EXTRACTION AND ISOLATION OF INDOLE ALKALOIDS FROM *Tabernaemontana catharinensis*: TECHNICAL AND ECONOMICAL ANALYSIS

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Leishmaniasis is a parasitic infection that is considered worldwide one of the most important public health problem, affecting more than 12 million people with an estimated number of 2 million new cases occurring annually. Among the compounds isolated from *Tabernaemontana catharinensis*, coronaridine and voacangine are the indole alkaloids which have antileishmanial activity. In this work, the technical and economical analysis of extraction and isolation of indole alkaloids from *Tabernaemontana catharinensis* is presented. The extraction was carried out using supercritical CO_2 as solvent and ethanol as cosolvent (9.2% m/m). The data were taken total solvent flow rate of 6.1×10^{-5} kg/s, pressures of 300 bar and temperature of 318.15 K. The extracts were fractionated and analyzed by Thin-layer chromatography and Gas-chromatography/Mass Spectrometry. The economical analysis was carried out considering that the cost of manufacturing can be obtained in terms of the costs of investment, operational labor, raw material, waste treatment and utilities.

Keywords: Indole alkaloids, *Tabernaemontana catharinensis*, leishmaniasis, supercritical fluids, technical and economical analysis

INTRODUCTION

Leishmaniasis is an endemic health problem in 88 countries distributed in 4 continents. Brazil, Bangladesh, India, Peru, Iran, Saudi Arabian, Syria, and some countries in Africa are the most affected ones. There are about 12 million of infected people in the world, and only in Brazil there are 20,000 new cases per year [1]. The leishmaniasis is caused by a parasitic protozoa transmitted to humans by sandflies. Another form of leishmaniasis transmission is the person to person contagion due to the sharing of needles among injected drug users co-infected with HIV [2]. Leishmaniasis can cause continuous fever, lost of appetite, liver overgrowth, skin ulceration, anemia, and death. There are two main forms of leishmaniasis: cutaneous and visceral. The first form is non-fatal but can form disfiguring lesions. The second one is very severe resulting in several thousands of deaths per year.

The traditional treatment is based on the administration of antimonial drugs such as meglumine antimonate or sodium stibogluconate to the patients. In general, the drug is administrated by intravenous or intramuscular injections for 20-80 days. This conduct cannot be used in pregnant woman or people with heart, hepatic, or renal failure. The therapeutical procedure can cause irregular heartbeat, fever, nausea, pain in the upper abdominal area extending to the back, vomiting, increased level of hepatic enzymes, muscle pain, general feeling of discomfort, drowsiness, headache, joint pain, and lost of appetite. Furthermore, there are several strains of the parasite that are resistant to antimonial drugs [3]. For these reasons, some other drugs have been developed to give alternative therapeutic choices for patients infected with leishmania parasite. The alkaloids extracted from *Tabernaemontana catharinensis* (syn. *Peschiera australis*), showed potent *in vitro* antileishmanial activity, and thus, is a potential candidate for leishmaniasis therapy [4].

The yields obtained by the low pressure organic solvent extraction are very low, thus supercritical extraction can be an advantageous process. In this work is presented one methodology to estimate the cost of manufacturing the alkaloidal fraction obtained from the *Tabernaemontana catharinensis.* The cost of fractionation of the extract was also take into account.

MATERIAL AND METHODS

Raw Material Preparation

Thin branches and leaves from *Tabernaemontana catharinensis* were collected by FIOCRUZ (RJ, Brazil) at Campinas region (SP, Brazil). The raw material was dried at ambient conditions under the shadow and subsequently triturated. Afterwards, the raw material was transferred to LASEFI – DEA / FEA – UNICAMP, conditioned under vacuum in plastic bags and stored in a domestic freezer (Metalfrio, double action, São Paulo, Brazil) at –15 ºC. The size distribution of the particles was determined using a mechanical agitator (Abrosinox, Granutest, Santo Amaro, Brazil) with the rheostat set at 10 during 10 minutes. Sieves of 24, 32 and 48 mesh (Tyler series) were used.

SFE Experimental Procedure

The experimental runs were conducted using a SFE unit containing of an extraction cell of approximately 221×10^{-6} m^3 (length of 37.5×10^{-2} m and inside diameter of 2.74×10^{-2} m) and maximum pressure of 400 bar described by Pasquel *et al.* [5]. The data were taken at total solvent flow rate of 6.1×10^{-5} kg/s, 300 bar, 45ºC, using ethanol as co-solvent (9.2% m/m), and the methodology described by Pereira [6].

Fractionation of the SFE extract

The SFE extract (CE) was dissolved in HCl 5% (fumigating 37%, Merck, P.A.) and washed three times with hexane (Merck, P.A., lot K26803774934), to remove wax and lipidic compounds (HE). The aqueous extract was alkalinized with NH_4OH (25%, P.A., Merck) and washed three times with chloroform (Merck, lot K2835045, P.A.), obtaining two fractions: the organic fraction or alkaloidal fraction (AF) and aqueous fraction (AqE). The alkaloidal fraction was evaporated using a rotary evaporator (Laborota, model 4001, Viertrieb, Germany), with vacuum control (Heidolph Instruments GMBH, model Rotavac control, Viertrieb, Germany), bath at 40ºC of the thermostatic.

ECONOMICAL ANALYSIS

The cost of manufacturing is influenced by a series of factors that can be divided into tree categories: direct costs, fixed costs, and general expenses. The direct costs take into account expenses that depend directly of the production rate. Some of the items that contribute to direct costs are the raw materials, utilities, and operating labor, among others. The fixed cost does not directly depend on the production rate and must be considered even if the operation is interrupted. Examples of the items that are included in this cost are the depreciation, taxes and insurance, etc. General expenses are overheads of the plant needed to maintain the business and consist of the administrative cost, sales expenses, research and development.

In this work the methodology of Turton et al. [7] was used to estimate the manufacturing cost (COM). The methodology defines COM as a weighed sum of three factors: fixed capital investment (FCI), the cost of labor (COL), and the third factor that includes the cost of the raw material (CRM), the cost of waste treatment (CWT), and the cost of utilities (CUT). The COM can be calculated using:

$$COM = 0.304\ FCI + 2.73\ COL + 1.23\ (CRM + CWT + CUT) \quad (1)$$

Where FCI is the fixed capital investment, COL is the operational cost, CUT is the utility cost, CWT is the waste treatment cost, and CRM is the raw material cost.

The FCI is the investment of the extraction plant and should take into account the cost of the property, the preparation of the ground to for the construction, the civil construction, and the equipments costs. The equipment cost can be obtained from companies that build supercritical extraction systems or estimated from the costs of the main equipment that composes the industrial installation [8], such as the extraction columns, the flash tanks, the heat exchangers, the CO_2 reservoir, and CO_2 pump or compressor.

In this work, the operational labor was estimated from the number of man-hours required to run each equipment of the supercritical extraction unit [8]. The waste treatment of the process was neglected in the calculation of the COM. The cost of the utilities used to perform the extraction was estimated from the pure CO_2 entropy-temperature diagram [9].

RESULTS AND DISCUSSION

Technical Analysis

The mean particle diameter of *Tabernaemontana catharinensis* used in the experiments was 0.648×10^{-3} m and the bed density was 322 kg/m^3. The extraction curve obtained is presented in Figure 1. The yield was practically constant after 120 minutes of extraction, nonetheless increased at the end of the experiment due to the depressurization of the system. The yield of extract at the end of the experiment was 0.96%. This extract was fractionated producing 4.29×10^{-3} kg of alkaloids/kg of raw material. The gas chromatography analysis of the alkaloidal fraction showed the presence of 1.37×10^{-5} kg of coronaridine/kg of raw material and 86.34×10^{-5} kg of voacangine/kg of raw material.

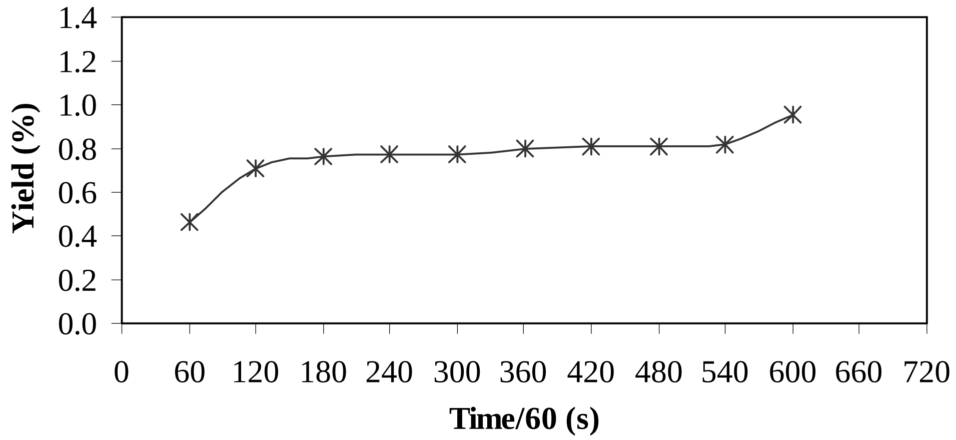

Figure 1. Supercritical CO_2 extraction curve of *Tabernaemontana catharinensis*: Experimental condition: P = 300 bar, T = 55 °C, Q_{CO2} = 6.1´ 10^{-5} kg/s, 9.2 % (m/m) of ethanol as co-solvent.

Economical Analysis

The manufacturing cost of the alkaloidal fraction was determined using the extraction and the fractionation costs estimated using the methodology proposed by Turton et al. [7]. The basis used in this calculation was 1 year of operation, or 7920 h (300 days, working 24 hours per day).

Extraction Section

Scale-Up Procedure. The scale-up procedure used assume that both yield and extraction time of the industrial process will be similar to the laboratorial scale if the ratio between the mass of particles inside of the extractor and solvent flow rate is kept constant. Thus, the extraction time was assume to be 2 hours, once after this period no increase in the yield (0.96%) of the process was observed (see Figure 1); the CO_2 mass flow rate was set at 400 kg/h (considering the bed density equal to the laboratorial scale). The number of extraction batches per year was 3960.

*Fixed Cost of Investment (FCI).*The investment of the extraction section is composed basically by the supercritcal extraction unit. In this work, it was considered an industrial scale unit containing two 400 liters columns and its estimated price was US$ 2,000,000.00.

Cost of Raw Material (CRM). Tabernaemontana catharinensis is a native tree in the southern part of Brazil and there is no commercial production of it. Thus, the cost of raw material was estimated considering its cost equal to the Maté Tea (*Ilex paraguariensis*) that has similar herbal structure and that grows at the same region. The commercialization cost of mate tea is US$ 169.70/ton of leaves and branches [10]. As the alkaloids are present mainly in the branches, the leaves should be separated and the cost of the branches should be around US$ 225.00/ton of branches. The pre-processing cost (drying and comminution) was estimated in US$ 30.00 using the software SuperPro Designer v4.7. Thus, the cost of the raw material was US$ 255.00/ton. The calculations were done using density of 322 kg/m^3 for a column volume of 0.4 m^3, CO_2 consumption make-up of 2% of the total used in the extraction, and ethanol consumption equal to 10% of the used.

Table 1.Raw material cost in the extraction section

Raw Material	Specific Cost (US$/ton)	Total Amount (ton/year)	Cost (US$)
T. catharinensis	255.00	510	130,050.00
CO_2	150.00	63.3	9,500.00
Ethanol	221.00 [11]	29.2	6,450.00
		Total (CRM)	146,000.00

Cost of Operational Labor (COL). The operational cost was calculated using the tables presented by Ulrich [8]. For a supercritical extraction unit containing two extraction columns, one CO_2 reservoir, one flash tank, one condenser, one heat exchanger, and one expansion valve will need 2 operators per shift. The cost of operational labor used was US$ 3.00/hour, and the total operational cost was US$ 47,520.00.

Cost of Waste Treatment (CWT). It was considered that the particles after extraction can be sold by the price to remove them from the extraction unit and, therefore, there will be no solid waste to be treated. The gas that leaves the extraction section is CO_2 and there will be no treatment on it. The liquid stream is the extract plus co-solvent that is the main product. Thus, there will be no waste treatment cost in the extraction section.

Cost of Utility (CUT). The cost of the utilities used in the extraction section is presented in Table 2. The main utility cost is the condensation of the CO_2 after the flash separation vessel.

Table 2. Cost of utilities used in the extraction section

Equipment	Energy (Mcal)	Specific Cost [7] (US$/Mcal)	Cost (US$)
Flash Distillation	97,380	0.0133 (steam)	1,295.00
Condenser	-161,700	0.0837 (cold water)	13,550.00
Pump	31,050	0.0703 (electricity)	2,200.00
Heat Exchanger	45,700	0.0133 (steam)	610.00
		Total (CUT)	17,655.00

Extraction Cost (COM1). The cost of manufacturing the extract (COM1) can be calculated using equation 1. The total value was US$ 940,000.00. In one year it is produced 4,900 kg of extract and, therefore, the specific cost of the extraction was US$ 191.84/ kg of extract. The main component of COM1 was the investment (64.8%) followed by raw material (19.1%), operational labor (13.8%), and utilities (2.3%).

Fractionation Section

The fractionation section can be divided in two parts: the hexane and the chloroform extraction. It was considered that the fractionation section operates to treat 1 hour of the extraction section production. The volume of the settler/mixer was 200 L constructed by carbon steal lined with glass. The cost of the equipment was US$ 32,000.00 [12]. The solvent is separated from the extract in flash tanks (stainless steal, US$ 3,000.00 [12]) and is recycled to the process. The total investment was US$ 35,000.00 that can be divided to the twos parts of the fractionation section. Thus, the investment per section was US$ 17,500.00.

Cost of the Alkaloidal extract. The manufacturing cost of each extract obtained in the fractionation section is presented in Table 3. In order to calculate the values presented in this table, the price of the chemicals were obtained from the software SuperPro Designer v4.7, 1 worker per shift in the fractionation section, recycle of 95% of the organic solvents, and utilities in the mixer and in the flash tanks. The main raw material in the hexane section was the crude extract (CE) obtained in the supercritical extraction and in the chloroform section was the aqueous fraction (AqE) produced in the hexane section (HE).

As the alkaloidal fraction represents 44.7% of the extract, the annual production of alkaloids will be 2,190 kg, and the specific cost will be US$ 732.18/ kg of alkaloids. In these fractions, the majority of the cost is based on the raw material, followed by operational labor, investment and utility. The price of the meglumine antimonate drug is approximately US$ 2,000.00/kg [13].

Table 3. Manufacturing cost of the extracts in the fractionation section

Section	COL (US$)	CUT (US$)	CRM (US$)	FCI (US$)	COM (US$)
Hexane	11,880.00	555,00	954,350.00	17,500.00	1,212,285.55
Chloroform	11,880.00	555,00	1,272,390.00	17,500.00	1,603,474.75

CONCLUSIONS

In this work, the methodology used to estimate the extraction and fractionation cost of alkaloids from *Tabernaemontana catharinensis*, that present antileishmanial activity, was presented. Tests *in vivo* in rats showed that the efficacy of the *Tabenaemontana catharinensis* alkaloid coronaridine against leishmaniasis is slightly lower than meglumine antimonate, but the efficacy of the methyl derivative of coronaridine is very similar to the commercial drug [14]. *In vivo* tests in humans should establish the dosage of alkaloid needed to cure the disease as well as the side effects of treatment. The dosage of alkaloid will determine if this treatment is economically feasible or not.

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