Chemical, physicochemical profile and dose-response effect of Hymenaea courbaril essential oil on bacteria

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Abstract

Hymenaea courbaril is a tree species belonging to the genus *Hymenaea* and family Fabaceae. This study aimed to extract the essential oil from the fruit peel and evaluate the physicochemical characteristics and bacterial activity. The fruits were collected in the municipality of Santa Helena de Goiás, Goiás, Brasil. The bark was crushed and the powder obtained used in the extraction of the essential oil in the Clevenger-type system. The physical-chemical evaluation was performed for organoleptic analysis, refractive index, relative density, optical rotation and solubility in 70% hydroethanolic solution. The chemical profile was evaluated by gas chromatography with a coupled mass emitter. The antibacterial assay was performed in a *Petri* dish at different oil concentrations and the antibiosis halo (mm) when present was evaluated with the aid of a caliper. The essential oil of the peel of the fruit of *H. courbaril* was aromatic, clear, slightly aromatic, presenting physicochemical characteristics similar to the several essential oils of plants. It demonstrated a chemical profile rich in molecules and effective antibacterial action.

Keywords: *Hymenaea* genus, *Staphylococcus aureus*, *Enterococcus faecalis*, Germacrene, Sesquiterpenes

Resumo

Hymenaea courbaril é uma espécie arbórea pertencente ao gênero *Hymenaea* e familia Fabaceae. Esse estudo teve por objetivo, a extraçã do óleo essencial da casca do fruto e avaliar as caracterísicas físico-químicas e atividade bacteriana. Os frutos foram coletados no municipio de Santa Helena de Goiás, Goiás, Brasil. A casca foi triturada e o pó obtido utilizado na extração do óleo essencial no sistema tipo Clevenger. A avaliação físico-química foi realizada para análise organoléptica, índice de refração, densidade relative, rotação óptica e solubilidade em solução hidroetanólica 70%. O perfil químico foi avaliado por cromatografia gasosa com emissor de massas acoplado. O ensaio antibacteriana foi realisado em placa de *Petri* em diferentes concentrações de óleo e o halo de antibiose (mm) quando presente, foi avaliado com auxílio de paquímetro. O óleo essencial da casca do fruto de *H. courbaril* apresentou ser aromático, límpido, levemente aromático, apresentando características físico-químicas similares aos diversos óleos essenciais de plantas. Demonstrou perfil químico rico em moléculas e efetiva ação antibacteriana.

Palavras-chave: Gênero *Hymenaea*, *Staphylococcus aureus*, *Enterococcus faecalis*, Germacreno, Sesquiterpenos

Resumen

Hymenaea courbaril es una especie arbórea perteneciente al género Hymenaea y a la familia Fabaceae. Este estudio tuvo como objetivo extraer el aceite esencial de la cáscara de la fruta y evaluar las características fisicoquímicas y la actividad bacteriana. Los frutos fueron recolectados en el municipio de Santa Helena de Goiás, Goiás, Brasil. La corteza fue triturada y el polvo obtenido se utilizó en la extracción del aceite esencial en el sistema tipo Clevenger. La evaluación físico-química se realizó para análisis organoléptico, índice de refracción, densidad relativa, rotación óptica y solubilidad en solución hidroetanólica al 70%. El perfil químico se evaluó por cromatografía de gases con un emisor másico acoplado. El ensayo antibacteriano se realizó en una placa de Petri a diferentes concentraciones de aceite y el halo de antibiosis (mm) cuando estaba presente se evaluó con la ayuda de un pie de rey. El aceite esencial de la cáscara del fruto de H. courbaril fue aromático, claro, ligeramente aromático, presentando características fisicoquímicas similares a los diversos aceites esenciales de plantas. Demostró un perfil químico rico en moléculas y una eficaz acción antibacteriana.

Palabras clave: Género *Hymenaea*, *Staphylococcus aureus*, *Enterococcus faecalis*, Germacreno, Sesquiterpenos

1. Introduction

Brasil in the environment is highlighted in terms of its number of biomes and Cerrado domain, presenting a rich diversity in species of fauna and flora in the Americas. In particular, the Cerrado domain is located in the central region, interconnecting all Brazilian biomes (Elias et al., 2019). Menezes Filho et al. (2022) and Bueno et al. (2018) characterize the Cerrado as a neotropical savanna, and this environment is the second largest in natural area in South America, behind only the Amazon biome.

The Cerrado has, in its various phytophysiognomies, a plurality of plant species obtaining recognized privilege of Hotspots (Vieira et al., 2019). In this environment, more than 11,000 species of native plants are described (Mendonça et al. 2008), of which 4,400 are endemic (Myers et al., 2000). In addition, it is estimated that 1/3 of the entire Brazilian biota and around 5% of the worlds fauna and flora is inserted in this environment of marked contrasts (Bueno et al., 2018).

Among this exuberant flora, Oliveira et al. (2020) highlights, among angiosperms, the family Fabaceae Lindl. belonging to the order Fabales (APG IV 2016), which is an important group of plants with about 727 genera, with 19,325 species, among which *Hymenaea courbaril* stands out (Souza et al., 2016). *H. Courbaril* is popularly known as "jatobá, jutaí, jataí-açu, jataí-bravo, jutaí-grande, jataí-peba, jataí-uba or jatobá-do-campo". This species has aroused both economic interest in civil and naval construction, in the ornamentation of parks and gardens, in reforestation and also in medicine (Santos et al., 2019).

H. courbaril positively influences data on phytochemical prospecting, having several groups belonging to special metabolism considered of medical interest, with important results on numerous biological activities, and among the population's knowledge of phytotherapy, in the treatment of bronchitis, cough, pneumonia and influenza (Silveira et al., 2020). In several scientific studies, such as de Silva et al. (2019), the researchers evaluated the extract obtained from the peel of the fruit of *H. courbaril* through response surface analysis, predicting to optimize the extraction of phenolic compounds, where it exhibited important content of phenolic compounds and extractable total flavonoids. In addition to extracts, fractions and subfractions, the essential oil can also be obtained from species of *Hymenaea*, Menezes Filho et al. (2020) evaluated this group of volatile molecules obtained from the bark of *H. stigonocarpa* and *H. courbaril*, where they exhibited an important dose-response on the antioxidant activities in reducing the DPPH free radical and antifungal on *Sclerotinia sclerotiorum*, *Colletotrichum acutatum*, *C. gloresporioides*, *Aspergillus flavus* and *A. niger*.

Essential oils are included among the important classes of special metabolites that the various plant groups produce, thus presenting, in their composition, terpenic, diterpenic, sesquiterpenic and phenylpropanoid compounds, oxygenated or hydrocarbons, with a physiological purpose in the protection of the plant, as a means of attraction pollinators, as well as, for medicine, in important acetylcholinesterase inhibitors, with antifungal, antibacterial, antiviral, cytotoxic, allelopathic activities, among others (Everton et al., 2020).

Volatile compounds extracted from different organs of plants have shown good inhibition results on important groups of pathogenic microorganisms such as *Candida* and *Salmonella*. Essential oils in several plant groups have been and are extensively tested for their important anti-*Candida* activity (Hamdy et al., 2020; Potente et al., 2020; Garcia et al., 2021) and on *Salmonella* cv. Enteritidis and Typhimurium, microorganisms that annually cause serious health problems in humans and animals, where in some cases deaths from contamination are recorded (Mortazavi; Aliakbarlu, 2019; Čabarkapa, et al., 2019; Corrêa et al., 2021). *H. courbaril*, as previously noted, already comprise a large number of studies, however, it still presents a vast field of research, mainly evaluating the volatile compounds of the fruit peel on important fungal and bacterial groups of medical interest.

Therefore, this study aimed to determine the chemical, physicochemical profile and to evaluate the dose response of the essential oil from the peel of the fruit of *Hymenaea courbaril* on bacteria. Acceptable citation models are: according to Menezes et al. (2020) or according to the data (Menezes et al., 2020).

2. Material and Methods

The peel of the fruit of *H. courbaril* was collected in January 2022 in a permanent protection area located in the municipality of Santa Helena de Goiás, State of Goiás, Brasil. The fruit peel was fragmented into pieces with a maximum of 2 cm^2, and then they were ground in a cyclone-type knife mill to obtain a fine and homogeneous powder. Voucher material (HRV 7.0005) was deposited at the Herbarium of the Department of Biology Science of Instituto Federal Goiano, Rio Verde, Goiás State, Brasil.

Essential oil extraction was performed with 200g of fruit peel powder in 3 replications. The essential oil from fruit peel was obtained by hydrodistillation for 3 h in a Clevenger-type apparatus. The essential oil percentage yield (%) was calculated as volume of oil and the weight of fresh material (*w/v*). The oil was recovered in dichloromethane and stored under refrigeration after removal of the solvent, according by Souza et al. (2021).

The color and appearance of the essential oil were evaluated by the organoleptic analysis method (Gomes et al., 2018). The essential oil solubility was determined in a 70% (*v/v*) hydroethanolic solution, as described by Alarcón et al. (2019). An aliquot of 100 μL of 70% hydroethanolic solution and 2 μL of essential oil was added to an *Eppendorf* tube. The tube was homogenized for 5 minutes.

The refractive index test was performed on a digital refractometer with a refraction range between (1.3330 - 1.5080) and a resolution of 0.0001 at 20 °C as described by Alarcón et al. (2019) modified. The optical rotation test was performed in a polarimeter with a 10 mL cell at a temperature of 25 °C and an α_D line and a sodium lamp at 589 nm with a measuring range (-180° to +180°) on the Vernier scale. The sample was prepared with 10% (*m/v*) of essential oil in 98% ethanol, proposed by Alarcón et al. (2019). The relative density was obtained in a 1 mL pycnometer. The pycnometer was cleaned (Isopropyl alcohol) and dried (25 ºC) and its mass determined. Then 1 mL of oil was added and the mass determined again. Density was expressed in g mL^{-1} at 20 °C according to Alarcón et al. (2019).

The chemical profile of the essential oil was also evaluated by GC-MS. In this experiment, a PerkinElmer GC Clarus 580 was used. Equipment coupled to MS Clarus SQ 8S was used, the temperatures of the injector, interface and source 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 rate of 12 °C min^{-1} and holding for 1 min, the helium gas flow was 1 mL min^{-1}. One μL of sample dissolved in ketone was injected. The column used was a DB-5MS (30 m, 0.25 mm ID, 0.25 μm). The identification of compounds was performed using the standard series of calibration of *n*-alkanes (C7 – C40), and the mass spectrum was compared with the literature by Adams (2007) according by Menezes Filho et al. (2020).

Bacterial strains were obtained from the authors' private microorganism bank. The microbiological assay followed was described by Vieira et al. (2021) adapted, using the paper disk diffusion technique. Strains of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (LB 29212), *Salmonella serovar* Enteritidis (ATCC 13076) and *Salmonella serovar* Thyphymurium (ATCC 14028) were used. The activation of the microorganisms was carried out in a sterile solution of NaCl concentration 0.85% (*m/v*) until reaching 0.5 on the MacFarland conc. ($1x10^8$ CFU mL^{-1}) in a UV-*Vis* spectrophotometer. *Petri* dishes (10 cm^2) were prepared with Colony Counting Agar (CCA) after sterilization. *Petri* dishes containing specific medium were inoculated with a sterile *Drigalski* loop soaked in microbial suspension and spread over the agar in the plate.

Filter paper discs with a diameter of 7 mm were impregnated with 100 μL of the essential oil at different concentrations (100, 50, 25, 5 and 2.5 mg mL^{-1}), as a negative control, saline and dimethyl sulfoxide were used. at 10% (DMSO) (*v/v*), and as a positive control, discs containing antimicrobial agents, Azithromycin (15 μg), Cephalexin (30 μg) and Tigecycline (15 μg). *Petri* dishes were incubated at 36 °C with an interval between 24-36 h, after this period, the halo of antibiosis, when present, was measured with the aid of a digital caliper. The minimum antibiosis halo was 5 mm. The test was performed in quadruplicate.

3. Results and Discussion

The essential oil from the peel of the fruit of *H. courbaril* showed an extraction yield of 0.086% ± 0.06. A similar result was obtained in the study by Menezes Filho et al. (2020) with *H. stigonocarpa* and *H. courbaril* where the researchers obtained yields of 0.04% and 0.06% for the skin of both fruits. According to Ozcan & Chalchat (2002) the yield of essential oil in different vegetables is dependent on seasonal variation, locality and we also complement that this variation also occurs regarding the type of plant organ, flower, bark, trunk, fruits, leaves, roots, among others. The organoleptic color was light yellow, aromatic, crystalline and limpid. Solubility was positive in 70% hydroethanolic solution. The refractive index was 1.5031 at 20 °C. Optical rotation was +96° α_D. The relative density was 0.981 g mL^{-1} at 20 °C. Due to the low content of data on essential oils of the genus *Hymenaea*, a comparison with other essential oils was performed. Therefore, Gomes et al. (2018) described for the essential oil of clove (*Syzygium aromaticum*) a transparent, limpid, aromatic color, with positive solubility for ethanol (90%), refractive index of 1.526 and relative density of 0.973 g mL^{-1}, similar and close to our findings.

Eighteen compounds were identified in the essential oil of the peel of the fruit of *H. courbaril*, the majority being α-copaene, caryophyllene E, valencene, α-chamigrene, δ-cadiene, *Trans*-dauca-4-11-7-diene, spathulenol and caryophyllene oxide (Table 1). Sales et al. (2014) describe for the essential oil of the peel of the fruit of *H. courbaril*, 23 compounds, all belonging to the class of sesquiterpenes. The major compounds were α-coapene 8.46%, (*Z*)-β-caryophyllene 17.56%, germacrene-D 17.61%, bicyclogermacrene 6.46% and caryophyllene oxide with 14.65%. Pereira et al. (2007) found the following major molecules in the essential oil extracted from the resin of *H. courbaril* β-caryophyllene 60.5%, caryophyllene oxide 20.7% and α-humulene 2.3%.

Table 1. Chemical composition of the essential oil from the peel fruit of *Hymenaea courbaril* L., analyzed by gas chromatography coupled with mass spectrometry (GC-MS).

Retention time (min)	**Compound name**	**Area (%)**
9.096	α-Ylangene	1.07
9.156	α-Copaene	**6.21**
9.306	β-Elemene	2.41
9.771	Caryophyllene E	**8.55**
10.017	Aromadendrene	2.17
10.202	α-Humulene	0.66
10.418	*Cis*-Muurola-4(14),5-diene	3.83
10.554	γ-Muurolene	0.17
10.603	Valencene	**7.29**
10.666	α-Amorphene	4.13
10.742	α-Chamigrene	**15.22**
10.816	β-Himachalene	4.44
10.983	δ-Cadiene	**10.73**
11.010	*Trans*-Cadina-1,4-diene	6,77
11.564	*Trans*-Dauca-4-11-7-diene	**11.00**
11.780	Spathulenol	**9.05**
11.877	Caryophyllene oxide	**5.51**
14.211	Amorpha-4,7(11)-diene-2-α-hydroxy	0.40
Total identified (%)	-	99.61

Source: Authors, 2022.

The bacteria *E. coli*, *S. aureus* and *E. faecalis* showed to be sensitive to the essential oil of the peel of the fruit of *H. courbaril* between the concentrations 100-2.5 mg L^{-1} (Table 2). The bacteria *S. serovar* Enteritidis and Typhymurium proved to be resistant, with only slight inhibition observed at the two highest doses 100-50 mg L^{-1}. All inhibition assays were compared to reference antibiotics, with our findings being inferior to commercial synthetic molecules. Statistically, our results demonstrate, in the entire trial, statistical difference according to *Duncan's* test with 5% significance applied. Although the essential oil has shown satisfactory bacterial inhibition action, further studies should be carried out isolating the major compounds, such as α-coapene, caryophyllene, valencene, α-chamirene, δ-cadiene, *Trans*-dauca-4-11-7-diene and caryophyllene oxide where we suggest that these molecules are responsible for the growth inhibition potential on this select group of Gram-positive and Gram-negative bacteria.

Our findings demonstrate that the essential oil of the peel of the fruit of *H. courbaril* presents greater activity in the Gram-negative group (*E. coli*, *S. serovar* Enteritidis and *S. serovar* Typhymurium). Although Burt (2004) and Sales et al. (2014) discuss both groups (Gram) in different bacterial genera and species, these researchers suggest that essential oils act mainly on Gram-positive bacteria.

Sales et al. (2014) evaluated two strains of *S. aureus* (ATCC 6538P and ATCC 14458) where they describe in a study with the essential oil of the peel of the fruit of *H. courbaril* for minimum inhibitory concentration (MIC) and lethal inhibitory concentration (LIC) of 0.28-0.28 and 0.28-0.56, respectively. Where the ATCC 1458 strain proved to be less sensitive to the action of the essential oil. The essential oil of *H. courbaril* resin exhibited antimicrobial potential against *Pseudomonas aeruginosa* with 13 mm, promising results were also described for *S. serovar* Typhymurium, *S. aureus, E. coli* and *Streptococcus haemolyticus* by the agar diffusion method in the study by Pereira et al. (2007).

Table 2. Antibacterial activity of essential oil from the peel of the fruit of *Hymenaea courbaril* L.

	Inhibition Zone (mm)				
Microorganisms	**100 mg mL^{-1}**	**50 mg mL^{-1}**	**25 mg mL^{-1}**	**5 mg mL^{-1}**	**2.5 mg mL^{-1}**
E. coli	13.07b	11.54c	9.04d	5.16e	0.00f
S. aureus	17.63b	15.41c	12.32d	8.60e	5.26f
E. faecalis	14.50b	12.91c	9.66d	5.71e	0.00f
S. Enteritidis	10.06b	5.78c	0.00d	0.00d	0.00d
S. Thyphymurium	8.70b	6.05c	0.00d	0.00d	0.00d

Note: Different lowercase letters on the same line differ statistically by Duncan's test with 5% probability. Azithromycin, Cephalexin and Tigecycline. Source: Authors, 2022.

4. Conclusions

The essential oil obtained from the peel of the fruits of *Hymenaea courbaril* showed good yield and physicochemical characteristics comparable to the other essential oils of several vegetables. As a bactericidal effect, the oil was able to effectively inhibit the main bacteria of the Gram-negative group (*Escherichia coli*, *Salmonella serovar* Enteritidis and *serovar* Typhymurium), in addition to having activity for the Gram-positive group (*Staphylococcus aureus* and *Enterococcus faecalis*).

This important biological data, allows that future works of isolation of molecules of this oil can be used with substitutes or used in synergism with the main groups of antibiotics of synthetic origin in the market.

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