

**Análise cromatográfica e avaliação físico-química do óleo essencial das flores de
Bauhinia monandra Kurz**

**Chromatographic analysis and physicochemical evaluation of the essential oil of
Bauhinia monandra Kurz flowers**

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Bauhinia monandra Kurz**

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Resumo

O estudo das técnicas cromatográficas, clássicas e modernas, descreve a simplicidade e, ao mesmo tempo, os avanços que essa área vem sofrendo nos últimos anos em pesquisas científicas de qualidade e também na aprendizagem de cursos de graduação e pós-graduação em todo o mundo. Este trabalho apresenta uma caracterização por cromatografia em camada delgada (CCD) e cromatografia gasosa acoplada à espectrometria de massa (CG-EM), como método desenvolvido por estudantes de pós-graduação que envolve a combinação de uma técnica clássica e moderna bem como propriedades físico químicas do óleo essencial das flores de *Bauhinia monandra*. O óleo essencial foi extraído por Clevenger, a CCD foi realizada em diferentes eluentes e reveladores, deste modo obteve-se os fatores de retenção R_f s e o perfil químico por CG-EM. O óleo essencial das flores apresentou rendimento de 0,06%, uma solubilidade positiva em etanol 70%, índice de refração de 1,3621, rotação óptica de +36,4 α_D e densidade relativa de 0,941 g mL⁻¹ a 20 °C e na CCD 18 R_f s foram observados após o uso de diferentes reveladores, com a classe predominante de oxigenados. Na análise por CG-EM, foram observados 7 compostos, sendo dois majoritários, caracterizados como panaxeno com 20,51% e α -guaieno com 33,39%. O óleo essencial da flor de *B. monandra* apresentou predominância de 70,22% dos compostos sesquiterpênicos. As técnicas aliadas, clássicas e modernas, demonstraram diferentes formas de avaliar o óleo essencial por meio de sua composição química, ambas com alta eficiência e precisão, além de ser um projeto apropriado para ser desenvolvido por estudantes de pós-graduação.

Palavras-chave: Cromatografia; Óleo essencial; Gênero *Bauhinia*.

Abstract

The study of chromatographic techniques, classical and modern, describes the simplicity and, at the same time, the advances that this area has undergone in recent years in quality scientific research and also in learning in undergraduate and postgraduate courses around the world. This paper investigate a characterization by thin layer chromatography (TLC) and gas chromatography coupled with mass spectrometry (GC-MS), as a method developed by graduate students that involve a combination of a classic and modern technique, as well as

results about the physicochemical properties of the essential oil of *Bauhinia monandra* flower. Essential oil was extracted by Clevenger, the TLC was performed in different eluents and developers, and thus the retention factors (*R_fs*), and the chemical profile by GC-MS were obtained. The essential oil of the flowers showed a yield of 0.06%, positive solubility in ethanol 70%, refractive index of 1.3621, optical rotation of +36.4 α_D and relative density of 0.941 g mL⁻¹ at 20 °C. In the TLC analysis 18 *R_fs* were observed after the use of different developers, with the predominant class of oxygenates compounds. In the GC-MS analysis, 7 compounds were observed, being two majorities, characterized as panaxene with 20.51% and the α -guaiene with 33.39%. The essential oil of *B. monandra* flower showed a predominance of 70.22% of sesquiterpenic compounds. The allied techniques, classic and modern, demonstrated different ways of evaluating the essential oil through its chemical composition, both techniques showed high efficiency and precision, in addition was an appropriate project developed by postgraduate students.

Keywords: Chromatography; Essential oil; Genus *Bauhinia*.

Resumen

El estudio de las técnicas cromatográficas, clásicas y modernas, describe la simplicidad y, al mismo tiempo, los avances que esta área ha sufrido en los últimos años en la investigación científica de calidad y también en el aprendizaje de cursos de pregrado y posgrado en todo el mundo. En este trabajo, se presenta una caracterización por cromatografía de capa fina (CCF) y cromatografía gaseosa con espectrometría de masas (CG-EM) como método desarrollado por estudiantes de posgrado, que implica una combinación de técnicas clásicas y modernas, así como propiedades físicas y químicas del aceite esencial de flores de *Bauhinia monandra*. El aceite esencial fue extraído por Clevenger, el CCF se realizó en diferentes eluyentes y desarrolladores, obteniendo los factores de retención *R_fs* y el perfil químico por CG-EM. El aceite esencial de las flores mostró un rendimiento de 0,06%, solubilidad positiva en etanol al 70%, índice de refracción de 1,3621, rotación óptica de +36,4 α_D y densidad relativa de 0,941 g mL⁻¹ a 20 °C y en CCF 18 *R_fs* se observaron después de usar diferentes desarrolladores, con la clase predominante de oxigenados. En el análisis CG-MS, se observaron 7 compuestos, dos de los cuales fueron principales, caracterizados como panaxeno con 20,51% y α -guaieno con 33,39%. El aceite esencial de la flor de *B. monandra* mostró un predominio del 70,22% de los compuestos sesquiterpénicos. Las técnicas aliadas, clásicas y modernas, demostraron diferentes formas de evaluar el aceite esencial a través de su composición química, ambas con

alta eficiencia y precisión, además de ser un proyecto apropiado para ser desarrollado por estudiantes graduados.

Palabras clave: Cromatografía; Aceite esencial; Género *Bauhinia*.

1. Introduction

Techniques, such as, thin layer chromatography (TLC) and gas chromatography coupled with mass spectrometry (GC-MS) are important subjects in the development of studies on separations of compounds in various areas of chemistry. Chromatography is a widely studied technique as a tool to solve different problems in research and industry, and it is taught in undergraduate and postgraduate courses (Dawan et al., 2017; Lebot; Legendre, 2016; Silva et al., 2009).

The TLC is one of the most common techniques in the separation of compounds, presenting low cost of material and reagent, being well discussed in textbooks and various scientific articles, it is also considered the basis for separation chromatographic studies. Another chromatographic technique of great importance is the GC, considered one of the modern techniques, has wide use in large research centers, University, pharmaceutical industries, forensic science, being a technique of separation, identification and determination of compounds with greater robustness and sensitivity, however, presents high costs with maintenance of the equipment, in the purchase of gases and reagents of high purity degree (Liu et al., 2018; Chiaradia et al., 2008).

However, the quality of scientific information has been demanding greater efficiency, accuracy and precision of data obtained in the area of chromatography, reducing the use of classical techniques, which are addressed in teaching and in simpler studies especially to phytocomposites. Advanced techniques such as high performance liquid chromatography (HPLC) and GC with detection by mass spectrometry (MS) are focused on high efficiency in the execution of experiments and in obtaining results with superior quality and reproducibility, which is not observed in classic techniques. (Tahtah et al., 2016; Da Silva; Collins, 2011; Lances, 2009).

Classical chromatographic techniques, classified as planar on paper (PC), thin layer (TLC) and liquid on column (LC) can use cellulose paper or silica gel as stationary phases, polar and apolar solvents as mobile phase, and several revealing compounds, among them iodine, ferric chloride, sulfuric vanillin, 2,4-dinitrophenylhydrazine (2,4-DNFH), and ultraviolet light (UV) in short and long wavelengths, these techniques cover the analysis a

wide range of compounds. However, after identification of the principal compounds, the next step is to start for more robust chromatographic analysis (Sfecci et al., 2017; Lanças, 2009; Pereira; Neto, 2000; Reynolds; O'Dell, 1992; Ruppel Jr. et al., 1971).

Traditional techniques provide great assistance, as previously mentioned, in studies with biopharmaceuticals in particular of vegetable origin. It is through the TLC that the researcher can elucidate the classes of compounds present in a specific plant organ, making a survey of a certain number of natural species and their biological activity. With these species is possible to produce in the laboratory, tinctures and extracts, using steam-dragging or hydro-distillation, crushing and supercritical fluid. This information can be confirmed when combined with the empirical knowledge of the rural population who use plants as a means of treating and curing countless forms of disease (Ghosh et al., 2019; De Oliveira et al., 2018; Fadel et al., 2018; Da Silva; De Oliveira, 2018; Yadav, et al., 2017; Nóbrega et al., 2017).

In this study, both TLC and GC-MS chromatographic techniques were used to characterize the essential oil extracted from *Bauhinia monandra* flowers, since does not have data about its characterization in the literature. The specie *B. monandra* is exotic in Brazil, and very important due to its medicinal use in the treatment of diabetes, and different biological activities such as antioxidant, antifungal, diuretic and hypoglycemic (Araújo-Melo et al., 2017; Fernandes et al., 2012; Menezes et al., 2007; Aderogba et al., 2006; Argolô et al., 2004). This specie is native to the tropical regions of Southeast Asia, such as China, India and Vietnam. In the Brazilian territory, it has adapted well to several regional climates, it is one of several species of the genus *Bauhinia* widely used in ornamentation, being a floristic option for the cities, avenues, parks and gardens, because it is an eccentric species due to the size of the numerous flowers, as well as the exhaled aroma produced from the essential oil (Anoliefo; Gill, 1992).

Generally, essential oils are composed of monoterpenes, sesquiterpenes and phenylpropanoids, and can be extracted by steam-dragging techniques, by pressing or supercritical fluid from any plant organ (Bezerra-Silva et al., 2014; Bizzo et al., 2009). They are usually used as flavors, fragrances, fragrance fixatives, pharmaceutical formulations both topical and endogenously administered as antifungal, antibacterial and antiviral agents, as well as marketed in their natural form (Lucena et al., 2019; Pombo et al., 2018; Rivera et al., 2017; Alarcón et al., 2017; Jaramillo et al., 2014; Silva-Santos et al., 2008).

The present paper aims to evaluate the classes of compounds present in the essential oil of *Bauhinia monandra* flowers by TLC and GC-MS and also to obtain information of its physicochemical properties.

2. Material and Methods

The study is an academic research that consist of a field and laboratory part realized in the first semester of 2019. It was developed as a part of the Advanced Chromatography discipline to three postgraduate students of Program in Agrochemistry “*stricto sensu*” offered by the Goiano Federal Institute (IF Goiano), Campus Rio Verde, Goiás, Brazil. The study is of a qualitative and quantitative “quali-quantit” nature based on the studies by Pereira et al. (2018).

The flowers of *B. monandra* were collected in the early hours of the morning, between 6-8 a.m., in the month of July 2019, from 20 individuals located in the municipality of *Rio Verde – Goiás* (GO), Brazil, with the following geographical coordinates: 17°43'25.9"S 50°52'55.5"W (GPS, Garmin, Mod. Glosnass). The floral material was stored in cooled thermal (Glacial Mor) packaging and sent to the laboratory of Technological Chemistry at IF Goiano. The species was identified by Msc Biologist. Antonio Carlos Pereira de Menezes Filho. An excicata was herborized and deposited in the Herbarium of the IF Goiano, with the following record, Voucher HRV: 1345.

The essential oil of *B. monandra* flowers was extracted in a Clevenger-type system (Marconi, Mod. MA553/2000) from aliquots of 500 g of inflorescence. Each aliquot was processed (Philips Walita, Mod. Viva Collection) with 500 mL of distilled water. The solution was transferred to the system, maintained under reflux for 3 hours. The hydrolat was collected and transferred to a 500 mL separation funnel (Laborglas, Borosilicate), and then washed three times with 10 mL of dichloromethane (Alphatec, P.A. – ACS, purity 99%). An organic fraction was collected and dried with anhydrous sodium sulfate (Vetec, P.A – ACS, purity 100%), and then filtered on qualitative filter paper (Unifil, C42). The supernatant was kept in a 125 mL becker bottle (Qualividros, Boron 3.3) wrapped with aluminum foil, in an environment protected from light and heat above 25 °C.

Then, the yield of essential oil mass obtained was calculated according to equation 1, described by Menezes Filho et al. (2020).

$$\%Yield = \frac{EO_{mass}}{Weigth_{fresh\ mass}} \cdot 100 \quad \text{Eq. [1]}$$

Where EO is the mass of essential oil obtained after extraction, and $Weigth_{fresh\ mass}$ is the mass of inflorescences.

The solubility of essential oil was determined in a solution of 70% (v/v) ethanol (LChemicals, P.A – ACS, purity 99.5%) as described by Alarcón et al. (2019). In an *Eppendorf* (Videplast) tube an aliquot of 100 μL of hydroethanolic solution 70% about 2 μL of essential oil was added. The tube was homogenized in Vortex type equipment (Labor Import, Mod. Multifunctional 220) at 100 rpm for 5 minutes.

The refractive index test was performed in a refractometer (Hanna Instruments, Mod. HI96800) with refractive indexes between (1.3330 to 1.5080) and resolution of 0.0001 to 20 $^{\circ}\text{C}$, according to Alarcón et al. (2019).

The optical rotation test was determined in polarimeter provided with a 10 mL cell at a temperature of 20 $^{\circ}\text{C}$ and line α_{D} of the sodium lamp at 589.3 nm (Novainstruments, Mod. WXG-4) with a measurement range of -180° to $+180^{\circ}$ on the Vernier scale. The sample was prepared with 10% (w/v) of essential oil in 96% ethanol (LChemicals, P.A – ACS, purity 99.5%), as described by Alarcón et al. (2019).

For relative density, a pycnometer (RBR, Mod. 0892) of 1 mL was used. The pycnometer was clean and dry and weighed empty and the mass determined. Then 1 mL of essential oil was added, and subsequently its mass was determined and annotated. The density was expressed in g mL^{-1} at 20 $^{\circ}\text{C}$ and determined according to equation 2 as described by Alarcón et al. (2019).

$$\text{gmL}^{-1}20^{\circ}\text{C} = \frac{[(\text{Pic} + \text{Sample}) - (\text{Picvz})]}{V(\text{mL})} \quad \text{Eq. [2]}$$

Where Pic means Pycnometer, Picvz is an empty pycnometer and V is the volume of EO in mL.

To identify the classes of essential oil compounds from *B. monandra* flower, TLC aluminum sheets of silica gel pre-coated (DC-Fertigfolien Alugram[®], Xtra SILG/UV₂₅₄) were used. Small strips of the chromatographic plate were cut to the following size of 2 cm wide x 10 cm high. Spots were applied using glass capillary, approximately 1 cm from the lower edge. After drying, the plates were eluted with different solvents in a chromatographic tank, such as acetone (Alphatec, P.A – ACS, purity 99,9%), hexane (Dinâmica, P.A – ACS, purity 100%) and dichloromethane (Alphatec, P.A – ACS, purity 99,9%), using 10 mL in proportions of hexane/dichloromethane (1:1) and acetone/petroleum ether (1:1). Spots were developer using, sulphur vanillin, ferric chloride, UV light in long and short wavelengths (365 and 254 nm) and exposure to Iodine steam as described by Li et al. (2019), Pitakpawasutthi et

al. (2016), and Silva et al. (2009). In each elution system, the retention factors (R_f s) of the observed spots were calculated using equation 3.

$$R_f = \frac{ds}{dm} \quad \text{Eq. [3]}$$

Where R_f is the retention factor, ds is the distance that the compound has moved from the base, dm is the distance traveled by the spot in the plate.

The composition of the essential oil was also evaluated by GC-MS. In this experiment a PerkinElmer GC Clarus 580 equipment coupled with MS Clarus SQ 8S was used, the injector, interface and source temperatures were 250, 270 and 270 °C respectively, using electron impact at 70 eV. The analysis was performed using the following heating method, 60 °C for 2 min., rising to 270 °C at a ratio of 12 °C min.⁻¹ and remaining for 1 min, helium gas flow was 1 mL min⁻¹, 1 µL of sample dissolved in ketone (Alphatec, P.A – ACS, purity 100%) was injected. The column used was a DB-5MS (30 m, 0.25 mm ID, 0.25 µm).

The identification of the compounds was performed from the *n*-alkane calibration standar series (C7 – C40) (Sigma-Aldrich), and the mass spectrum was compared to the literature of Adams (2007) and by the Nist 11 Spectroteca.

The statistical analysis consisted of triplicates from the arithmetic mean, followed by (±) standard deviation. The statistical software used was PAST 3 (free version, 2019).

3. Results and Discussion

The essential oil of *B. monandra* flower showed a yield of 0.06 ± 0.09%, the organoleptic characteristics showed as a translucent, homogeneous, crystal clear, aromatic and oil liquid to the touch.

It is remarkable the number of studies on the essential oil yield of the genus *Bauhinia* in the literature as shown in the Table 1. Sharma et al. (2013) in their study found an essential oil yield of 0.3% for *Bauhinia variegata* flowers obtained by hydrodistillation. However, Da Silva et al. (2020) extracted the essential oil of two species, *Bauhinia rufa* and *Bauhinia dumosa*, and found yield of 0.01 and 0.03%, respectively. Table 1 present different yields found to genus *Bauhinia* in the different places in Brazil.

Table 1. Essential oil yield to genus *Bauhinia* in different states of Brazil.

Specie	Yield (%)	State (Brazil)	Reference
<i>Bauhinia unguolata</i>	0.01	Ceará	De Sousa et al., 2016; Gois et al., 2011
	0.07	Roraima	Medeiros et al., 2016
<i>Bauhinia unguulate</i>	0.02	Ceará	De Sousa et al., 2016; Gois et al., 2011
<i>Bauhinia acuruana</i>	0.02	Ceará	De Sousa et al., 2016; Gois et al., 2011
<i>Bauhinia pentandra</i>	0.01	Rio de Janeiro	De Almeida et al., 2015
<i>Bauhinia forficata</i>	0.02	São Paulo	Sartorelli; Correa, 2007

Source: De Sousa et al. (2016), Medeiros et al. (2016), De Almeida et al. (2015), Gois et al. (2011), e Sartorelli; Correa (2007).

The solubility analysis showed that it was positive in 70% hydroethanolic solution, and a refractive index of 1.3621 ± 0.07 , optical rotation of $+36.4 \pm 0.02 \alpha_D$ and the relative density of $0.941 \pm 0.05 \text{ g mL}^{-1}$ were obtained.

Very little are known about physicochemical properties of essential oils of *Bauhinia* flower in the literature, thus the *B. monandra* flower essential oil was compared with other essential oils. Méndez et al. (2015) evaluated the essential oil of *Plectranthus amboinicus* in two extraction processes and obtained refractive indexes of 1.4753 and 1.5065, positive solubility for both samples, optical rotation of -0.283 and -0.267 and density of 0.947 and 0.947 g mL^{-1} . Santos et al. (2012) found relative density of 0.924 g mL^{-1} and optical rotation of $+36.8 \alpha_D$ for the essential oil of *Piper malacophyllum* leaves.

Experiments to evaluate the composition of the essential oil were performed and Table 2 presents the results of the analysis by TLC and respective retention factors (R_fs) for compounds detected in the analysis of essential oil of the flowers of *B. monandra* using different elution systems and developers.

Table 2. Results of TLC of essential oil from *B. monandra* flowers, collected in Rio Verde - GO, Brazil, in 2019.

Eluentes	UV 254nm	UV 365nm	Vanillin Sulfuric	Iron Chloride	Iodine	Classes Chemicals
Acetone	0,86*	-	0,80* 0,90*	0,84*	-	Hydrocarbons
Hexane	-	0,06*	-	-	0,12* 0,13*	Oxygenates
Dichloromethane	0,58*	0,11* 0,18* 0,39*	0,15*	-	0,33* 0,49*	Oxygenates
Petroleum Ether	0,58*	0,55*	-	-	0,07*	Oxygenates
Acetone/petroleum ether	0,81*	-	0,80*	0,86* 1,0*	-	Phenolic
Hexane/dichloromethane	0,59*	0,56*	-	-	0,24* 0,29* 0,34* 0,39* 0,49* 0,50*	Oxygenates

*R_fs Holding factor, determined in millimeters (mm). Source: Own, (2020).

The results obtained in the analysis by TLC shown that the eluent dichloromethane presented a greater number of compounds (7) using different revelation systems. However, it was observed a variation in the R_fs, which is related to the interaction of each compound with the stationary phase and denotes the presence of compounds of different polarity (polar compounds, medium polarity and apolar) and this analysis, can give important contribution about number of spots present in the essential oil. Compared to hexane, the elution with dichloromethane showed better separation, which makes it easier to identify the presence of different constituents in the essential oil. Silva et al. (2009) evaluating by TLC the essential oil of lemon observed the same behavior, however the researchers obtained for the eluent dichloromethane the highest R_fs.

In the TLC analysis, mainly considering monoterpenes, it was identified only 3 compounds for the eluent hexane, for dichloromethane 7 compounds and especially for the solution of hexane/dichloromethane 8 compounds were observed in the separation. The eluted compounds in dichloromethane were revealed under UV₃₆₅ nm light which suggests the presence of aromatic compounds, and the other 6 compounds were revealed using a dichloromethane/hexane solution combined with exposure to Iodine steam, which is considered one of the universal revealers. This may be related to a rapid reaction, where the Iodine atom is complex to oxygenated and/or aromatic functional groups (Collins et al., 2006).

The results obtained using the developers Iodine, UV light and sulfuric vanillin, revealed the largest number of compounds, especially for Iodine, 11 compounds was observed. Ferric chloride has not been shown to be the best option evaluated separately, as only 3 compounds were observed after discoloration. When the plates was submitted to UV lights in short UV₂₅₄ nm and long UV₃₆₅ nm, 5 and 6 separate compounds were revealed suggesting the presence of conjugation and fluorescence, respectively.

An interesting feature was observed during the analysis by TLC, most of the compounds visible under UV light were observed due to the previous use of ferric chloride or sulphuric vanillin in the eluent dichloromethane. The application of sulphuric vanillin and ferric chloride in combination with hexane: dichloromethane eluents and in petroleum ether even after exposure to UV light did not reveal any class of compounds. However, the developers vanillin sulphur and ferric chloride combined with eluent acetone or acetone/petroleum ether and exposed to UV₂₅₄ nm, showed different plots suggesting the presence of classes of phenolic compounds.

From Table 1, it is observed that the oxygenated compounds presented *R_fs* between 0.58 and 0.59 revealed with UV₂₅₄ nm light, between 0.06 and 0.56 revealed with UV₃₆₅ nm light, and with Iodine steam between 0.12 and 0.50. With this evaluation, it can be discussed that polar compounds with lower *R_fs* interacted more with silica, and that for the higher *R_fs* did not present such interaction, this is due to the interactions of polarities of the eluents used, and of the groups of compounds evaluated.

Although the TLC does not provide specific measures, only knowledge about class of compounds and number of spots, as a preliminary analysis, it is effective when used in combination with other techniques such as GC-MS. Several chromatographic methods are developed to standardize the analysis mainly for compounds originating from medicinal plants, which include GC-MS to ensure that there is quality, efficacy and safety in the results with the aim of developing new drugs for pharmacological use (Valle Jr. et al., 2016).

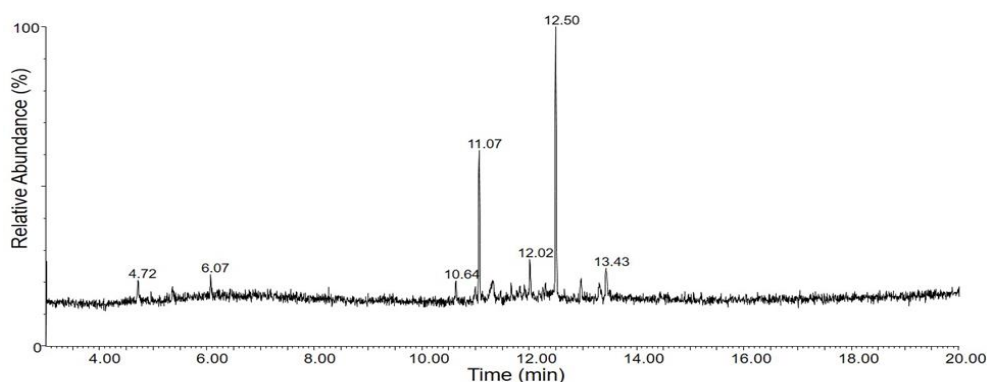
According to Chiaradia et al. (2008), Andrey (2003), and Kitson et al. (1996), the combination of GC and MS is relatively simple, where the gas chromatograph is compatible with the use of the vacuum produced for MS. The ionization methods with the highest employability in GC-MS are electronic impact (EI) and chemical ionization (CI). In EI the analyte, in gas phase, is bombarded with high energy electrons, with a power of 70 eV, producing fragmented ions, which help in the identification of compounds. In this technique, molecules in the form of gas absorb this energy by triggering several processes, and the analyte is ionized by removing a single electron (M^+ or M^-). In this process, the energy use is

10 eV and the rest of the energy generates the fragmentation in the analyte. However, there is a disadvantage in the application of IE, the fragmentation of a compound occurs with rapid reaction, the molecular ion may not be observed in the GC-MS total ion chromatogram (TIC), losing important information in MS.

Besides the analysis by TCL, the essential oil of the *B. monandra* flowers was evaluated by GC-MS. After the chromatographic analysis, the GC-MS total ion chromatogram (TIC) and fragmentogram or mass spectrum are obtained.

Figure 1 shows the TIC obtained for the essential oil of *B. monandra* flowers from GC-MS analysis. By the TIC it is possible to observe two peaks of maximum intensity with retention times of 11.07 and 12.50 minutes, which when compared with the equipment library and with data of alkane patterns, correspond to the panaxene and α -guaiene compounds, respectively.

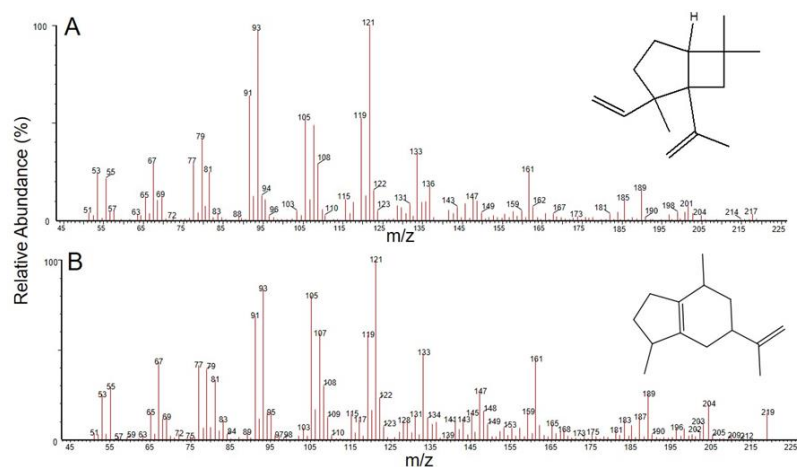
Figure 1. GC-MS total ion chromatogram of essential oil from *Bauhinia monandra* flower.



Source: Authors, (2020).

In Figure 2 the characteristic GC-MS mass spectra of the panaxene and α -guaiene majority compounds are presented, the comparison between the mass spectrum fragments obtained with that of the NIST 11 library showed a similarity of 84.5 and 83.6 % as shown in Table 3, both compounds present $m/z = 204$, characterized by similar fragment ions as $m/z = 93$, $m/z = 105$ and $m/z = 121$.

Figure 2. GC-MS mass spectrum of compounds with a retention time of (A) 11.07 min as panaxene, and in (B) 12.50 min as α -guaiene.



Source: Authors, (2020).

Table 3 presents the compounds with retention times, Kovats' literary indexes, relative percentage areas for the peaks and the similarity with the library for each compound. Only 7 compounds were observed, panaxene with 20.51% and α -guaiene with 33.39%, as the two highest peaks detected in relation to the total area of compounds analyzed. The essential oil of *B. monandra* flower showed about 70.22% sesquiterpene compounds and only 7.13% monoterpenes. These data are in accordance with the analyses made by TLC where 7 compounds were detected.

Table 3. Chemical profile obtained by GC-MS of the essential oil from *Bauhinia monandra* flowers.

Compounds	TR*	IKlit	AR (%)	Similarity (%)
3-Carene	4,72	1004	3,14	86,6
D-Limonene	6,07	1031	3,99	80,2
1,5-Cyclodecadiene,1,5-dimethyl-8-(1-methylethylthienyl)-,[S-(Z,E)]	10,64	1099	3,29	83,3
Panaxene	11,07	1312	20,51	84,5
1-Isopropyl-4,7-dimethyl-1,2,3,4,5,6-hexahydronaphthalene	12,02	1406	5,99	74,3
α -Guaiene	12,50	1436	33,39	83,6
<i>Epi-Zonarene</i>	13,43	1499	7,04	61,4
Monoterpenes			7,13%	
Sesquiterpenes			70,22%	
Total			77,35%	

*TR = Retention Time. **IKlit = Literary Kovats Index, Adams (2007). ***AR = Relative Area. Source: Authors, (2020).

Corroborating to this study, Gois et al. (2011) analyzing essential oil of *Bauhinia acuruana* leaves detected 91.4% of sesquiterpenes; Gramosa et al. (2009) for essential oil of *Bauhinia unguolata* leaves detected 74.3% sesquiterpenes; and Duarte-Almeida et al. (2004) for essential oils of *Bauhinia aculeate*, *Bauhinia brevipes*, *Bauhinia forficata*, *Bauhinia longifolia*, *Bauhinia pentandra*, *Bauhinia rufa* and *B. variegata* found 100%, 83.97%, 88.4%, 89.0%, 100%, 100% and 90.5% of sesquiterpenes, respectively. The genus *Bauhinia* presents rich essential oil in the class of sesquiterpene compounds, however, the species *Bauhinia pulchella* (De Sousa et al., 2016) presented for the essential oil of leaves, higher percentage of monoterpenes of 55.8% and sesquiterpenes of 39.8%.

We can emphasize here that the developed work besides giving a scientific contribution and showing the capacity of different techniques of analysis, passing through a classic technique and being confirmed by a modern one, still allows the students the knowledge of the techniques and the development of the work as participation in the course of chromatography. Having direct contact with the materials and methods, the students were able to assemble the experiments and discuss theories and practices, and it was possible to develop a work that resulted in a publication.

4. Conclusions and Suggestions

This is the first study reporting the essential oil of *Bauhinia monandra* flowers, an exotic species in Brazil. As for the essential oil yield, it presents similar to the few data in the literature consulted for this genus. The organoleptic and physicochemical properties are similar to other essential oils of numerous plant species assessed. However, the focus of the study was to characterize the essential oil and compare the techniques by TLC and GC-MS among the study of numerous chromatographic techniques.

In this study, it is possible to conclude that the analysis by TLC presents relative ease acquisition of the commercial ready plate and the reagents. However, the technique by GC-MS presents greater robustness and greater efficiency in the separation of compounds, which were identified and characterized. The study of chromatographic separations described in this study presents two proposal, characterization of a new essential oil and elucidate the teaching of chromatography in higher education and in postgraduate studies, mainly in the area of natural products.

To future work, experiments should be carried out, evaluating the essential oil of *B. monandra* toward different types of oxidizing agents to application in the pharmaceutical and

chemical industry, also as an antioxidant in the food industry and, as a biological agent in the form of micro and nanocapsules, as well nanospheres incorporated with chitosan or calcium chloride in phytopathogens and pathogens also to application in the agricultural and biotechnology industries.

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