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Extraction and isolation of indole alkaloids from *Tabernaemontana catharinensis* A.DC: Technical and economical analysis

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Abstract

In the present 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 (5%, v/v). The global yield isotherms were determined at 35 and 45 °C for pressures of 150–350 bar. The mass transfer rate for the constant extraction rate period (CER), the duration of the CER period, and the mass ratio of solute in the fluid phase at the bed outlet were calculated. The extraction curves were adjusted by Crank, Goto et al. and Esquível et al. models. 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. The higher global yields were obtained at 350 bar (1.30×10^{-2} and 1.54×10^{-2} kg/kg, at temperatures of 35 and 45 °C, respectively). The Goto's model was able to quantitatively describe the experimental data. The cost of manufacturing the extracts obtained at 350 bar, 45 °C, using 5% (v/v) of ethanol was US$ 79.35 kg^{-1} of extract. Using previous experimental data obtained at 300 bar, 55 °C, using 10% of ethanol (v/v), the cost of manufacturing for the fractionation process to obtain a rich alkaloidal fraction (AF) was US$ 440.31 kg^{-1} of alkaloids.

Keywords: Indole alkaloids; Leishmaniasis; Supercritical fluid extraction; *Tabernaemontana catharinensis*; Technical and economical analysis

1. Introduction

Several separation techniques have been developed for food, pharmaceutical and chemical industries, due to the imposed environmental regulation, the necessity of minimizing energy and public health requirements. The interest in supercritical fluid process is the recovery of functional ingredients with high-purity [2]. Supercritical extraction has been shown to be a technically viable process to obtain several natural products. In spite of that, there are only few industrial applications of this technology. The reason for this is the high investment cost associated with the high pressures requirements for the extraction process, which results in a high cost of manufacturing [3]. The great improvement of the SFE processes is the properties of the supercritical solvent: it presents high mass transfer capacity, the solvent power can be altered by process conditions (temperature and pressure), and is easily removed from final extract. So, these SFE characteristics reduce the cleaning and purification step, common in conventional processes [4].

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 people infected in the world, and only in Brazil there are 20,000 new cases per year [5]. The leishmaniasis is caused by a parasitic protozoa transmitted to humans by sandflies. In the last years have been notified an increase of persons with HIV co-infection [6]. 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 of this disease 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,

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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 [7]. 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 *in vitro* antileishmanial activity, and thus, is a potential candidate for leishmaniasis therapy [8]. *T. catharinensis* is a native tree in the southern part of Brazil that have been studied due to its biological activities: antitumoral, anti-inflammatory, analgesic [9], anticancer [10], antioxidant and antimycobacterial [11], trypanocidal [12] and antileishmanial activities [8,13]. The yields of indole alkaloids obtained by the low pressure organic solvent extraction are very low, beside the organic solvent consumption in the conventional process is very high, then supercritical extraction can be an advantageous process.

In order to transfer this technology to the industry an analysis of the economical feasibilities must be done. The cost of manufacturing (COM) 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, among others [14].

The purposes of this work were to determine the global yields isotherms and extraction kinetics parameters and to calculate the manufacturing cost of extracts and indole alkaloids from *T. catharinensis*.

2. Material and methods

2.1. Raw material preparation

Thin branches from *T. catharinensis* were collected at Campinas region (SP, Brazil), from the Guará/Ribeirão Anhumas in November 2002 (spring in the South hemisphere). The raw material was dried at ambient conditions under the shadow, subsequently triturated, packed under vacuum in plastic bags and stored in a domestic freezer (Metalfrio, double action, São Paulo, Brazil) at −15 °C. 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 min. Sieves of 24, 32 and 48 mesh (Tyler series) were used.

2.2. SFE experimental procedure

The experimental runs were conducted using a SFE unit containing a nylon extraction cell (80 mesh) of approximately 274×10^{-6} m^3 and maximum pressure of 400 bar described by Pasquel et al. [15]. The data were taken at temperatures of 35 and 45 °C, pressures of 150, 200, 250, and 300 bar and total solvent flow rate of $3.7 \pm 0.2 \times 10^{-5}$ kg/s, using ethanol as co-solvent (5.0%, v/v), $12.15 \pm 0.01 \times 10^{-3}$ kg of raw material and the methodology described by Pereira et al. [1]. The mass transfer kinetics experiments were carried out at the conditions that maximize the global yield using a total solvent flow rate of $3.5 \pm 0.2 \times 10^{-5}$ kg/s. The raw material mass used in these experiments were $85.00 \pm 0.01 \times 10^{-3}$ kg.

2.3. Fitting of the overall extraction curves to models

The overall extraction curves (OECs) were fitted to the models of Crank [16], Goto et al. [17], Esquível et al. [18], using the Tecanalysis software [19]. For the Crank [16] model was fitted the mass coefficient of diffusion (D). The Goto et al. [17] model required the fitting of the desorption equilibrium constant (k) and the dimensionless parameter ϕ, related to the mass transfer resistance in the system. For the Esquivel et al. [18] model was fitted the empirical constant (k).

2.4. Economical analysis

In the calculation of the COM, two experimental conditions were considered: one is extracts from *T. catharinensis* obtained by SFE as described in the previous section (denoted as condition 1), another is extracts obtained by Pereira et al. [1] at 300 bar, 55 °C, using 10% (v/v) of ethanol as co-solvent (denoted as condition 2). In the first case, the COM was calculated for the operational condition (temperature and pressure) that resulted in the higher global yield. For extracts obtained by Pereira et al. [1] the manufacturing cost of the fractionation process to obtain a rich alkaloidal fraction (AF) was also calculated.

The methodology of Turton et al. [14] was used to estimate the manufacturing cost (COM). This methodology defines COM as a weighed sum of five factors: capital investment (FCI), the cost of operational labor (COL), the cost of the raw material (CRM), the cost of waste treatment (CWT), and the cost of utilities (CUT). The details and considerations of each cost are presented by Rosa and Meireles [3]. The COM can be calculated using:

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

For the manufacturing cost of the rich alkaloidal fraction (AF) the fractionation process described by Pereira et al. [1] was considered. In this, the SFE extract is dissolved in HCl 5% (fumigating 37%) and washed with hexane to remove wax and lipidic compounds. The aqueous extract is alkalinized with NH_4OH (25%) and washed with chloroform (Merck, lot K2835045, PA), obtaining two fractions: the organic fraction or alkaloidal fraction (AF) and aqueous fraction. The solvent of the organic fraction is evaporated using a rotovap, with vacuum control, at 40 °C.

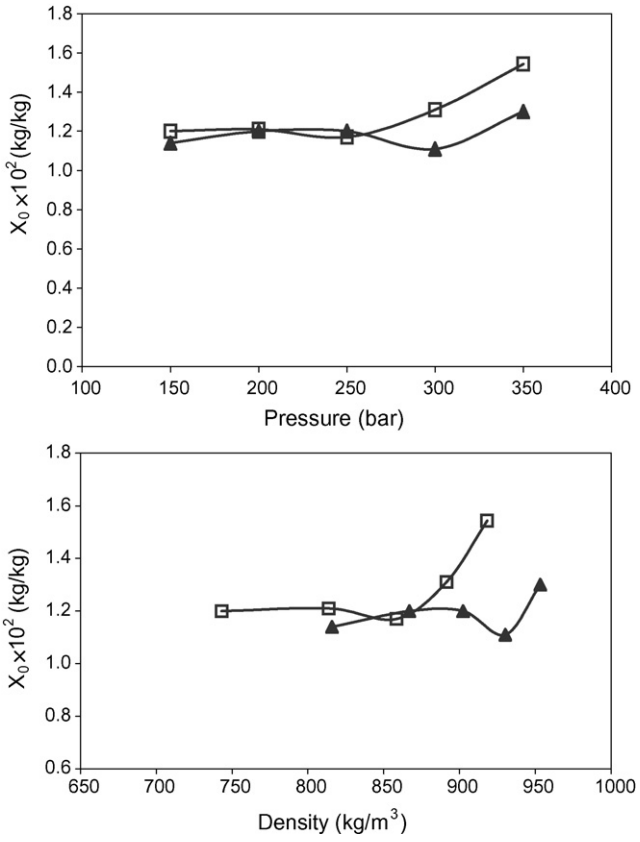

Fig. 1. Global yield (X_0) isotherms of *T. catharinensis* obtained at 35 °C (▲) and 45 °C (□), 350 bar using 5% (v/v) of ethanol.

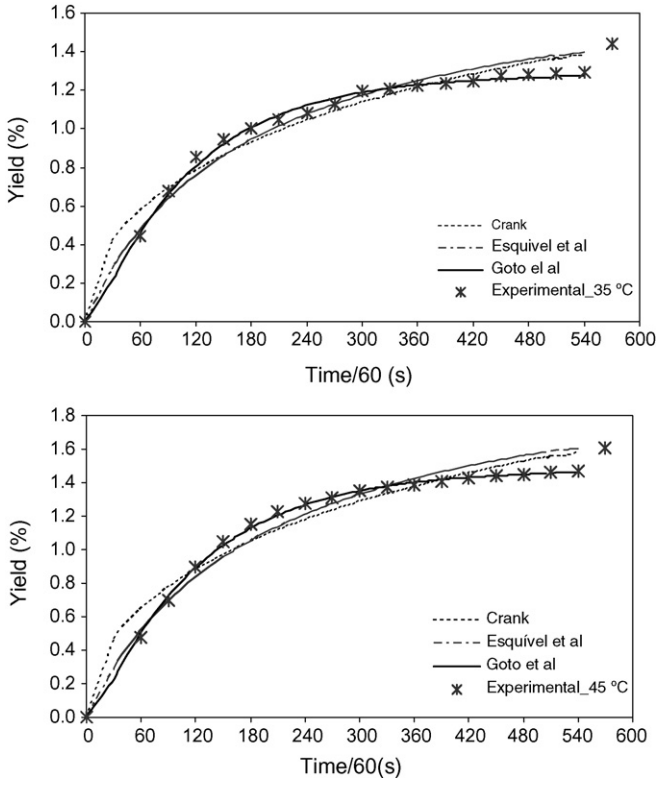

Fig. 2. Comparison between experimental and fitted OECs for SFE process at 35 and 45 °C, 350 bar using 5% (v/v) of ethanol. The last two points at 570 min represent the increase in the yield obtained during the depressurization of the SFE unit.

3. Results and discussion

3.1. Technical analysis

Fig. 1 shows the global yield (X_0) isotherms for 35 and 45 °C. It is verified that above 870 kg/m^3, the increase of temperature at constant pressure caused a decrease in the solvent density; however the global yield increased. This indicates that the predominant effect on the solubility, in this region, is the solute's vapor pressure. Higher global yields were obtained at 350 bar. Fig. 2 presents the OECs at 35 and 45 °C, 350 bar, 5% of ethanol. The last two points indicate the increase in yield obtained during the depressurization of the SFE unit. The fitted curves for the models of Crank [16], Goto et al. [17], and Esquivel et al. [18] are shown for comparison. The parameters fitted to the various models are in Table 1. The overall extraction curves were better fitted by the Goto's model [17] in both conditions. According to Fig. 2, at 45 °C, the constant extraction rate period (CER) continued up to 167 min (t_{CER}). The mass transfer rate for the CER (M_{CER}) was 9.0×10^{-8} kg/s. At 35 °C, although the M_{CER} had been very similar (9.2×10^{-8} kg/s) to that at 45 °C, the CER period was shorter and continued up to 137 min, and as a result the yield at CER period was larger than that at 35 °C. For this reason, the manufacturing cost of *T. catharinensis* extracts (COM_E1) was calculated for the process at 350 bar/45 °C (condition 1).

In order to compare the manufacturing cost of *T. catharinensis* extracts, data from Pereira et al. [1] (condition 2) was considered. The manufacturing cost (COM_E2) was estimated for the process carried out at 300 bar, 55 °C and 6.1×10^{-5} kg/s of total solvent flow rate, using 10% of ethanol (v/v). The OEC obtained is presented in Fig. 3. At the end of the experiment the yield increased due to the depressurization of the system; this was also observed in this work (Fig. 2). The yield of extract at the end of the experiment was 1.04%. Pereira et al. [1] fraction-

Table 1
Model parameters for the system *T. catharinensis*/CO_2/ethanol in SFE at 45 °C/350 bar, 5% (v/v) of ethanol

T (°C)	Models						
	Crank [16]		Goto et al. [17]			Esquível et al. [18]	
	D ($\times 10\,m^2/s$)	SSD[a]	k	Φ	SSD[a]	k ($\times 10^{-2}$)	SSD[a]
35	1.47	0.060	2.361	1.061	0.004	1.73	0.031
45	1.29	0.101	2.591	1.141	0.003	1.95	0.053

[a] Sum of the square deviation.

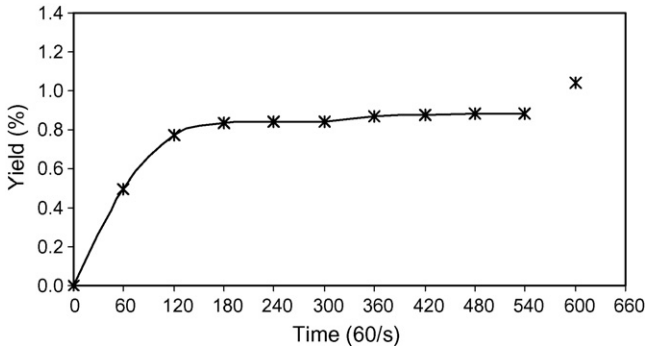

Fig. 3. Overall extraction curve of the system *Tabernaemontana catharinensis* + CO_2 + ethanol. Experimental condition: 300 bar, 55 °C, 6.1×10^{-5} kg/s, 10% (v/v) of ethanol [1]. The last point at 600 min represents the increase in the yield obtained during the depressurization of the SFE unit.

ated this extract 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.

3.2. Economical analysis

3.2.1. Extraction section

3.2.1.1. 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 are kept constant. The considered industrial unit was equipped with two extraction columns with 0.4 m^3 of internal volume. The two columns is necessary to maintain the unit to operate constantly; when the extraction is carried out in one column, the other one is depressurized, the exhausted particles are unload, the column is cleaned, the fresh particles are loaded, and the column is pressurized. Thus, considering that the laboratorial and industrial scale bed densities are identical, the scale-up factor for conditions 1 and 2 were 1460 and 1810 folds, respectively. The experimental bed density for the condition 1 (350 bar, 45 °C, 5% (v/v) of ethanol, 3.5×10^{-5} kg of solvent/s) was 310 kg of raw material per m^3 of extraction column. As this value was assumed to remain constant in the industrial scale, the amount of raw material used per industrial scale column per extraction cycle was 124 kg. The solvent mass flow rate per mass of raw material ratio in the laboratorial scale unit was 1.48 kg of solvent/(kg of raw material × h), and consequently the solvent flow rate in the industrial scale was 184 kg/h. For condition 2 (300 bar, 55 °C, 10% (v/v) of ethanol, 6.1×10^{-5} kg of solvent/s) [1], the laboratorial bed density was 322 kg/m^3, and the solvent (CO_2 + ethanol) mass flow rate was 400 kg/h using the same previous considerations. The difference in bed densities was caused by the characteristics of two sets of raw materials used in conditions 1 and 2.

3.2.1.2. Fixed cost of investment (FCI). The investment of the extraction section is composed basically by the supercritical extraction unit. It was considered an industrial scale unit containing two 0.4 m^3 columns and its estimated price was US$ 2000000.00. The annual depreciation was considered to be 10%.

3.2.1.3. Cost of raw material (CRM). *T. 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 grows at the same region. The commercialization cost of mate tea is US$ 62.63 t^{-1} [20]. As the alkaloids are present mainly in the branches, the leaves should be separated and the cost of the branches should be around US$ 83.00 t^{-1} 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$ 113 t^{-1}. The calculations were done using bed density of 310 and 322 kg/m^3 for raw materials of conditions 1 and 2 respectively, and using a CO_2 consumption make-up of 2% of the total used in the extraction, and ethanol consumption equal to 10% of the used. The specific cost of solvent was US$ 150.00 t^{-1} of CO_2 [3] and US$ 221.00 t^{-1} of ethanol [21].

3.2.1.4. Cost of operational labor (COL). The operational cost was calculated using the tables presented by Ulrich [22]. 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 two operators per shift will be needed. The cost of operational labor used was US$ 3.00 h^{-1}, and the total operational cost was US$ 47,520.00, considering 330 days, working 24 h per day.

3.2.1.5. 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.

3.2.1.6. 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.

3.2.1.7. Extraction cost (COM_E). For condition 1 it was evaluated the influence of the extraction time on the specific cost (Fig. 4). Although t_{CER} in this condition had been equal to 167 min, according to Fig. 4 the smaller manufacturing cost was obtained in 90 min of process. Thus, an extraction time of 90 min was assumed in the further calculations. Using this value of extraction time, the number of extraction batches per year was 5280, considering 330 days, working 24 h per day. For condition 2 [1], although the yield was practically constant after 120 min of extraction, t_{CER} was 90 min for this condition, and the specific cost of manufacturing was 23% lower than that at 120 min. In order to estimate the extract manufacturing cost it was considered the extract obtained after 90 min of process and

Table 2
Cost of utilities used in the extraction section

Equipment	Energy (Mcal)		Specific cost [14] (US$ Mcal^{-1})		Cost (US$)	
Condition	1	2	1	2	1	2
Flash	50834	88434	0.01	0.01 (steam)	676.10	1176.18
Condenser	−70588	−146838	0.08	0.08 (cold water)	5908.28	12290.31
Pump	16326	28200	0.07	0.07 (electricity)	1147.70	1982.48
Heat exchanger	9959	41483	0.01	0.01 (steam)	132.45	551.72
				Total (CUT)	7864.53	16000.69

Condition 1: 45 °C/350 bar/5% ethanol (v/v); condition 2 [1]: 55 °C/300 bar, 10% ethanol (v/v).

the material recovered during the depressurization step, for both conditions.

Using Eq. (1) the values of COM_E1 in condition 1 was US$ 79.35 kg^{-1} of extract and for condition 2 the COM_E2 was US$ 121.79 kg^{-1} of extract. The main component of COM_E1 was the investment (72.86%) followed by operational labor (14.41%), raw material (11.56%), and utilities (1.16%). On the other hand, for the condition 2, the main component of COM_E2 was the investment (71.23%) followed by operational labor (14.08%), raw material (12.37%), and utilities (2.32%).

The reason for smaller manufacturing cost in the condition 1 (US$ 79.35 kg^{-1} of extract) is the operational conditions that caused higher M_{CER} ($9.0 \pm 0.8 \times 10^{-8}$ kg/s). In condition 2 (300 bar/55 °C, 10% ethanol), the M_{CER} was 7.6×10^{-8} kg/s [1]. This change of process condition (from 300 bar, 55 °C, 10% ethanol to 350 bar, 45 °C, 5% ethanol) permitted a reduction in 35% of COM_E2 value.

Other factor is that in both COM_E was considered the cost of raw material similar to the cost of Mate Tea (*I. paraguariensis*). The COM_E value can have been under or overestimated, because *T. catharinensis* is a native tree that has not a developed agriculture. Then, instead mate tea, it was considered other species for the manufacturing cost calculation as: "mangabeira" (*Hancornia speciosa*), and "maniçoba" *(Manihot glaziowii* Muell. Arg). These plants had been chosen due to their commercial importance. The "mangabeira" is a tree with

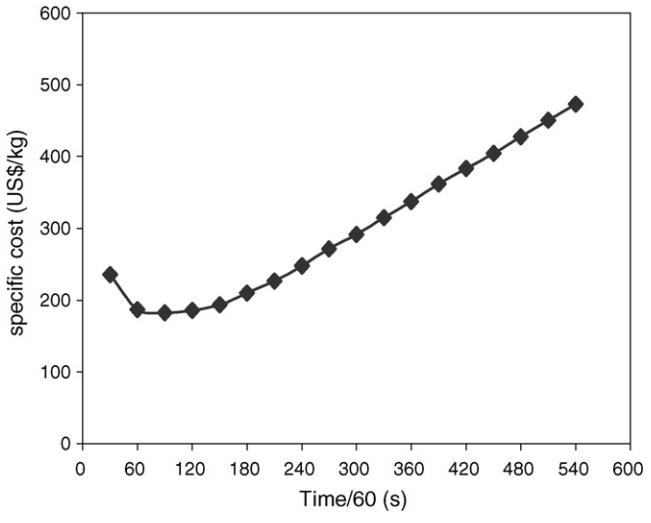

Fig. 4. Specific manufacturing cost by the process time, in SFE at 45 °C, 350 bar, 5% (v/v) of ethanol.

Table 3
Specific cost, raw material cost and manufacturing cost of extracts *T. catharinensis* using several plants

Plant	Specific cost (US$ t^{-1}) [23]	CRM (US$)	COM_E3 (US$ kg^{-1} of extract)
Erva mate	113.0	79858.3	79.35
Mangabeira	256.0	173573.9	90.32
Maniçoba	110.6	78272.6	79.17

5–10 m of height and is a latex producer. It occurs exclusively in Brazil, where in the Northeast is more abundant. Its fruit, the "mangaba", is used in the industry as frozen pulp and in formulating ice cream, candies, wines, vinegars and others [26]. The "maniçoba", native in the Northeast and Central Brazil region, has up to 15 m of height. These trees produce latex and seed oil useful in the rubber and dyes industries [27]. Table 3 shows the CRM and COM_E3 values of *T. catharinensis* extracts using the cost of maniçoba and mangaba [23]. It was considered the process to be performed at 45 °C/350 bar with conditions given previously during 90 min.

From Table 3, although the specific cost of raw material had been changed in up to 100%, the range COM_E3 costs were from US$ 79.17 and 90.32 kg^{-1} of the extract. In other words, the specific cost of raw materials varied from 9 to 22% of COM_E3 values. These values are low if compared with the manufacturing cost of other materials obtained by supercritical fluids, as ginger oleoresin extracted from *Zingiber officinale* Roscoe [3]: the COM_E value reported was US$ 99.80 kg^{-1} of ginger oleoresin, with 2.7% of global yield in the CER period (165 min). Although the global yield of ginger oleoresin had been higher (2.3 time) than that obtained with *T. catharinensis* (R_{CER} = 1.19%), according to Table 3, the difference between the ginger oleoresin cost and *T. catharinensis* extracts cost was only US$ 9.5 and 20.6 kg^{-1}, respectively. In addition, the duration of the extraction process for ginger oleoresin was longer (165 min) than that for *T. catharinensis* extracts (90 min). Although these materials are different with respect to the specie and the composition of extracts, it is observed that the estimative for COM of *T. catharinensis* extracts are coherent.

4. Fractionation section

The fractionating of the extracts cost was calculated for the alkaloidal fraction obtained by Pereira et al. [1] at 300 bar/45 °C, 10% of ethanol. The fractionation section can be divided in two

Table 4
Manufacturing cost of the alkaloidal fraction (COM_AF) per batch from *T. catharinensis* extracts obtained by Pereira et al. [1]

Section	COL (US$)/batch[a]	CUT (US$)/batch[a]	CRM (US$)/batch[a]	FCI (US$)/batch[a]	COM_AF (US$)/batch[a]
Hexane	1.5	0.07	101.43	2.21	130.12
Chloroform	1.5	0.07	137.90	2.21	175.17

[a] Hour-batch.

parts: the hexane and the chloroform extraction. It was considered that the fractionation section operates to treat 1 h of the extraction section production. The volume of the settler/mixer was 200 L constructed of stainless steel lined with glass. The cost of the equipment was US$ 32000.00 [24]. The solvent is separated from the extract in flash tanks (stainless steel, US$ 3000.00 [24]) and is recycled in the process. The total investment was US$ 35000.00 that can be divided into the two parts of the fractionation section. Thus, the investment per section was US$ 17500.00.

4.1. Cost of the alkaloidal fraction (COM_AF)

The manufacturing cost of each extract rich in alkaloids obtained after the fractionation section is presented in Table 4. 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 obtained in the supercritical extraction and in the chloroform section was the aqueous fraction produced in the hexane section. Considering that the bed density of the industrial SFE unit would be equal to the laboratorial scale, for extractor vessels' of 0.4 m^3, in each batch (90 min) it would be produced 1.29 kg of crude extract, and in an year approximately 7072.67 kg of this extract. As the alkaloidal fraction represents 44.7% of the extract, the annual production of alkaloids would be 3161 kg. Using Eq. (1), it is possible to calculate the cost of each section (Table 4); the cost of manufacturing for the fractionation section would be US$ 175.17 h^{-1}-batch. If in each hour-batch 0.86 kg would be produced, and 44.7% of it is alkaloids, so the specific cost would be US$ 440.31 kg^{-1} of alkaloids. In these fractions, the major part of the cost is the raw material, followed by operational labor, investment and utility.

The great importance of *T. catharinensis* extracts in public health is its activity against Leishmaniasis [8,13]. The antimonial drugs are the main medicines used in the treatment. However, beside high toxicity these drugs can cause several adverse reactions, as irregular heartbeat, fever, nausea, vomiting, others [8,7]. The price of the meglumine antimonate drug is approximately US$ 2000.00 kg^{-1} [25]. Tests *in vitro* in rats showed that the efficacy of the *T. 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 [8]. The dosage of alkaloid will determine if this treatment is economically feasible or not. Recently, a study evaluated the leishmanicidal effect of alkaloidal fraction from the *T. catharinensis* obtained by SFE at 250 bar/45 °C and 300 bar/55 °C, both using ethanol at 5%, as well as their cytotoxicity to mouse macrophages. It was verified that 10 and 100 μg of AF/mL inhibited 20–26 and 80–100%, respectively, of parasite growth; in addition, tests showed that AFs were not toxic for macrophages [13]. However, *in vivo* tests in humans should establish the dosage of alkaloid needed to cure the disease.

It is possible to estimate the amount necessary in the human treatment using a correlation with experimental data of literature. Delorenzi et al. [8] studied the leishmanicidal activity of alkaloid fraction (or chloroformic fraction: CLF). In this study, the alkaloid fraction was obtained by conventional extraction method. For our discussion, we will use AF for alkaloid fraction obtained by SFE and CLF for alkaloidal fraction obtained by the conventional method. Delorenzi et al. [8] showed that the treatment with 100 μg/mL of CLF reached 100% lethality of parasite after 24 h. Besides, Soares et al. [13] showed that AF had similar potential to CLF in the same concentration and analysis period. Delorenzi et al. [8] also calculated the IC_{50} (50% inhibitory concentration) for CLF and glucantime when administrated in different concentrations. In this analysis, demonstrated that adding CLF or glucantime in the culture infected in first day of culture or once a day for 3 days, the IC_{50}s were 2.6 and 15 μg/mL for CLF and glucantime, respectively. When the infected macrophages were treated 3 days with the drugs, the IC_{50}s were 1.25 and 6.6 μg/mL for CLF and glucantime. This indicated that the IC_{50} of CLF or AF is about 5.52 higher than the IC_{50} of glucantime.

The meglumine antimonate drug is presented in ampoules of 5 mL containing 425 mg of pentavalent antimonie. The dose recommended is 20 mg of antimonie/kg weight/day, during from 20 to 40 days [28]. Considering a patient with 60 kg, this will be used about 2.8 doses/day. At the end of treatment, in 30 days, he should be used about 85 doses. The price of the meglumine antimonate drug is approximately US$ 2000.00 kg^{-1} or US$ 0.85 per dose [25]. Then, the cost of treatment will be US$ 72.00 per infected person. In a population of 12 million of infected, the cost for the treatment will be US$ 864000000.00. Beside, more than 400,000 new cases worldwide per year are detected. To control these new cases the government will be spent US$ 28800000.00 per year.

On the other hand, using the same the relation for IC_{50} of glucantime/AF (5.52) in the human treatment, about 3.62 mg of AF/kg weight/day will be sufficient to generate the same effect that glucantime. Considering the same amount of drug in the ampoule, at the end of treatment, in 30 days, the patient should be used about 15 ampoules. The price of each ampoule with AF would be US$ 0.19 per dose or US$ 440.31 kg^{-1}. The cost of treatment would be US$ 3.22 per infected person. Then, the manufacturing cost to produce the amount of AF to be used in for

the treatment of 12 million of infected will be US$ 34428719.52. To treat the 400,000 of new cases per year, the government will be spent US$ 1147623.98 $year^{-1}$, representing a reduction in cost of about 96%.

In this work, it was demonstrated that the specific cost was US$ 440.31 kg^{-1} of alkaloids, smaller than the antimonial drugs used for treatment against Leishmaniasis. This shows that it is possible to obtain a high potency medicine using supercritical fluid process.

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