microwave-assisted extractions of active ingredients from plants, Journal of Chromatography A 1218, 6213-6225.

Copson D., 1975, Microwave heating. The AVI Publishing Company, Westport, Connecticut.

Costa C. M. L., 2010, Characterization and experimental analysis of fluidized bed coating of jambu seeds (Spilanthes oleracea), PhD Thesis, University of Campinas, Campinas, São Paulo (in Portuguese).

Costa S.S.; Gariepy Y.; Rocha S.C.S.; Raghavan G.S.V., 2012, Influence of the extraction temperature and extraction time on the composition of mint essential oil using microwave extraction, 62nd Canadian Chemical Engineering Conference, Vancouver, Canada, October.

Costa S.S.; Moris V.A.S.; Rocha S.C.S., 2011, Influence of process variables on granulation of microcrystalline cellulose in vibrofluidized bed, Powder Technology 207, 454-460.

Dai J.; Orsat V.; Raghavan G.S.V.; Yaylayan V., 2010, Investigation of various factors for the extraction of peppermint (Mentha piperita L.) leaves, Journal of Food Engineering 96, 40-543.

Dias A.M.A.; Santos P.; Seabra I.J.; Júnior R.N.C.; Braga M.E.M.; Sousa H.C., 2012, Spilanthol from Spilanthes acmella flowers, leaves and stems obtained by selective supercritical carbon dioxide extraction, The Journal of Supercritical Fluids 61, 62-70.

Hind N.; Biggs N., 2003, Acmella oleracea: compositae, Curti's Botanical Magazine 20, 31-39.

Jacobs M.B., 1973, The chemical analysis of foods and food products ($2^{nd}$ edition). D. Van Nostrand Company, New York.

Nascimento A. M.; Souza L.M.; Baggio C.H.; Werner M.F.P.; Maria-Ferreira D.; Silva L.M.; Sassaki G.L.; Gorin P.A.J.; Iacomini M.; Cipriani T.R., 2013, Gastroprotective effect and structure of a rhamnogalacturonan from *Acmella oleracea*, Phytochemistry 85, 137-142.

Ratnasooriya W.D.; Pieris K.P.P.; Samaratunga U.; Jayakody J.R.A.C., 2004, Diuretic activity of *Spilanthes acmella* flowers in rats, Journal of Ethnopharmacology 91, 317-320.

Sanga E.; Mujumdar A.S.; Raghavan G.S.V., 2000, Principles and applications of microwave drying, Mujumdar A.S., Drying Technology in Agriculture and Food Sciences. Elsevier Science Publishers: NY.

Simas N.K.; Dellamora E.C.L.; Schripsema J.; Lage C.L.S.; Oliveira Filho A.M.; Wessjohann L.; Porzel A.; Kuster R.M., 2013, Acetylenic 2-phenylethylamides and new isobutylamides from *Acmella oleracea (L.) R.K. Jansen*, a Brazilian spice with larvicidal activity on *Aedes aegypti*, Phytochemistry Letters 6, 67-72.

Singh M.; Chaturved R., 2012, Screening and quantification of an antiseptic alkylamide, spilanthol from in vitro cell and tissue cultures of Spilanthes acmella Murr, Industrial Crops and Products 36, 321-328.

Torres J.M.; Chávez A.G., 2001, Alcamidas em plantas: distribución e importancia, Avance y Perspectiva 20, 377-387.

UNDP (United Nations Development Programme), 2010, Latin American and the Caribbean: A biodiversity Superpower <www.undp.org> accessed 21.11.2012

Venkatesh M.S.; Raghavan G.S.V., 2004, An overview of microwave processing and dielectric properties of agri-food materials, Biosystems Engineering 88,1-18.