Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.1590/fst.18718

Phytochemical screening, Sun Protection Factor (SPF) and sugar analysis of jatobá fruits (*Hymenaea martiana* Hayne): A native medicinal plant from the San Francisco Valley

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Abstract

Hymenaea martiana is a native medicinal plant from the Caatinga, but biochemical studies of the fruit have not yet been reported. Thus, this study aimed to determine sugars and secondary metabolites, as well as assess the sunscreen potential of *H. martiana* fruits. The fruits were collected in Petrolina and separated into pulp and seeds. The sugar analysis investigated the presence of glucose, xylose, cellobiose, arabinose and xylitol. The determination of secondary metabolites was made through phytochemical screening and sunscreen activity was assessed with the spectrophotometric method. In the fruit pulp, carbohydrates with a great biotechnological potential were identified. The substances found in the phytochemical screening showed great antioxidant, photoprotective and medicinal potential. With the pulp extract, the sun protection factor values obtained were not significant and for the extract from the seeds, the values were 4.54 ± 0.11. Although the values found are below the recommended, the fruit extracts of *H. martiana* could be used in future development of sunscreen products providing several benefits to the formulation.

Keywords: biotechnological potential; fruits; *Hymenaea martiana*.

Practical Application: Determination of sugars, metabolites and sunscreen activity from Jatobá fruits. Application in food and cosmetics industries.

1 Introduction

Hymenaea is a genus of the Fabaceae family, highly distributed from Central America to South America, mainly in the Amazon basin, with about 25 species described in the Americas. Plants of the this genus are known in Brazil as "jatobá", "jetaí", "jataí-uva", "jetaíba" and are largely distributed in Brazil, especially in the Brazilian biome called "cerrado" (savanna-like vegetation) (Minas Gerais, Bahia, Goias, and Tocantins) and the Amazon forest (Boniface et al., 2017). One species of this genus can be found in Brazilian Caatinga, *Hymenaea martiana*, with a great phytochemical and medicinal potential (Oliveira et al., 2016; Oliveira et al., 2018).

Being characterized as a region of economic and agricultural importance in the Brazilian Caatinga Biome, the San Francisco Valley is a region in the west of the state of Pernambuco and north of Bahia, bordering the San Francisco River, with semiarid vegetation and climate (Sá et al., 2009). The particular characteristics of this biome, like the high solar incidence and long periods of drought, can interfere directly in the vegetation morphology and especially with its secondary metabolites composition, since some phenolic compounds such as flavonoids may play a special role in the chemoprotection against ultraviolet radiation and external aggressions (Markham et al., 1998).

Among the native medicinal plants found in the São Francisco Valley, with importance in the local traditional medicine, *Hymenaea martiana* Hayne (Fabaceae) can be highlighted, popularly known as "jatobá" in Northeastern Brazil. Shanley & Medina (2005) characterize this plant as a large tree, 15-20 m high, with dense foliage and thick bark, straight trunk, about 2 m in diameter.

The traditional use of jatobá has been reported, and the alcoholic extract from the barks of *H. martiana* has been used in the treatment of inflammations and rheumatism, and also as antinociceptive and analgesic (Neves et al., 1993; Gazzaneo et al., 2005). Some substances that have been linked to the pharmacological activities are flavonoids (Carneiro et al., 1993; Silva et al., 2012).

Fruits of other species of the genus were analyzed and in the pulp, rutin (Peres et al., 2013), fiber, ascorbic acid, α-tocopherol, β-sitosterol, oleic and linolenic acid (Dias et al., 2013), *D*-glucose, *D*-glucuronic acid and sucrose (Chung et al., 1997) were identified. In the seeds, *D*-glucose, galactose, xylose (Busato et al., 2001), arabinose (Omaira et al., 2007), hymenain and ipomopsin (Simões et al., 2009) were important substances found. These literature data demonstrate the great medicinal and chemical

Received 31 July, 2018
Accepted 11 Feb., 2019
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potential of the fruits and seeds of *Hymenaea*, with important sugars and phenolic compounds in the chemical composition.

Phenolic compounds, such as flavonoids, can absorb ultraviolet radiation (UV) and this class of substance can play an important role against UV radiation from sunlight (Oliveira-Júnior & Almeida, 2012). The UV radiation can be subdivided into three bands: UVA, UVB and UVC. The UVA radiation can be classified as an inducer of oxidative processes in the skin and the use of photoprotectors against UVA radiation has been stimulated, aiming at protection against skin diseases (Popim et al., 2008).

Sunscreens derived from natural products and cosmetic products have been developed using plant extracts and oil as raw material due to their photoprotective activity, and the popular acceptance has been positive (Oliveira-Júnior & Almeida, 2012).

Nevertheless, according to a literature review, neither phytochemical nor biochemical study on the fruit from *Hymenaea martiana* was found, and the photoprotective activity was not evaluated. In light of this, this study aimed to determine sugars and secondary metabolites, as well as analyze the sunscreen potential of *Hymenaea martiana* fruits.

2. Materials and methods

2.1 Plant material

The fruits of *Hymenaea martiana* Hayne were collected in Petrolina, Pernambuco, Brazil, in July 2015, and were identified by the Federal University of São Francisco Valley Herbarium (HVASF), with a voucher specimen n° 6444, coordinates 09”11’04.30 ° S, 040”18’05.40 ° W, 357 m high. The fruits were separated into two plant materials (pulp and seeds) and were pulverized using a mill (Quimis®, SP 31, Diadema, Brazil).

Table 1. Chromatographic conditions of the sugar analysis of jatobá fruits.

Parameters	Conditions
Detector	Refractive index, model ProStar 355 (Varian®, Palo Alto, USA)
Column	Hi-Plex® H 8 µm (300 x 7.7 mm) (Agilent®, Santa Clara, USA)
Column temperature	60 °C
Mobile phase	Sulfuric acid solution (0.005 mol/L)
Flow rate	0.6 mL/min
Analysis time	15 minutes

2.2 Sugar analysis using High-performance Liquid Chromatography (HPLC)

The determination and quantification of sugars (glucose, xylose and arabinose) were carried out through HPLC, using a liquid chromatograph equipped with a ProStar 210 pump model (Varian®, Palo Alto, USA), model 7725 manual injector (Rheodyne®, Sigma-Aldrich, St. Louis, USA), with a 20-µL loop. The chromatographic conditions are presented in Table 1.

The sample preparation was carried out with a dilution of 1 g of each sample (pulp and seed) with distilled water, in the rate of 1:20 (pulp/seed: solution) (dilution factor = 20). The solution was stirred, allowed to stand for a few minutes and filtered; then, 20 µL was injected into the equipment.

2.3 Phytochemical screening of the fruits of H. martiana

Initially, 100 g of pulp and seeds was added to 500 mL of ethanol 95% in amber flasks, which were then kept in the dark for three consecutive days at room temperature, shaken daily. After the maceration time, the residue was filtered. The procedure was repeated three times. After the process, the extraction solution was concentrated under vacuum (Fisatom®, model 801, São Paulo, Brazil) at 50 °C (Silva et al., 2012).

An aliquot of the extracts from each plant material was solubilized in chloroform and subjected to analysis through thin-layer chromatography (TLC) with silica gel 60 F_{254}, with aluminum support, applied with a micropipette and eluted with different solvent systems, according to Wagner & Bladt (1996), seeking to highlight the main groups of secondary metabolites (Table 2).

2.4 Assessment of sunscreen activity

The sunscreen activity was performed using the spectrophotometric analysis of the diluted solutions, according to Mansur et al. (1986). The extracts were previously dried in an oven at 40 °C for 60 minutes. Dilutions were prepared with concentrations of 5, 25, 50 and 100 mg.L^{-1}. Readings from 290 to 320 nm with 5-nm intervals were carried out in a spectrophotometer (Quimis®, model Q898UVDB, Diadema, Brazil), with quartz cells of 1-cm optical path.

Calculations of the Sun Protection Factor (SPF) were made considering the intervals λ determined using the following the equation described by Mansur et al. (1986): SPF = Correction Factor x Amount of absorbance 290-320 nm x Erythemogenic

Table 2. Elution systems and revelators used in the phytochemical screening.

Secondary metabolites	Elution Systems	Revelators
Alkaloids	Toluene: ethyl acetate: diethyl amine (70:20:10, v/v)	Dragendorff reagent
Anthracene derivatives	Ethyl acetate: methanol: water (100:13.5:10, v/v)	KOH 10% ethanolic reagent
Coumarins	Toluene: ethyl ether (1:1 saturated acetic acid 10%, v/v)	KOH 10% ethanolic reagent
Flavonoids and tannins	Ethyl acetate: formic acid: acetic acid glacial: water (100:11:11:26, v/v)	NP + PEG reagent
Lignans	Chloroform: methanol: water (70:30:4, v/v)	Vanilin phosphoric reagent
Mono and diterpenes	Toluene: ethyl acetate (93:7, v/v)	Vanilin sulfuric reagent
Naphthoquinones	Toluene: formic acid (99:1, v/v)	KOH 10% ethanolic reagent
Triterpenes and steroids	Toluene: chloroform: ethanol (40:40:10, v/v)	Lieberman-Burchard reagent

Effect of radiation (λ) x Spectrophotometric reading of sample absorbance (λ).

The EE (λ) (Eritemogenic Effect) and I(λ) (Solar Intensity) used for the calculation of the SPF (Sun Protection Factor) were the same found in the literature, and the abs (λ) was the spectrophotometric reading of the absorbance of the sunscreen solution (Mansur et al., 1986). The dilution factor was applied for equivalence correction and CF (Correction Factor) was 10 and the determinations were conducted in triplicates.

3 Results and discussion

3.1 Sugar analysis using High-performance Liquid Chromatography (HPLC)

The concentrations of sugars found in the pulp and the seeds of *H. martiana* are displayed in Table 3.

Glucose and xylose have been identified previously in *Hymenaea* in seeds (Omaira et al., 2007; Lima et al., 1993). A study on the pericarp of *Hymenaea oblongifolia* identified fructose, glucose, glucuronic acid, sorbose and sucrose (Chung et al., 1997).

A polysaccharide has been studied in *Hymenaea*, xyloglucan, which has been extracted from cotyledons (Buckeridge et al., 1997), seeds (Lima et al., 1993) and leaves (Busato et al., 2001). As one of the main hemicellulosic polysaccharides, xyloglucan is present in primary cell walls of dicotyledonous plants (Mcneil et al., 1984; Fry, 1989; Carpita & Gibeaut, 1993), but it was also found in the gymnosperm (Kakegawa et al., 1998). This carbohydrate is related to important functions such as the control of cell expansion, effects on growth and as a reserve of carbohydrate in seeds (Fry, 1989; Hayashi, 1989; Kai & Petkowicz, 2010). Galactose, glucose, xylose and arabinose were identified in this species, derived from the degradation of this polysaccharide present in seeds of this species (Omaira et al., 2007).

Regarding the measurements, the pulp has a higher amount of glucose, xylose and xylitol, as found in other fruits (Roesler et al., 2007). The seeds showed other types of sugars, such as arabinose, which have been previously described in *H. courbaril* (Omaira et al., 2007), and cellobiose, not yet reported in *Hymenaea*. According to the literature, this is also the first report of the presence of xylitol in species of this genus.

According to Mussatto & Roberto (2002), xylitol is a non-toxic polyol, classified by the FDA as a safe additive that may be used as a substitute for sugar (sucrose) and that has nutritional properties and benefits to the human health. It may also act in the cure or prevention of diseases, with various applications in the dental and medical fields and reports on the efficacy in the prevention and control of dental caries. Being a natural sweetener well tolerated by diabetics, it can be used as an adjuvant in the treatment of lipid metabolism disorder and renal injuries. In addition, xylitol can prevent lung infections, otitis and osteoporosis. In addition to these actions, it has the advantages of high chemical and microbiological stability, being a potential additive for food (Mussatto & Roberto, 2002). Thus, the biotechnological potential of the fruits of *H. martiana* is evident because of its important carbohydrates in the chemical composition.

3.2 Phytochemical screening of the fruits of H. martiana

A phytochemical screening was carried out with the extracts and the TLC plates were analyzed. The maceration of the fruits indicated the presence of anthracene derivatives, flavonoids, monoterpenes, diterpenes and naphthoquinones; the maceration of the seeds indicated the presence of anthracene derivatives and flavonoids (Table 4).

The analysis of the pulp extract indicated the presence of anthracenic derivatives, flavonoids, monoterpenes, diterpenes, naphthoquinones, triterpenes and steroids. Although there are no studies with the fruits of *H. martiana*, previous studies on fruits of other species of the genus identified substances with important pharmacological activities, such as the flavonoid rutin (Peres et al., 2013), the carotenoids β and α-tocopherol (Dias et al., 2013) and diterpenes such as spathulenol, stigmasterol and β-sitosterol, among other terpenes (Aguiar et al., 2010; Dias et al., 2013). The analysis of the extract from the seeds indicated the presence of anthracenic derivatives and flavonoids. However, studies were found in other species of the genus that only indicated the presence of coumarin (ipomopsin and himenain) (Simões et al., 2009) and xyloglucans (Lima et al., 1993; Buckeridge et al., 1997).

3.3 Assessment of sunscreen activity

The Sun Protection Factor (SPF) values were calculated according to Mansur et al. (1986) (Table 5).

Table 3. Sugars found in the fruits of *H. martiana*.

Sugars	Pulp ($mg.L^{-1}$)	Seeds ($mg.L^{-1}$)
Glucose	82,464.8	9,961.8
Xylose	61,472.8	13.6
Xylitol	27.4	1969.2
Cellobiose	N.D.	666
Arabinose	N.D.	5.4

Legend: N.D.: Not Detected.

Table 4. Phytochemical screening of the fruits of *Hymenaea martiana* submitted to maceration.

Secondary metabolites	Pulp extract	Seed extract
Alkaloids	-	-
Anthracene derivatives	+	+
Coumarins	-	-
Flavonoids and tannins	+++	+
Lignans	-	-
Mono and diterpenes	+++	-
Naphthoquinones	+	-
Triterpenes and steroids	-	-

Table 5. Sun Protection Factor (SPF) values in the UVA range (290-320 nm) from the extracts of the fruits of *Hymenaea martiana* (100 $mg.L^{-1}$).

Sample	SPF
Pulp extract	0.66 ± 0.41
Seed extract	4.54 ± 0.11

With the extract obtained from the pulp, SPF values obtained were not significant, with 0.66 ± 0.41. But the extract obtained from the seeds was 4.54 ± 0.11. According to the National Health Surveillance Agency (Brasil, 2012), the minimum value for SPF for sunscreen products is 6.0. Although the values found are lower than the recommended by the national health surveillance agency, the extracts obtained from the fruits of *H. martiana* could still be used for the future development of sunscreen products, as for example together with chemical filters, which would bring protection within the minimum values recommended, while also providing several benefits to the formulation with the supply of different secondary metabolites containing important medicinal properties. Currently, the inclusion of natural products in the photoprotective formulations is a tendency (Oliveira-Júnior & Almeida, 2012) and studies have focused on the analysis of chemical constituents containing chromophores and compounds with antioxidant activity (Polonini et al., 2011) such as the phenolic compounds, mainly the flavonoids (Oliveira-Júnior et al., 2012), substances found in phytochemical screening of the fruits and seeds of the species under study.

Despite the seed extract showed higher value for SPF then the pulp extract, it presented qualitatively less presence of flavonoids and tannins, as shown in Table 4. This fact may be due to the chemical composition of the seeds, with may include wax, oil and fatty acids. Previous study presented the cosmetic potential of the lipid fraction of seeds, as skin moisturizers and suscreens, with significant SPF values related to fatty acids as linoleic and oleic acid in the seeds of coffee beans (Wagemaker et al., 2011). So, the SPF values for the seed extract of *Hymenaea martiana* may be related not only to the phenolic compounds, but also to the lipid fraction of the seeds. Therefore, more in-depth studies are necessary to quantify and identify the major constituents of these materials, to improve the phytochemical knowledge of the species.

4 Conclusion

This study shows the great biochemical and medicinal potential of the fruit from *Hymenaea martiana*. The sugar analysis showed that several important carbohydrates are found in the pulp and seeds. The phytochemical screening identified important bioactive secondary metabolites and the sunscreen activity showed a great potential for the future development of cosmetic formulations.

Thus, bringing new data on the fruits of *Hymenaea martiana*, this study emphasizes the biotechnological potential of the species, aggregating chemical and medicinal value to a species native to the Caatinga of the São Francisco Valley, an important step for the conservation of its biodiversity.

Acknowledgements

The authors thank the collaboration of the Brazilian agencies CAPES and FACEPE for the financial support, and the cooperation of Biochemical Engineering Laboratory from the Federal University of Campina Grande. We also would like to thank teacher Abilio Borghi for the grammar review of the manuscript.

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