Vol. 18(21), pp. 462-471, 22 May, 2019
DOI: 10.5897/AJB2019.16762
Article Number: 1B85DD461005
ISSN: 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Chemical study of *Peganum harmala* seeds

Isabela Araujo e Amariz*, Jacyara Pereira da Silva, Emanuella Chiara Valença Pereira, Nathália Andrezza Carvalho de Souza, José Marcos Teixeira de Alencar Filho, Renan Nunes Pereira, Ana Paula de Oliveira and Larissa Araújo Rolim

Center for Drug, Food and Medicine Analysis, Federal University of São Francisco Valley - Petrolina (PE), Brazil.

Received 5 February 2019; Accepted 12 April, 2019

Peganum harmala **belongs to the family Zygophyllaceae and is popularly known as "Arruda of Syria". It presents a broad therapeutic potential, highlighting the anti inflammatory, anticancer, analgesic, antiseptic and antibacterial activities. It is used in the preparation of religious rituals beverages with effects on the central nervous system as an inhibitor of the enzyme monoaminoxidase, whose action is due to the presence of harmonic alkaloids of the β–carbolines group, with harmine and harmaline being the most found in** ***P. harmala*** **seeds. The objective of this study was to evaluate the chemical and pharmacognostic characteristics of** ***P. harmala*** **seeds with the aid of gas chromatography coupled to mass spectrometry. Pharmacognostic characterization was performed following the 5th edition of the Brazilian Pharmacopoeia. Scanning electron microscopy coupled to X-ray dispersive energy spectroscopy was performed for the seeds elemental identification. The compounds present in the species were identified by gas chromatography coupled to mass spectrometry. Phytochemical evaluation demonstrated the major secondary metabolites. The scanning electron microscopy coupled to X-ray dispersive energy spectroscopy analysis showed the morphology of the surface and interior of the seeds, as well as the atomic chemical analysis of the structures. By gas chromatography coupled to mass spectrometry, the chemical profile of** ***P. harmala*** **seeds was identified and the β–carbolines (harmaline and harmine) were identified, which are compounds of great pharmacological importance for the species. Therefore, it is concluded that this study is of great contribution to the plant material standardization that will serve as the basis for the future development of a pharmaceutical product.**

Key words: Arruda of Syria, characterization, gas chromatography, β-carbolines, *Peganum harmala*.

INTRODUCTION

The use of natural products for therapeutic purposes comes from the beginning of the civilizations. A large portion of the world's population, especially from developing countries, makes use of herbal medicines relying on them for the cure, prevention and treatment of diseases. In addition, it is a source of bioactive compounds, which contributes to the development of new therapeutic strategies (Firmo et al., 2018).

Peganum harmala popularly known as "Syrian rust", belongs to the family Zygophyllaceae, being well adapted

*Corresponding author. E-mail: Isabela.amariz26@gmail.com. Tel: +55 87 99993-4030.

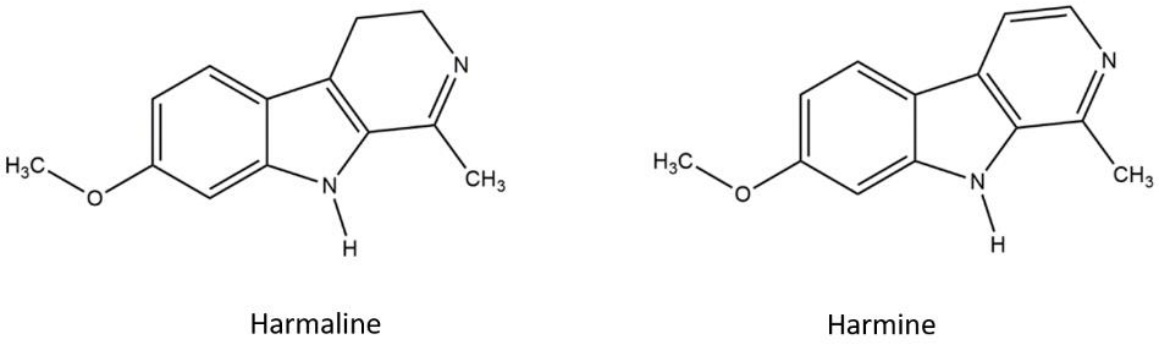

Figure 1. Chemical structure of β–carbolines: Harmaline and Harmine, respectively.

to the climate of dry regions mainly found in North Africa, Middle East, India and Mongolia (Herraiz et al., 2010). This species has several therapeutic purposes, mainly highlighting the neuropharmacological, antidepressive and hallucinogenic activities. These activities come from β-carbolines, a group of harmonic alkaloids found in a variety of plants (Sassoui et al., 2005). In the seeds of *P. harmala* the most commonly β-carbolines (Figure 1) found are: harmine, harmaline and tetrahydroharmine (Rodrigues, 2013; Muller, 2006).

The β-carbolines action is similar to monoamine oxidase (iMAO) inhibitor drugs, which act by deactivating this enzyme responsible for the monoamines degradation (serotonin and catecholamines), and thus act by regulating and / or increasing their levels in the synaptic cleft (Herraiz et al., 2010; Moloudizargari et al., 2013).

In view of these pharmacological actions, *P. harmala* is an important source of β-carbolines and it can be used as a vegetable raw material for the development of phytotherapeutic drugs or phytomedicines containing these active substances. Therefore, the objective of this study was to evaluate the chemical and pharmacognostic characteristics of *P. harmala* seeds with the help of gas chromatography coupled to mass spectrometry (GC-MS).

MATERIALS AND METHODS

Collection of plant material

The *P. harmala* seeds were purchased in local commerce in the city of Petrolina-PE, in July 2016 and were identified based on herbarium specimens in Herbarium Alexandre Leal Costa – ALCB located in the Department of Botany of the Institute of Biology of the Federal University of Bahia (UFBA), under the code ALCB 12384 (Code: ALCB014643, Type of record: preserved specimen).

Solvents

The solvents used were: Ultra purified water (Milli–Q), Absolute Ethyl Alcohol P.A (Synth®), Acetonitrile (Merck®), Formic acid (Dinâmica®), Methanol (Merck®), Acetate of ethyl (HPLC grade).

Obtaining powder and preparing extracts

The seeds of *P. harmala* were oven dried in circulating air at 40°C for 96 h and then pulverized in a knife mill. Then, the dried and pulverized seeds were subjected to thorought maceration with absolute ethyl alcohol -99.5% PA (ethanol) with renewal of the extracting liquid every 72 h. The solutions were filtered and concentrated on a rotary evaporator (50°C) to obtain the crude ethanolic extract of *P. harmala* (EtOHB of *P. harmala*).

Pharmacognostic characterization of vegetable drug

P. harmala seed powder was used to perform the physical-chemical tests: loss determination by desiccation, the powders granulometry, total ashes, acid insoluble ash, sulfated ash, foam index and extractable substances through alcohol as described and recommended in the Brazilian Pharmacopoeia 5th Edition (Brazil, 2010; Luis et al., 2015; Kaskoos, 2014). For the determination of the powders granulometry, a set of five previously weighed sieves with mesh diameters of 500, 425, 250, 180 and 150 µm and a collector were selected. Physical-chemical tests were performed in triplicate and the results were expressed as mean ± standard deviation.

Electronic scan microscopy (SEM) of the seeds of *P. harmala*

The sample was prepared on double carbon tape contained in copper and nonmetallized stub. SEM analyzes of *P. harmala* seeds were obtained by JOEL® scanning electron microscope, model JSM–5900, coupled to a dispersive energy analyzer (EDS) using increases of 300 and 750 times.

Preliminary phytochemical assessment

The phytochemical evaluation of the crude ethanolic extract of *P. harmala* (EtOHB of *P. harmala*) was carried out using silica gel chromatography plates with aluminum support, applied with micropipette and eluted in different solvent systems, according to Wagner and Bladt (1996) to show the main groups of secondary metabolites.

Analysis by gas chromatography coupled to mass spectrometry (GC–MS)

The gas chromatography coupled to mass spectrometry (GC-MS)

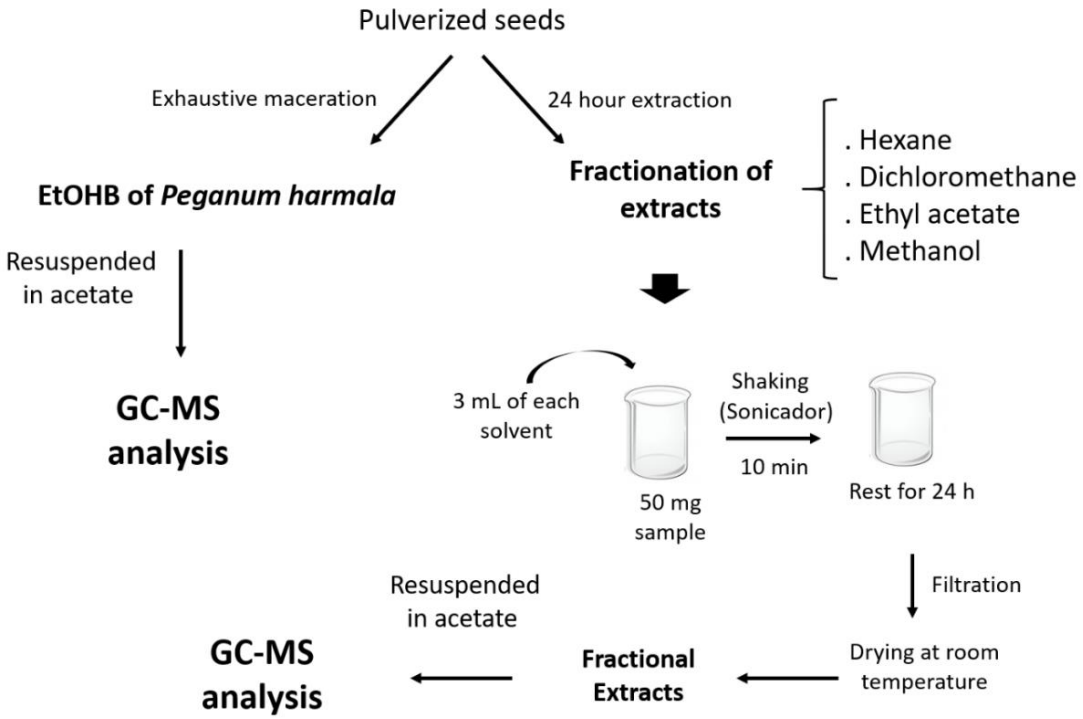

Figure 2. Schematic representation of *P. harmala* extracts by GC-MS.

was used to evaluate the chemical profile of the plant species from the analysis of EtOHB of *P. harmala* and the analysis of the fractionated extracts based on the increasing polarity of the solvents: hexane, dichloromethane, ethyl acetate and methanol, respectively.

P. harmala seeds were divided into two groups: Group A obtained from whole seeds; and Group B obtained from seeds divided in half. An amount of 50 mg was weighed, and then 3 ml of each solvent (in increasing order of polarity) was added and brought to sonicator shaking for 10 min with each solvent. It was allowed to stand for 24 h. After this time, the sample was filtered and added to the next solvent. The extract was dried at room temperature (25°C) by air circulation. This process was repeated for each new solvent and performed in triplicate. EtOHB of *P. harmala* was obtained according to the methodology described for obtaining and preparing the extracts described above. For the samples analysis resuspension was necessary in ethyl acetate (Grade High Performance Liquid Chromatography (HPLC)) in concentration of 10 mg/ml and then analysis on a gas chromatograph was performed coupled to a Shimadzu® mass spectrometer (QP-2010) and coupled to a self injector (AOC 20i) was used employing the following chromatographic conditions: RESTEK® RTX–5MS column (30.0 mm × 0.25 mm × 0.25 mm) using helium gas (99.99%) transported with a constant flow of 1.4 mL / min, sample injection volume of 1.0 µL, split mode with ratio 5 (split 1: 4 discard), injector temperature 260°C; electron impact mode at 70 eV; ion source temperature of 250°C. The oven temperature was programmed to 80°C (isothermal for 3 min), increasing from 5°C/min to 285°C (isothermal for 15 min) and 10°C/min to 320°C (isothermal for 20 min). A linear hydrocarbon mixture (C 9 H 20 – C 40 H 82) was injected under the same conditions as the samples under analysis, and the compunds identification was by comparison of the mass spectra obtained with the spectra presented by the SHIMADZU® database (GCMS Solution) (Wiley 7lib and NIST08lib) (Carvalho et al., 2014). The compound was considered as identified when it presented similarity index greater than or equal to 90% (Figure 2).

RESULTS AND DISCUSSION

Pharmacognostic characterization of vegetable drug

Weight loss

To determine the weight loss of the seeds of *P. harmala*, the gravimetric method described in the Brazilian Pharmacopoeia (2010) (Brasil, 2010) was used. The results presented a moisture content of 7.41 ± 0.02 in the seeds of *P. harmala*, this value is close to previous studies in which the results were 6.94 ± 1.05 (Kaskoos, 2014).

Granulometric determination of the powders

After the sieving process, it was observed that the largest amount of *P. harmala* powder was retained in the 850 µm mesh, as shown in the histogram of Figure 3A. The determination of the average particle size was carried out by calculating the percentage of the passage fraction and the fraction of powder retained in each of the sieves. The results obtained in graphical representation showed that the average particle size of *P. harmala* represented in the graph was 473 µm (Figure 3B), being classified as coarse

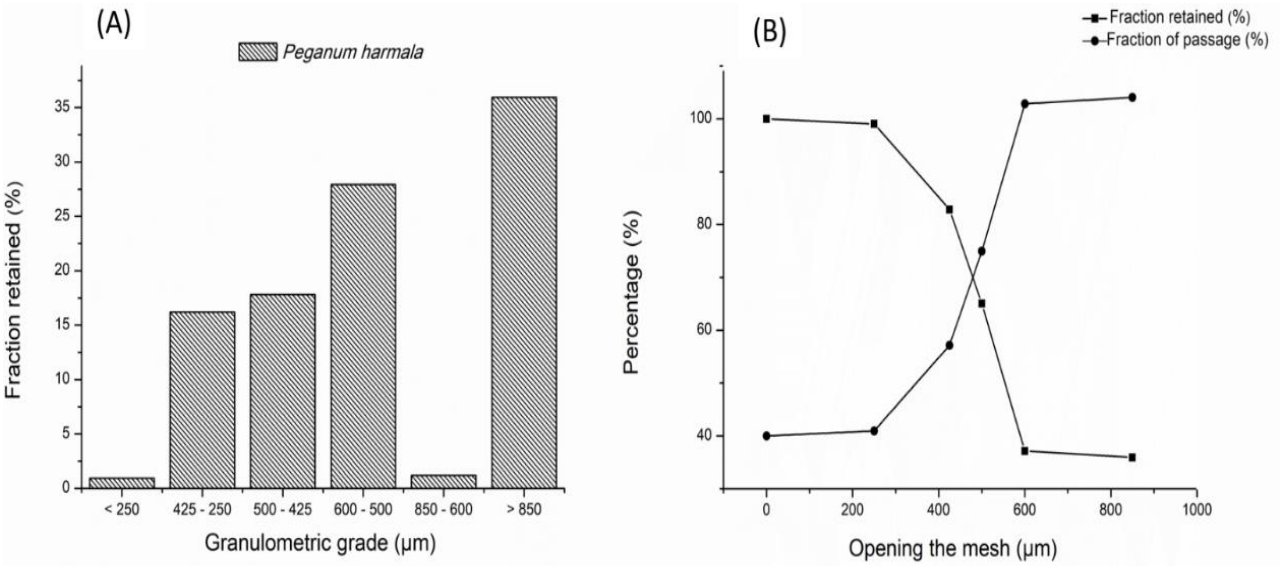

Figure 3. (A) Histogram of granulometric distribution of *P. harmala* seeds powder, (B) Graphical determination of the particle size of the obtained powder of *P. harmala*.

powder according to the Brazilian Pharmacopoeia (2010) (Brasil, 2010).

Determination of total ashes, acid insoluble ash and sulphated ash

The results of total ashes, acid insoluble ash and sulfated ashes of the plant drugs are shown in Table 1. The Brazilian Pharmacopoeia recommends the maximum values of 8 % for total ashes and 1.5 % for acid insoluble ashes. As it is possible to see in Table 1, the results obtained are in disagreement with this recommendation (Brasil, 2010). Comparing the results found with the studies by Kaskoos (2014), it was observed that the total ash content is very high (7.51 ± 1.16), whereas for the acid insoluble ash content the value was 1.56 ± 0.61 (Kaskoos, 2014). As the analyzes performed above have the purpose of performing the sample characterization, the results of this study indicate that this species has higher values than those commonly found. Such a characteristic condition of this plant drug may be related to changes in the conditions of cultivation, processing, transport and/or harvesting.

These quality parameters are important, being possible to evaluate the amount of contaminants present in the sample, such as sand, stone, bad processing and others. These results, when found in the sample, lead to changes in values (Couto et al., 2009). The high content of sulphated ash in the *P. harmala* sample may be indicative of the high amount of crystalline content in the seeds of this species. In plants, the crystalline mineral inclusions contribute to the ashes increase, mainly influencing the values of sulfated ash (Tengku et al., 2013).

Determination of foam index

The presence of saponins in the plant drug can be determined from the foam index test. This is due to the ability of saponins to form persistent and abundant foams in aqueous solution because of their amphiphilic nature, where sapogenin (lipophilic part) interacts with apolar compounds and sugar chains, interact with polar substances (Podolak et al., 2010).

According to the results of the performed test, after 15 min of analysis the foam formation was less than 1 cm in height, being thus the obtained index less than 100 demonstrating absence of saponins in *P. harmala.* Studies of Kaskoos (2014) also evidenced the absence of saponins when using the same test described by the Brazilian Pharmacopoeia (Kaskoos, 2014). Some studies report the presence of saponins in this species, but by using this test it was not possible to identify the same. Preliminary phytochemical evaluation was performed using the Wagner and Bladt (1996) methodology to verify the presence of secondary metabolites of the species and consequently the presence of saponins.

Determination of substances withdrawn by alcohol

For the determination of the content of extractable substances in ethanol the result obtained was 35.72 ± 0.52%. The extractive content provides primordial information on the substances that can be extracted with a certain solvent, and consequently indicate the yield of the different types of extraction used in the plant material technological transformation (Couto et al., 2009; Simões et al., 2017).

Table 1. Determination of ash content in *P. harmala* seeds

Species	Total ashes	Insoluble sshes in acid	Sulphated ashes
P. harmala	10.12 ± 1.55%	9.41± 1.29%	12.08 ± 0.15 %

Table 2. Phytochemical profile of the crude ethanolic extract of *P. harmala.*

Chemical class	EtOHB - PH
Alkaloids	++
Anthocyanins	+++
Aglycone Anthraquinone	+
Flavonoids	+++
Cumarines	+
Anthracene Derivatives	+
Lignans	-
Mono, sesqui and diterpenes	++
Napthtoquinones	-
Saponins	+++
Condensed tannins	++
Hydrolysable tannins	++
Triterpenes and steroids	+++
Xanthines	-

Preliminary phytochemical assessment

The results of the phytochemical analysis of EtOHB of *P. harmala* are shown in Table 2. The phytochemical analysis of EtOHB of *P. harmala* demonstrated the presence of alkaloids, saponins, coumarins, anthraquinones, anthocyanins, anthracene derivatives, monoterpenes, sesquiterpenes, diterpenes, triterpenes and tannins (condensed and hydrolysable). The result was negative for the presence of lignans, naphthoquinones and xanthines. These results are presented in Table 2.

In the genus *Peganum* compounds are found such as, alkaloids, triterpenoids, anthraquinones, flavonoids, carbohydrates, amino acids, volatile oils, sterols, vitamins among others. The alkaloids β-carbolines and quinazoline are characteristic of this genus and some of them are bioactive constituents exerting some type of therapeutic effect. Some examples of β-carbolines are harmalol, harmaline, harmine, harmana, tetrahydroharmine (Li et al., 2017).

Electronic scan microscopy (SEM) of the seeds of *P. harmala*

Scanning electron microscopy (SEM) coupled with X-ray dispersive energy (EDS) spectroscopy, was carried out to provide a quantitative chemical characterization of *P. harmala* seeds (Figure 4), as well as to aid in taxonomic identification. For better visualization, the seeds were distributed in order to visualize the seeds interior morphology. From these analyzes it is possible to observe the seed anatomical structure, observing the three main structures: tegument endosperm and embryo (Figure 4), whose functions are protection, nutrition and development, respectively.

The EDS evaluates the characteristic X-rays that are generated from the beam-electron interaction, informing the elements that compose the sample in the form of spectra (histograms) and it is also possible to identify the individual elements. Currently, most scanning electron microscopes are coupled to a dispersive energy spectrometer (Cruz et al., 2006). The elements identification present in the seeds of *P. harmala*, among them, carbon (C), oxygen (O), phosphorus (P), potassium (K), sulfur (S), chlorine (Cl), calcium, is shown in Figure 5. The presence of macronutrients in plants is important both for the primary metabolism and for the production of different secondary metabolites (Gobbo-Neto and Lopes, 2007). When the plant undergoes nutritional stress, it is common to increase the secondary metabolites concentration.

Analysis by gas chromatography coupled to mass spectrometry (GC-MS)

The GC-MS analysis aimed to evaluate the chemical profile of *P. harmala* seeds by observing the main

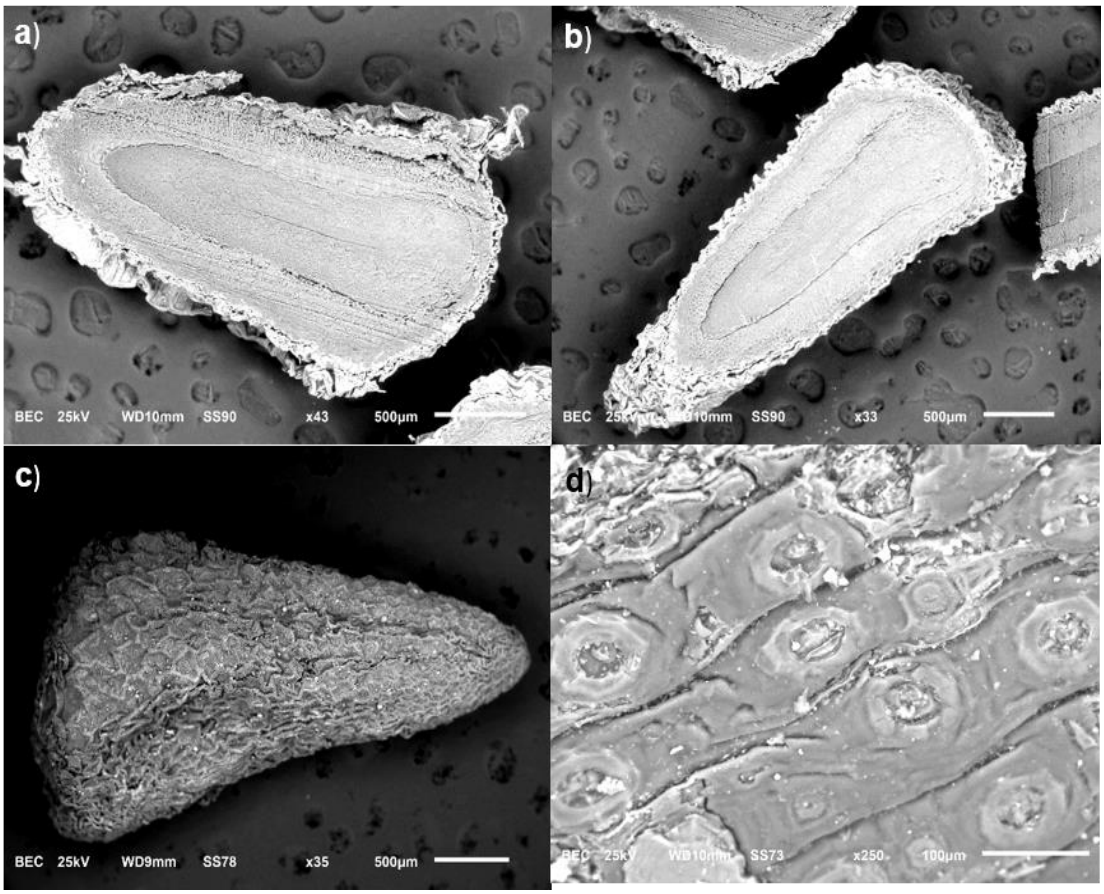

Figure 4. Photomicrographs of *P. harmala* seeds. (a) and (b) internal structure by longitudinal section; (c) and d) external structure evidencing the seed surface.

chemical constituents present. The identification occurs through molecular mass and the fragmentation profile generated by mass spectrometry, being in this technique the compounds separated from the volatization capacity of each one (Pavia et al., 2010).

In the chromatogram obtained after the GC-MS analysis of EtOHB of *P. harmala* (Figure 6), the presence of 18 peaks was observed, two β-carbolines were identified: peak 12 at harmaline (abundance of 0.29%-retention time 33.1 min) and peak 13 at harmine (abundance 7.34%-retention time 34 min). The identified compounds are shown in Table 3.

According to studies by OTT (1994), it was verified that harmine is found in greater quantity, in relation to harmaline in the seeds of *P. harmala*, which can justify the more effective action concerning iMAO by Harmine (Santos, 2007).

According to the studies of Tavares (2014), the determination of the harmaline and harmine alkaloids by gas phase chromatography allows better separation and identification of a more selective form. Further, according to their studies, the identification ions for harmaline are m / z 170 in / z 198, while for harmine, m / z 169 are in z / z 198 (Tavares, 2014), confirming what is demonstrated in the mass spectra of Figures 7 and 8, respectively. The results found corroborate with the data described in the literature and confirm the presence of the two compounds (harmaline and harmine), in the seeds of *P. harmala*.

In addition to these compounds, other hydrocarbons and their derivatives and fatty acids were identified, corresponding in the average identification of 80% of the chemical composition of the sample.

In the analysis performed by solvent partitioning, the objective was to show the chemical profile of *P. harmala* based on the affinity for the solvent used. The four extractions obtained with solvents in increasing order of polarity were analyzed: hexane, chloroform, ethyl acetate and methanol. From this, it was possible to visualize the chemical profile and the identification of the compounds present in greater quantity in the respective extracts. The compounds identification was performed according to the comparison of the similarity index of the compounds found in the software library. The two groups of the analysed seeds were: group A (whole seeds) and group B (seeds shaved in the middle), in order to evaluate if the extractive process would be more efficient with the seeds

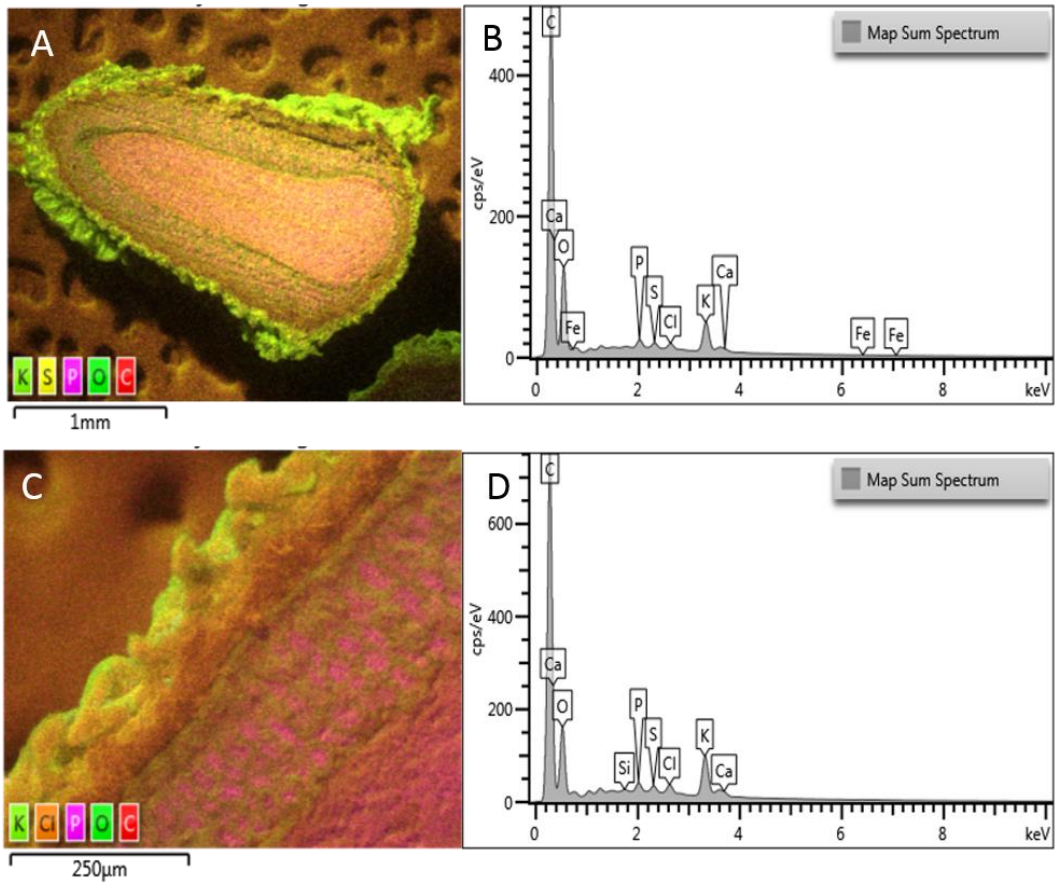

Figure 5. Photomicrographs A and C, respectively; and EDS B and D, corresponding to the seeds of *P. harmala*.

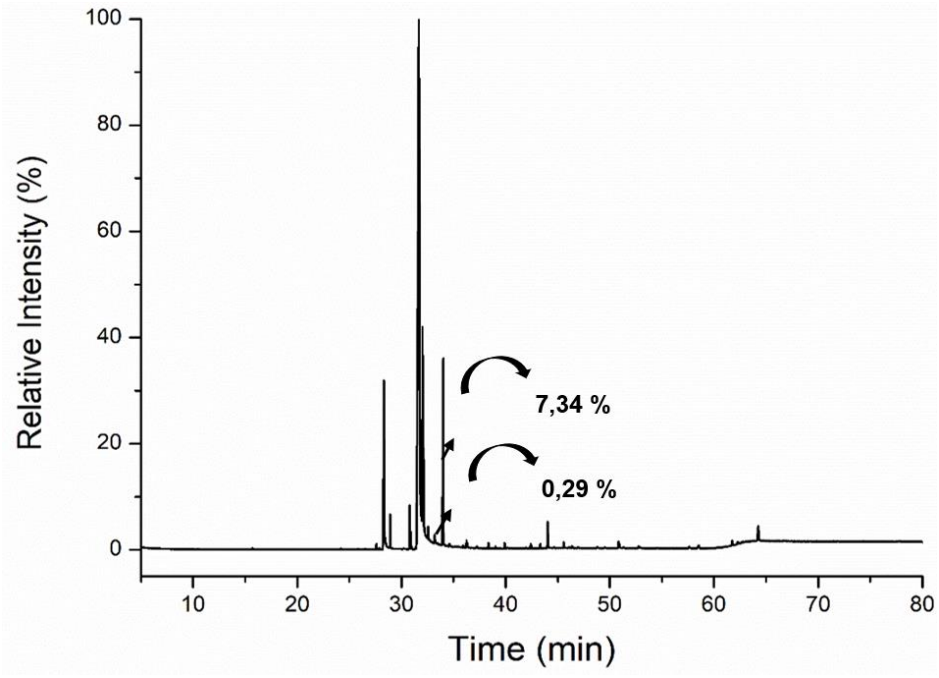

Figure 6. Chromatogram obtained after GC-MS analysis of EtOHB of *P. harmala* seeds.

Table 3. Identification of chemical constituents of crude ethanolic extract of *P. harmala* seeds by CG–MS. ND: not detected.

Retention time	Area	Area (%)	Name	m/z
28.315	17161385	8.48	Hexadecanoic acid	256
30.793	2388497	1.21	9,12-Octadecadienoic acid, methyl ester	294
30.901	983298	0.48	9-Octadecenoic acid (Z)-, methyl ester	278
31.667	93971890	46.44	Oleic acid, methyl ester	280
31.737	35831922	17.71	Octadec-9-enoic acid	282
32.019	14696352	7.26	Linoleic acid ethyl ester	279
32.112	7363111	3.63	Ethyl Oleate	310
32.216	2645169	1.31	ND	ND
32.355	706059	0.34	ND	ND
32.435	1006238	0.51	ND	ND
32.560	1745026	0.86	Octadecanoic acid, ethyl ester, Ethyl stearate	312
32.675	598424	0.29	ND	ND
33.193	637104	0.31	Harmaline	213 / 215
34.007	15845150	7.83	Harmine	212
36.249	440816	0.21	ND	ND
39.890	486937	0.24	ND	
44.028	1911462	0.95	Delta-tocopherol	402
45.578	446436	0.22	ND	ND
50.856	889410	0.44	ND	ND
58.526	470374	0.23	ND	ND
61.746	470308	0.23	ND	ND
64.249	1645318	0.82	ND	ND

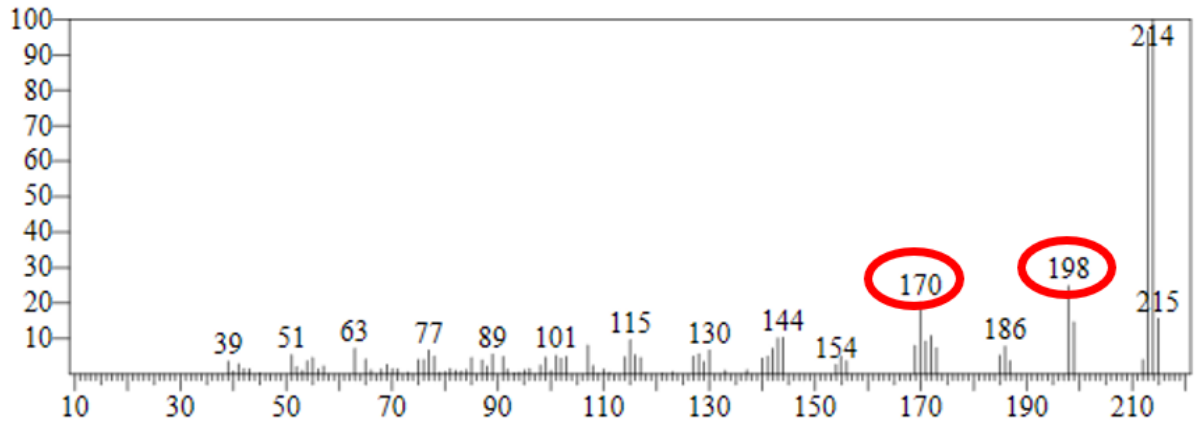

Figure 7. Mass spectrum obtained for harmaline in the EtOHB of *P. harmala*.

shaved in the middle or if it would be possible to extract the β-carbolines with the whole seeds, which facilitates the samples processing.

In the analysis of the seeds of group A with the solvent hexane, the presence of 17 peaks was observed, only one peak (retention time 42.0 min) was identified as the pentacosan hydrocarbon, it was not possible to identify the β-carbolines harmaline and harmine. The extraction with the solvents dichloromethane and ethyl acetate allowed the harmine compound identification, in which in the extraction with the dichloromethane harmine represented 81.0% of the chemical composition of the sample and in the extraction with ethyl acetate represented 91.04% of the sample chemical composition. In the analysis of the extraction with methanol the presence of 22 peaks was detected, among them it was possible to identify the presence of β-carbolines harmine (27.5%) and harmaline (1%), hydrocarbons and their derivatives, fatty acids, besides triterpenes betulin (11%) and lupenone (3.5%). The seeds analysis of group B with

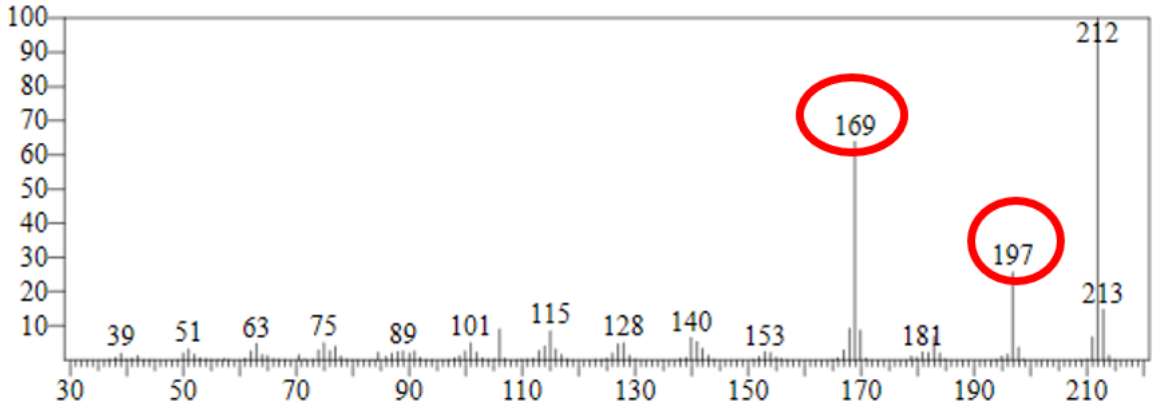

Figure 8. Mass spectrum obtained for harmine in the EtOHB of *P. harmala*.

Table 4. Quantitative relationship of harmaline and harmine composition present in the fractionated extracts of *P. harmala*.

		Hexane		Dichloromethane		Ethyl acetate		Methanol	
		A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Harmaline	RT 32.0 min	ND	ND	ND	ND	ND	ND	1.0%	ND
	m/z 213	ND	ND	ND	ND	ND	ND	213	ND
Harmine	RT 33.0	ND	ND	81.0%	18.59%	91.04%	81.0%	27.5%	11.72%
	m/z 212	ND	ND	212	212	212	212	212	212

ND: not detected. RT: retention time.

the solvent hexane demonstrated the presence of 18 peaks, of which 12 were identified and included hydrocarbons and fatty acids. In the extraction analysis with dichloromethane the presence of 28 peaks was observed, among which it was possible to identify the presence of β-carboline harmine (18.59%), hydrocarbons and stigmasterol fatty acids (6.5%). In the extraction with ethyl acetate solvent, 10 peaks were observed, of which 11% corresponded to fatty acids and 81% corresponding to β-carboline harmine.

In the analysis of the extraction with methanol the presence of 25 peaks was detected, being possible the identification of β-carboline harmine (11.72%), hydrocarbons and their derivatives, stigmasterol fatty acids (2%), as well as triterpene betulin (2%), the latter was also observed in the methanol extraction of whole seed (group A seeds). Table 4 shows the quantitative relationship of the harmaline and harmine compounds present in the fractionated extracts, according to the retention time (RT) and the mass / charge (m/z) ratio of the respective compounds of interest.

Considering the results presented on the analysis performed by solvent partition, it was noticed that the chemical profile of the extraction of the whole or shaved seeds presented the same aspects.

It was possible to identify the β-carbolines harmine and harmaline that have great pharmacological importance. It was observed that after extraction with the solvent methanol was more efficient when compared to the other analyzed solvents (hexane, dichloromethane and ethyl acetate), mainly due to the purity of the peak observed in the chromatogram and the greater amount of the extracted compound. The high polarity of the solvent methanol and its affinity with β-carboline molecules, justifies the most significant separation efficiency when the methanol solvent was used (Martins et al., 2013).

Conclusion

The results obtained on the pharmacognostic evaluation of *P. harmala* showed that the vegetable drug analyzed was of satisfactory quality. The phytochemical evaluation showed the main groups of secondary metabolites present in the species. The chemical characterization of the species performed using GC-MS techniques demonstrated the main compounds present in the *P. harmala* seeds. From the GC-MS it was possible to identify β-carbolines (harmaline and harmine), which have important pharmacological properties, mainly highlighting the iMAO activity and the central nervous system. It was still possible to observe that in the

extraction with the solvent methanol there was a greater efficiency of the compounds separation, and that the harmine compound is found in greater quantity, confirming with information already described. Therefore, this information is important for the standardization of the plant drug, contributing to the use of this data in the future development of a product based on plant material.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. Dr. N.P. Lopes and I. C. C. Turatti of the University of São Paulo (Ribeirão Preto), Brazil, for support with the GC-MS experiments. We also thank Prof. Dr. J. R. G. S. Almeida from Federal University of São Francisco Valley (UNIVASF), Brazil, as well as CAPES, CNPq, FINEP, FAPESB and FACEPE for financial support.

REFERENCES

Brazil (2010). Pharmacopeia Brazilian, 5. ed. Agência Nacional de Vigilância Sanitária. Brasília: Agência Nacional de Vigilância Sanitária 1:91-199.

Carvalho CC, Turatti ICC, Lopes NP, Salvador MJ, Nascimento AM (2014). Chemical composition and antimicrobial activity of essential oil from Brazilian plants Acanthospermum australe, Calea fruticosa and Mikania glauca. African Journal of Pharmacy and Pharmacology 8(14):392-398.

Couto R, Valgas A, Bara M, Paula J (2009). Physicochemical characterization of leaf dust of *Eugenia dysenterica* Dc. (Myrtaceae).

Cruz H, Gonzalez C, Juárez A, Herrera M, Juarez J (2006). Quantification of the microconstituents formed during solidification by the Newton thermal analysis 60 method. Journal of Materials Processing Technology 178(1-3):128-134.

Firmo W, Menezes V, Passos C, Dias C, Alves L, Dias I (2018). Historical context, popular use and scientific conception on medicinal plants. Caderno de Pesquisa 18 (especial):90-95.

Gobbo–Neto L, Lopes NP (2007). Medicinal plants: factors influencing the content of secondary metabolites. Quimica Nova 30(2):374-381.

Herraiz T, González D, Ancín-Azpilicueta C, Arán VJ, Guillén H (2010). Beta–Carboline alkaloids in Peganum harmala and inhibition of human monoamine oxidase (MAO). Food Chemical Toxicology 48(3):839-845.

Kaskoos RA (2014). Physico–chemical Parameters, Phytochemical Screening and Antioxidant Activity of Seeds of Peganum harmala Collected from Iraq. Asian Journal of Biomedical and Pharmaceutical Sciences 28(4):20-24.

Li S, Chenga X, Wang C (2017). A review on traditional uses, phytochemistry, pharmacology, pharmacokinetics and toxicology of the genus Peganum. Journal of Ethnopharmacology 203:127-162.

Luis GP, Cristina CBT, Virgínia CSM, Flores NF (2015). Quality Control and phytochemical screening of the plant drug of Morus nigra L. leaves (MORACEAE). Journal of Basic and Applied Pharmaceutical Sciences 36(2):90-95.

Martins C, Lopes W, Andrade J (2013). Solubility of organic substances. Quimica Nova 36(8):1248-1255.

Moloudizargari M, Mikaili P, Aghajanshakeri S, Asghari M, Shayegh J (2013). Pharmacological and therapeutic effects of Peganum harmala and its main alkaloids. Pharmacognosy Review 7(14):199-212

Muller SD (2006). Determination of Alkaloids and Flavonoids through HPLC and UV of extracts of Passiflora alata curtis, Passifloraceae–Passionfruit. Itajaí. Dissertation [Pharmaceutical Sciences] – University of the Vale does Itajaí.

Pavia D, Lampman G, Kriz G, Vyvyan J (2010). Introduction to Spectroscopy. 4. ed. São Paulo: Cengage Learning.

Podolak I, Galanty A, Sobolewska D (2010). Saponins as cytotoxic agents: A review. Phytochemistry Reviews 9(3):425-74.

Rodrigues TR (2013). Study of harmonic alkaloids in Passiflora edulis Sims f. flavicarpa Degener (MARACUJÁ AZEDO) by SBSE / CLAE–Dual Flu. São Paulo. Dissertation [Analytical and Inorganic Chemistry] – Institute of Chemistry of São Carlos of the University of São Paulo;.

Santos RG (2007). AYAHUASCA: neurochemistry and pharmacology. Mental Health Alcohol Alcohol Drugs 3(1). http://pepsic.bvsalud.org/scielo.php?script=sci_arttext&pid=S1806-69762007000100007&lng=pt&nrm=iso

Sassoui D, Seridi R, Azin K, Usai M (2005). Evaluation of phytochemical constituents by GC–MS and antidepressant activity of *Peganum harmala* L. seeds extract. Asian Pacific Journal of Tropical Disease 5(12):971-974.

Simões CMO, Schenkel EP, Mello JCP, Mentz LA, Petrovick PR (2017). Pharmacognosy: from the natural product to the drug. Porto Alegre: Artmed,. 486p.

Wagner H, Bladt S (1996). Plant drug analysis: a thin layer chromatography atlas. 2 ed. Berlim Heidelberg: Springer Verlag.

Tavares LS (2014). Investigation of alkaloids B–carbolines, Tryptamine, present in Ayahuasca (Santo Daime) in a sweat sample. Ribeirão Preto. Dissertation.

Tengku MT, Naz H, Jalal R, Hussin K, Abd RM, Adam A (2013). Chemical and pharmacognostical characterization of two Malaysian plants both known as Ajisamat. Brazilian Journal of Pharmacognosy 23:724-730.