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ORIGINAL ARTICLE

Influence of the Extractive Method on the Recovery of Phenolic Compounds in Different Parts of *Hymenaea martiana* Hayne

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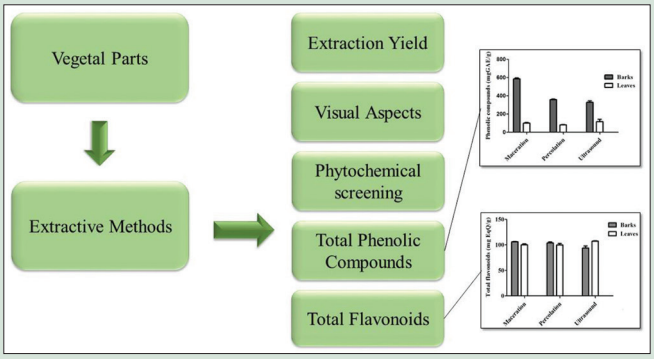
ABSTRACT

Background: Popularly known as "jatobá," *Hymenaea martiana* Hayne is a medicinal plant widely used in the Brazilian Northeast for the treatment of various diseases. **Objective:** The aim of this study was to evaluate the influence of different extractive methods in the production of phenolic compounds from different parts of *H. martiana*. **Materials and Methods:** The leaves, bark, fruits, and seeds were dried, pulverized, and submitted to maceration, ultrasound, and percolation extractive methods, which were evaluated for yield, visual aspects, qualitative phytochemical screening, phenolic compound content, and total flavonoids. **Results:** The highest results of yield were obtained from the maceration of the leaves, which may be related to the contact time between the plant drug and solvent. The visual aspects of the extracts presented some differences between the extractive methods. The phytochemical screening showed consistent data with other studies of the genus. Both the vegetal part as the different extractive methods influenced significantly the levels of phenolic compounds, and the highest content was found in the maceration of the barks, even more than the content found previously. No differences between the levels of total flavonoids were significant. The highest concentration of total flavonoids was found in the ultrasound of the barks, followed by maceration on this drug. According to the results, the barks of *H. martiana* presented the highest total flavonoid contents. **Conclusion:** The results demonstrate that both the vegetable and the different extractive methods influenced significantly various parameters obtained in the various extracts, demonstrating the importance of systematic comparative studies for the development of pharmaceuticals and cosmetics.

Key words: Extractive methods, *Hymenaea martiana*, phenolic compounds

SUMMARY

- The phytochemical screening showed consistent data with other studies of the genus *Hymenaea*
- Both the vegetable part and the different extractive methods influenced significantly various parameters obtained in the various extracts, including the levels of phenolic compounds
- The barks of *H. martiana* presented the highest total phenolic and flavonoid contents.



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INTRODUCTION

Hymenaea martiana Hayne popularly as "jatobá" is a native Caatinga tree of the *Fabaceae* family. Distributed all over the Brazilian territory including the Northeastern region, this medicinal plant has a rounded crown with dense foliage and barks and straight trunk, about 2 m in diameter, and can be characterized as a large tree, with 15–20 m high.[1,2]

The extract of the bark and stem bark of *H. martiana* has been used commonly in the treatment of respiratory problems, inflammation, and pain, and the resin is used as cicatrizing.[3] Some studies have shown their antimicrobial,[2] antinociceptive, and analgesic[4,5] activities, and some authors related the activities of this species to the presence of phenolic compounds, the flavonoids specifically.[6-8] Some studies have revealed the presence of these substances on the barks of *H. martiana*, as astilbin, eucryphin, engelitin,[6-8] and daucosterol.[6]

With simple and complex structures, and constituted by at least one aromatic ring substituted by at least one hydroxyl,[9] the phenolic compounds are products of the secondary metabolism of plants and fungi. Among these compounds, the flavonoids may be highlighted for the various biological activities proven such as antioxidant, anti-inflammatory, and anticancer.[10]

For the development and production of pharmaceutical products obtained from active vegetal raw materials, the phytotherapics, the preparation of the active drugs is a critical step. Its importance is due to the influence of some factors, as the variation on the vegetal production of the active substances, the extractive conditions, the solvent properties, as well as the multiple extraction techniques available.[11]

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According to the literature consulted, no comparative systematic study of the extractive methods for the recovery of phenolic compounds of this species was found. In this context, the aim of this study was to evaluate the influence of the different extractive methods on the recovery of phenolic compounds from different parts of *H. martiana* Hayne (Fabaceae).

MATERIALS AND METHODS

Plant material

The barks, leaves, fruits, and seeds of *H. martiana* Hayne were collected in the city of Petrolina, Pernambuco, Brazil, in May and July 2013, and identified by the Federal University of São Francisco Valley Herbarium (HVASF), with voucher specimen n° 6444, coordinates 09”11’04.30° S, 040”18’05.40° W, 357 m high. The plant materials were dried at 40°C for 72 h in air circulation oven (ETHIKTECHNO®, Model TD 420). After complete stabilization and drying, the material was pulverized using a mill (QUIMIS®).

Preparation of the extracts

All the vegetal drugs were submitted to three different extraction methods (maceration, percolation, and ultrasound).

For maceration, 100 g of each vegetal drug was mixed with 500 ml of ethanol 95% and maintained for 3 days with daily agitation at room temperature, protected from light. Then, the extractive solution was filtered and concentrated under vacuum.[8,11]

For percolation, 100 g of each vegetal drug was moistened with 3 L of ethanol 95% and were allowed to stand for 2 h. The percolator was prepared with cotton and paper filter. The vegetal drugs and the solvent were transferred to form a layer on the drug. The preparation was allowed to stand for 24 h. Then, the percolation was initiated adding ethanol 95% constantly. After the process, the extractive solution was filtered and concentrated under vacuum.[11]

For the ultrasound extraction, 10 g of each vegetal drug was maintained with 100 ml of ethanol 95%, and submitted to ultrasound (LOGEN®), for 30 min at 25°C. After the process, the extractive solution was filtered and concentrated under vacuum.

Extraction yield

The extraction yield was expressed as the percentage calculated by the weight of the obtained extract divided by the weight of the plant drug, multiplied by 100.

The visual aspects

The visual aspects were evaluated for color and texture of the extracts.

Phytochemical screening

An aliquot of each extract was solubilized in chloroform and submitted to analyses by thin layer chromatography with silica gel 60 F_{254} plates with aluminum support and eluted with different solvent systems, as described by Wagner and Bladt,[12] seeking to highlight the major groups of secondary metabolites [Table 1].

Determination of total phenolic compounds

The content of total phenolic compounds was measured by the colorimetric method, using the Folin–Ciocalteu reagent (SIGMA), and Gallic acid as the standard, based on the method described, only the volumes were adjusted.[13] For this, an aliquot (40 µl) of the diluted extract was added to 3.16 ml of distilled water and 200 µl of Folin–Ciocalteu reagent, being immediately mixed. The mixture was allowed to stand for 6 min, and after that was added 600 µl of a stock solution of Na_2CO_3 and well mixed. The final solutions were allowed to stand for 2 h at 25°C. The absorbance of each solution was obtained in using a spectrophotometer (QUIMIS) at 765 nm against the blank. Total phenolic contents of the extracts were expressed as mg Gallic acid equivalents per gram of the sample (mg GAE/g), through the calibration curve with Gallic acid. The calibration curve range was 50–1000 mg/l (R^2 = 0.9975). All samples were performed in triplicates.

Determination of total flavonoids

The content of total flavonoids was determined using the colorimetric method by metallic complexation described,[14] using quercetin as the standard. A sample solution of 5 mg/ml was prepared with absolute ethanol and was added 0.2 ml of $AlCl_3$ 2.5% alcoholic solution and 3.80 ml of absolute ethanol. The solutions were allowed to stand for 30 min at room temperature. The absorbance of each solution was obtained in using a spectrophotometer (QUIMIS) at 408 nm against the blank. Total flavonoid content of the extracts was expressed as mg quercetin equivalents per gram of the sample (mg QE/g), through the calibration curve with quercetin. The calibration curve range was 2.5–20 µg/ml (R^2 = 0.9930). All samples were performed in triplicates.

Statistical analysis

All determinations were performed in triplicate. Values were considered significantly different at $P < 0.05$. GraphPad Prism® software 5.0 (GraphPad Software Inc.) was used, using the two-way ANOVA test with Bonferroni post-test.

RESULTS

Extraction yield

The extracts were obtained with the following yields (% of dry weight of the plant) as shown in Table 2. The extractive methods showed no statistical differences for yields; thus, there was no influence. However, statistical differences were considered significant for the different plant parts ($P < 0.05$, two-way ANOVA, Bonferroni *post hoc*) [Table 2].

Visual aspects

The extracts obtained from the plant materials from *H. martiana* by different methods were analyzed in relation to the visual aspects

Table 1: Elution systems and revelators used in the phytochemical screening of *Hymenaea martiana* by thin layer chromatography

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)	Vanillin phosphoric reagent
Mono and diterpenes	Toluene: ethyl acetate (93:7, v/v)	Vanillin 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

NP: Natural products; PEG: Polyethylene glycol

[Table 3]. For the barks, maceration was presented as a crystallized material, with rigid consistency, dark brown to reddish, whereas the other methods presented with malleable consistency. For the leaves, the methods presented in a similar aspect as a liquid material with oily consistency, of dark green color, except for percolation that presented with gelatinized appearance. The extracts obtained by maceration of the fruits were presented as granular and gelatinized materials, hardened, dark brown color, and with a sweet smell, whereas the percolation was a caramelized liquid material, with yellow color and sweet smell. This indicates differences in the aspects of the extracts obtained by different methods for this plant material. For the seeds, the extracts showed a similar appearance, presented as a gelatinized material, hardened, dark brown color [Table 3].

Phytochemical screening

The phytochemical screening with the extracts was performed, and the chromatographic thin layer plates were analyzed. The analysis of the extracts obtained by maceration with *H. martiana* barks indicated the presence of anthracene derivatives, flavonoids, monoterpenes, diterpenes, naphthoquinones, saponins, triterpenes, and steroids. The maceration of the leaves indicated the presence of anthracene derivatives, flavonoids, saponins, and naphthoquinones. The maceration of the fruits indicated the presence of anthracene derivatives, flavonoids, monoterpenes, diterpenes, saponins, and naphthoquinones. The maceration of the seeds showed anthracene derivatives and flavonoids [Table 4].

The analysis of the extracts obtained by the percolation of the barks of *H. martiana* indicated the presence of anthracene derivatives, flavonoids, monoterpenes, diterpenes, naphthoquinones, and saponins, not indicating the presence of triterpenes and steroids as in the maceration method. The percolation of the leaves indicated the presence of the anthracene derivatives, flavonoids, monoterpenes, and diterpenes (compounds not showed in the maceration) and naphthoquinones, not indicating the presence of saponins. The fruits percolation indicated the presence of flavonoids, monoterpenes, diterpenes, naphthoquinones, saponins, triterpenes, and steroids (compounds not showed in the maceration), not indicating the presence of the anthracene derivatives as in the maceration method. The percolation of the seeds showed the presence of the anthracene derivatives, flavonoids, and saponins (compounds not showed in the maceration) [Table 5].

The analysis of the extracts obtained by the ultrasound method with the barks of *H. martiana* indicated the presence of anthracene derivatives, flavonoids, monoterpenes, diterpenes, naphthoquinones, and saponins, as observed in the maceration. The leaves ultrasound indicated the presence of flavonoids, monoterpenes, diterpenes (compounds not found in the maceration), and naphthoquinones, not indicating the presence of some compounds found in the other methods. The extracts obtained by ultrasound with the fruits indicated the presence of flavonoids, monoterpenes, diterpenes, naphthoquinones, and saponins, not indicating the presence of anthracene derivatives as in the maceration, triterpenes, and steroids as in the percolation. The ultrasound of the seeds indicated the presence of anthracene derivatives, flavonoids, and saponins (compound not found in the maceration) [Table 6].

Table 2: Extraction yield from different parts of *Hymenaea martiana*

Vegetal drug (%)	Maceration (%)	Percolation (%)	Ultrasound (%)
Barks	17.51	17.94	13.52
Leaves	39.34	37.39	38.32
Fruits	24.63	15.15	22.53
Seeds	26.27	14.18	29.67

Determination of total phenolic compounds

The influence of the extractive method on the total phenolic compounds content is showed in Figure 1. The vegetal part and the different methods showed significant influence on the contents ($P < 0.05$, ANOVA, Bonferroni, *post hoc*). Total phenolic content was not detected in extracts of fruits or seeds, probably due to limitations of the analytical method, which was not able to detect the amounts shown in these samples.

According to the data [Table 7], the highest content of phenolic compounds found in the maceration of the barks with 586.50 mg GAE/g of the extract [Figure 1].

Determination of total flavonoids

The interaction between the extractive method and vegetal parts on total flavonoid content is shown in Figure 2. No significant differences were found between the contents ($P < 0.05$, ANOVA, Bonferroni, *post hoc*). The highest content [Table 8] was found in the ultrasound of the barks, with 107.29 mg QE/g of extract, followed by the maceration of this drug. No total flavonoids content was found in the fruits and seed extracts [Figure 2 and Table 8].

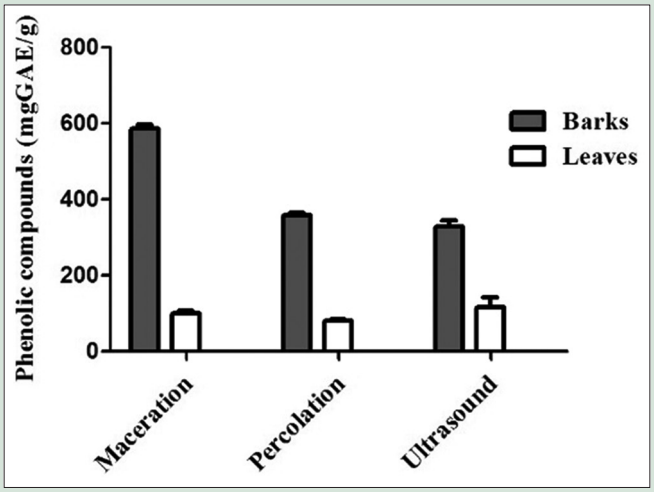

Figure 1: Histogram of total phenolic compound contents in different extracts of bark and leaves from *Hymenaea martiana*

Table 3: Visual aspects of the extracts from *Hymenaea martiana*

Vegetal drug	Maceration	Percolation	Ultrasound
Barks	Crystallized material with brown to reddish color	Pasty material, with malleable consistency, with dark brown to reddish color	Pasty material, with malleable consistency, with dark brown to reddish color
Leaves	Liquid and oily material, with dark green color	Gelled material, oily, with dark green color	Liquid and oily material, with dark green color
Fruits	Granular material, with dark brown color	Caramelized liquid material, with dark yellow color	Gelled material, with dark brown color
Seeds	Gelled and hardened material, with dark brown color	Gelled and hardened material, with dark brown color	Gelled and hardened material, with dark brown color

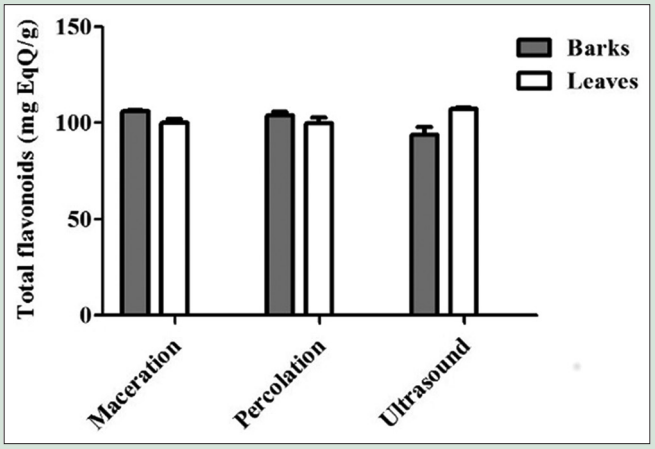

Figure 2: Histogram of total flavonoids contents in different extracts of bark and leaves from *Hymenaea martiana*

DISCUSSION

The extraction of secondary metabolites process involves complex mechanisms and several methods can be used. In this study, the techniques used were maceration, percolation, and ultrasound from different plant parts of the species under study. The difference between methods may lead to differences in the extracts, due to differences between the contact time of the plant drug and solvent, and peculiarities of each method.

The yields show the influence of the vegetative organs in this parameter. According to the obtained data, the highest yields were observed with extracts prepared from the maceration of the leaves of *H. martiana*. The maceration method is widely used, and the longer contact time of the plant drug with the solvent may favor the extraction of secondary metabolites. The ultrasound method presented interesting results because this method is fast and simple with 30 min duration, which can be an advantage. The percolation method, procedure referred in the Brazilian Pharmacopoeia, produced the lowest yields for plant materials. This may be related to the contact time between the plant drug and the solvent, which is larger in maceration, whereas in the percolation is relatively low.

The visual aspects of the extracts presented some differences between the extractive methods, but some plant materials such as leaves and seeds showed similar visual aspects. This indicates that each plant part behaves differently when subjected to different extractive methods.

The phytochemical screening showed differences between the obtained extracts, making evident the importance of selecting the method according to the substances of interest. The maceration of the barks showed data in accordance with the literature since secondary metabolites have been isolated and identified in the barks of *H. martiana* such as the flavonoids astilbin, eucryphin, engelitin, and taxifolin,[6,15] and the steroid daucosterol.[6]

Although there is no phytochemical studies with leaves of *H. martiana*, other species of the genus presented terpenoids, flavonoids,[16-19] sesquiterpenes,[20] and xyloglucans.[21]

There was not any available study with the fruits *H. martiana*, but previous studies with other plants from the genus showed carbohydrates *D*-fructose, *D*-glucose, *D*-glucuronic acid, *L*-sorbose, sucrose, and also diterpenes (5*R*-8*S*-10*R*)-cleroda-3-trans-13-dien-15-oic acid, (-)-kovalenic acid, ozic acid, and iso-ozic acid.[22,23]

Table 4: Phytochemical screening of the extracts of *Hymenaea martiana* obtained by maceration

Secondary metabolites	Barks	Leaves	Fruits	Seeds
Alkaloids	-	-	-	-
Anthracene derivatives	+++	++	+	+
Coumarins	-	-	-	-
Flavonoids	+++	+++	+++	+
Lignans	-	-	-	-
Monoterpenes and diterpenes	+	-	+++	-
Naphthoquinones	+++	+	+	-
Saponins	+	+	+	-
Triterpenes and steroids	+	-	-	-

-: Nondetected; +: Weakly positive; ++: Moderately positive; +++: Strongly positive

Table 5: Phytochemical screening of the extracts of *Hymenaea martiana* obtained by percolation

Secondary metabolites	Barks	Leaves	Fruits	Seeds
Alkaloids	-	-	-	-
Anthracene derivatives	+++	++	-	+
Coumarins	-	-	-	-
Flavonoids	+++	+++	+++	+
Lignans	-	-	-	-
Monoterpenes and diterpenes	++	++	+++	-
Naphthoquinones	+++	++	+	-
Saponins	+	-	+++	++
Triterpenes and steroids	-	-	++	-

-: Nondetected; +: Weakly positive; ++: Moderately positive; +++: Strongly positive

Table 6: Phytochemical screening of the extracts of *Hymenaea martiana* obtained by ultrasound

Secondary metabolites	Barks	Leaves	Fruits	Seeds
Alkaloids	-	-	-	
Anthracene derivatives	+++	-	-	+
Coumarins	-	-	-	-
Flavonoids	+++	+++	+++	+
Lignans	-	-	-	-
Monoterpenes and diterpenes	++	+	+	-
Naphthoquinones	+++	+	+	-
Saponins	++	-	++	+
Triterpenes and steroids	-	-	-	-

-: Nondetected; +: Weakly positive; ++: Moderately positive; +++: Strongly positive

Table 7: Total phenolic compounds in extracts from different parts of *Hymenaea martiana*

Vegetal drug	Total phenolic compounds (mg GAE/g of extract)		
	Maceration	Percolation	Ultrasound
Barks	586.50±9.61	359.28±5.09	327.89±16.84
Leaves	180.25±7.74	151.08±5.09	116.50±24.88
Fruits	ND	ND	ND
Seeds	ND	ND	ND

GAE: Gallic acid equivalents; ND: None detected

Table 8: Total flavonoid content in extracts obtained from different parts of *Hymenaea martiana*

Vegetal drug	Total flavonoid content (mg QE/g of extract)		
	Maceration	Percolation	Ultrasound
Barks	106.20±0.37	103.85±1.98	107.29±0.86
Leaves	100.02±1.95	99.97±2.82	92.48±3.44

QE: Quercetin equivalents

The analysis of the seed extracts indicated the presence of some substances, but the studies found with other plants from the same genus showed coumarins (ipomopsin and himenain)[24] and xyloglucans.[25,26]

According to the data [Table 8], the highest content of phenolic compounds found in the maceration of the barks with 586.50 mg GAE/g of extract, data greater than previously found in crude ethanolic extract obtained by the same method.[8]

Maceration is a widely used method for extraction of phenolic compounds.[8,27,28] This method showed to be efficient for the extraction of the barks of this species, which is used for medicinal purposes and this may explain much of the reported activities.[2,4,6,7,15]

Flavonoids are secondary metabolites from plants biosynthesized from the phenylpropanoids way[9] and can be defined as chemical substances containing a common nucleus of phenylchromanone with one or more hydroxyl groups, including derivatives linked to sugars.[29] This group of secondary metabolites has shown biological activities, and can be highlighted with a high therapeutic potential. Important activities as antioxidant, anti-inflammatory, and inhibiting unregulated cell proliferation are already related to the flavonoids,[10] demonstrating its importance in medicinal plants.

The highest contents of total flavonoids were found in the ultrasound of the barks of *H. martiana*. The ultrasound method, as described before, is a fast technique and is based on the high-frequency ultrasonic waves, which promotes the break of the cellular wall from the vegetal matrix. This feature can afford the better dissolution of the secondary metabolites within the solvent and the mass transference, which may favor the extraction of compounds.

The total flavonoid content found in the studied extracts is lower than that reported in the previous studies.[8] This data can show the difference between the analytical methods for the determination of the total flavonoids since the previous study was conducted using catechin as a standard.

Therefore, the total flavonoid content found in *H. martiana* is still relevant since the QE content 107.29 mg/g extract corresponding to approximately 10.73% of the sample.

CONCLUSION

According to the results of this study, among other plant materials, the barks of *H. martiana* showed the highest contents of total phenolic compounds and total flavonoids. The most efficient method for the extraction of these metabolites was maceration. The results demonstrate that both the plant parts as the different extractive methods significantly influenced various parameters in the various extracts obtained.

These data indicate the barks of *H. martiana* as the most suitable part for the extraction of these drug classes of chemical compounds, as well as for the further development of pharmaceuticals and cosmetics products.

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Conflicts of interest

There are no conflicts of interest.

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