

Phytochemical Profile of Seed Extracts of Plants Typical of the Brazilian Semiarid and their Potential Application in Brackish Water Desalination

Tadeu A. C. Costa, Vânia P. Campos,* Joilma S. Menezes, Sergio T. Oliva and Chirlene B. West

Departamento de Química Analítica, Instituto de Química, Universidade Federal da Bahia, 40170-290 Salvador-BA, Brazil

The phytochemical profile of typical seeds from semiarid areas of Bahia was explored. The desalination capacity of the seeds discovered in previous studies considered the seeds potentially suitable for desalination of water. Coals composed of crushed seeds of umbu (*Spondias tuberosa* Arr. Cam.) and umburana (*Amburana cearensis* A. C. Sm.) were prepared by heating at 250 °C. As the desalination process using these materials involves ion exchange besides adsorption, it became necessary to study their chemical composition to check the possibility of transferring undesirable chemical species to the water during the contact between material and water. None of the tested metabolites were found in umbu coal, which enabled its use in desalination. However, the presence of coumarin and alkaloids in the umburana seeds coal indicated that this material is not suitable for that use.

Keywords: phytochemical profile, seed extracts, semiarid regions

Introduction

Researchers at the Universidade Federal da Bahia in northeastern Brazil have gathered water quality data since 2001 in districts with semiarid climates, allowing identification of the main problems regarding water use and assessment of impacts on the health of the population in the region. Water quality data generated between 2001 and 2003 at twenty-three points along the Salitre River Basin, covering nine districts, showed that an average of 42% of the points presented brackish water, which is unsuitable for household consumption.¹

Although groundwater is an alternative source of drinking water in northeastern Brazil, there are restrictions associated with its use, as the water can become brackish due to the weathering of existing rocks, minerals, and soil features. However, the government has devoted significant attention to desalination programs, which have allowed the exploration of desalination processes based on reverse osmosis. This is the most widely used method in northeastern Brazil for water desalination. Among its advantages is the excellent quality of the treated water. However, reverse osmosis also involves the generation of waste (i.e., wastewater with a high ionic concentration).

There are other processes being used on a larger scale around the world that operate within different ranges of salinity.² Each process has advantages and disadvantages, but they mostly rely on large investments and complex technological resources for large-scale production. It is essential that the society, governments, and the various social, political and economic agents be mobilized toward viable solutions to water scarcity problems. A simple and efficient solution that may allow the semiarid communities to gain access to good quality drinking water may be brackish water desalination processes through contact with biological materials, such as plant seeds, which can withdraw part of the salt from the water. This process can be used in homemade devices for brackish water desalination.^{3,4} Menezes *et al.*^{3,4} tested different biological materials common to the semiarid region for the sorption capacity of salts contained in brackish water. Among the materials that were considered more efficient are the umbu (*Spondias tuberosa* Arr. Cam.) and umburana (*A. cearensis* A. C. Sm.) seeds. The algaroba (*Prosopis juliflora* D. C.) seed also showed a sorption capacity for salts contained in water, but its use was not considered because its toxicity to goats is well reported in the literature.⁵⁻⁸ Experiments conducted in that study showed that during the contact time between the water and the material prepared from the seeds, chemical species

*e-mail: vaniaroc@ufba.br

such as calcium and magnesium were transferred from the biological material into the water.

The *Spondias tuberosa* Arr. Cam. species, belonging to the family Anacardiaceae and popularly known as “umbuzeiro”, is native to Brazilian semiarid regions. Fruits and roots of this species have the potential for incorporation into the diet of humans and animals, and according to Kitts,⁹ they are rich in minerals and vitamin C (*L*-ascorbic acid). In addition to preventing diseases, vitamin C serves as an antioxidant and is therefore extremely important in the interception of free radicals derived from oxidative processes.

Very little is known about the composition of umbu seeds. It is known that they contain oil, proteins and certain minerals; however, their composition requires further investigation. Borges *et al.*¹⁰ physically and chemically characterized umbu seeds obtained from fruits at different maturation stages and of different varieties. These authors found significant amounts of lipids and fatty acids in the seed oil, which also had a high mineral content. The authors suggest the use of umbu seeds in the production of edible oil or in food if the absence of toxic substances can be demonstrated.

Considered native to the Brazilian semiarid regions, the species *Amburana cearensis* A. C. Sm. is found almost everywhere in South America and is known as umburana, “imburana”, “cumaru” and other designations. This species is widely used in folk medicine and for commercial purposes, highlighting the importance of further studies, especially on its seed, as there is almost nothing reported in the literature in terms of the chemical composition of *A. cearensis* seeds. According to Leal *et al.*,¹¹ umburana seeds contain approximately 23% oil, which consists mainly of glycerides of palmitic, linoleic, oleic and stearic acids. Coumarin and 6-hydroxycoumarin are also found in the seeds. Regarding the shells of the *A. cearensis* stalk, Canuto and Silveira¹² reported a methodology for isolation and identification of 12 chemical constituents present in the ethanol extract. According to Rossi,¹³ various substances were already isolated from the shells, including coumarin, isokaempferide, fisetin, alfolon and amburosides.

The objective of this work was to study the composition of the materials, which in earlier studies^{3,4} were identified as having desalination capacity and being potentially applicable in homemade device for brackish water desalination, targeting human watering. The authors found that umbu seeds as well as umburana seeds have the ability to remove about 25% of salts of low salinity brackish water (1 to 5‰) after contact for 10 min. The efficiency doubles with heating the water to 50 °C. However, the desalination process using these materials involves ion exchange besides

adsorption. Thus, it became necessary to study the chemical composition of the materials to check the possibility of transfer of chemical species to water during the contact between material and brackish water in the desalination process. The phytochemical profile of these biological materials, including materials in the coal form (which is the most efficient form for the desalination process), was then investigated. Initially, the main secondary metabolites were investigated (alkaloids, flavonoids, tannins, coumarins, and others) in these seeds. Additionally, the saturated fatty acid composition was determined.

Experimental

Biological materials were purchased at free fairs, and they originated from the semiarid region of Bahia. The fruits were washed and dried at room temperature, and the seeds were then removed. The *in natura* seeds were ground in a mill and sieved through 0.8 mm mesh. Figure 1 shows adult trees, fruits and seeds of both species.

The moisture content of the ground *in natura* seeds was determined drying the material at 110 °C in an oven to constant weight.

Coals of the seeds were obtained by heating the material in an oven at 250 °C for 1 h using the best conditions for maximum brackish water salt sorption capacity in a homemade desalination device.^{3,4}

During storage, crushed seeds of umburana formed a large amount of white crystalline solid, which was mixed with the sample and was also present in the storage container lid, indicating volatilization and crystallization processes. This material was subsequently identified as coumarin (1,2-benzopyrone) by solubility and melting point tests and by infrared (IR) and mass spectra.

Qualitative phytochemical analysis of the major classes of secondary metabolites present in the extracts

The purpose of this step was to identify the presence of chemical groups in the biological material prepared with umbu and umburana seeds using specific reagents according to procedures described in the literature.¹⁴⁻¹⁷

Preparation of the extracts for chromatographic profile determination

The extracts were prepared at 10% (m/v) by macerating the sample followed by sequential extraction with solvents of increasing polarity: hexane, dichloromethane (DCM), ethyl acetate and ethanol, using 15 g of the material prepared with the seeds and 150 mL of solvent. The solid phase was dried at 40 °C for a period of 48 h; this was followed by a new



Figure 1. Mature tree, fruits and seeds of (a) umbu and (b) umburana.

extraction cycle. The extracts were identified with the codes Ac (*in natura* umburana) and Ac-C (as coal) in the order of elution solvents as follows: Ac1 and Ac-C1 (hexane extract of *in natura* umburana seeds and as coal, respectively); Ac2 and Ac-C2 (dichloromethane extract of *in natura* umburana and as coal, respectively); Ac3 and Ac-C3 (ethyl acetate extract of *in natura* umburana and as coal, respectively); and Ac4 and Ac-C4 (*in natura* umburana ethanolic extract and as coal, respectively). Analogously, the extracts of umbu seeds were identified as follows: St-1 and St-C1; St-2 and St-C2; St-3 and St-C3; St-4 and St-C4.

Identification of the crystallized solid produced during the storage of milled *in natura* umburana seeds

Solid crystals were placed into 4 test tubes into which the following were added (in order): water, diethyl ether, chloroform and ethanol. The solubility of the solid in these solvents was observed.

The melting point of the crystals was determined in triplicate using a Microquímica melting point apparatus (model MQAPF 301).

The solid was analyzed in KBr tablets with an Fourier transform infrared (FTIR) spectrometer (Bomem, model MB100).

The determination of the mass spectra was carried out by injecting 1 L of methanol solution containing 1 mg mL⁻¹ of the solid into a gas chromatograph for mass spectrometric detection (GC-MS) (Shimadzu, Model GC-2010 Plus). An Rtx-5MS column was used (Restek; 30 m × 0.25 mm i.d.; 0.25 mm film thickness), and the oven was set at 50 °C for 1 min with a heating ramp of 5 °C min⁻¹

to 130 °C and 10 °C min⁻¹ to 300 °C for 2 min. The injector was maintained in splitless mode at 250 °C. The carrier gas was helium with a flow rate of 1.2 mL min⁻¹. The analysis was performed in triplicate, and the results were interpreted using the National Institute of Standards and Technology (NIST) library (spectra 05, 21, 27, 107 and 147).

Analysis of the extract constituents of the studied seeds by high performance liquid chromatography (HPLC)

Preparation of the standards for analysis

A total of 12.5 mg of each compound (coumarin, gallic acid and *L*-ascorbic acid) was weighed in an Eppendorf microcentrifuge tube, and 5 mL of methanol chromatographic standard was added. The tube was then placed on a vortex-type agitator for 60 s. The contents were transferred to a 25 mL volumetric flask to yield stock solutions of 500 µg mL⁻¹.

Method development

Using a liquid chromatograph (HP 1090, Agilent) and a reverse-phase column (Zorbax Eclipse XDB, C18, 150 × 4.6 mm, 5 µm), different combinations of mobile phases and flows were evaluated (Table 1) to optimize the running time associated with the resolution of the chromatographic peaks.

UV spectrometric analysis of gallic acid, *L*-ascorbic acid and coumarin in the extracts of the studied seeds

Verification of the absorption of each compound and the consequent optimization of the wavelength for identification of the compounds by HPLC-UV were performed using a scanning spectrum test in a UV spectrophotometer (Varian/

Table 1. Combinations of mobile phases and different flows to optimize the analysis of the extract constituents of the studied seeds by HPLC (running time 15 min)

Experiment	Mobile phase	Flow / (mL min ⁻¹)
1	H ₂ O/acetonitrile, 60:40	0.8
2	H ₂ O/acetonitrile, 70:30	0.8
3	H ₂ O/acetonitrile, 80:20	0.8
4	H ₂ O/acetonitrile, 60:40	1.0
5	H ₂ O/acetonitrile, 70:30	1.0
6	H ₂ O/acetonitrile, 80:20	1.0
7	H ₂ O/MeOH, 60:40	1.0
8	H ₂ O/MeOH, 70:30	1.0
9	H ₂ O/MeOH, 80:20	1.0
10	H ₂ O/MeOH/acetonitrile, 80:10:10	1.0

Cary) using standard solutions containing 30 µg mL⁻¹ coumarin, gallic acid and *L*-ascorbic acid, diluted separately in methanol. These solutions were subjected to scanning in the region from 200 to 400 nm using methanol as a chromatographic standard.

Figure 2 shows the overlapping patterns of UV spectra with the chosen wavelength that provided adequate sensitivity with the three compounds ($\lambda = 254$ nm). The analysis was performed with isocratic elution (water/acetonitrile, 80:20), a flow rate of 1.0 mL min⁻¹, an injection volume of 20 µL, and a running time of 15 min. The chromatographic conditions were the same as those used for the analysis of the DCM, ethyl acetate and ethanol extracts of the *in natura* umburana and umbu seeds and their respective coal forms. The thin layer chromatography (TLC) profiles of these extracts were obtained.

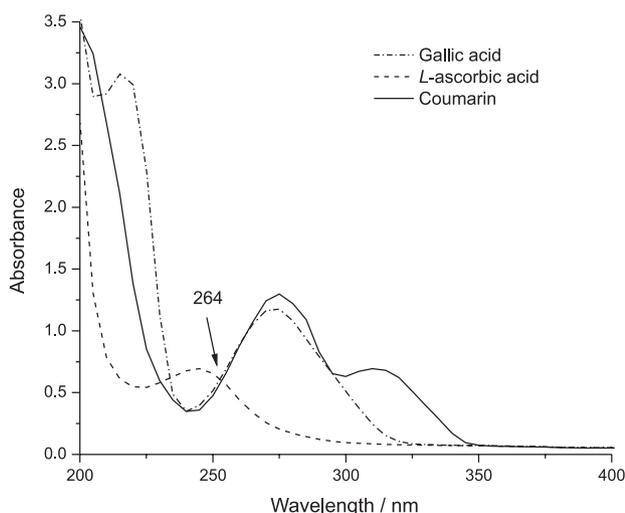


Figure 2. Overlap of UV spectra of gallic acid, *L*-ascorbic acid and coumarin in methanol.

Sample preparation for analysis

A total of 5 mg of each dried extract (DCM, ethyl acetate and ethanol) was weighed in Eppendorf microcentrifuge tubes. Next, 1 mL of methanol (chromatographic grade) was added following the same procedure for preparation of the standards, and the solution was transferred to a 10 mL volumetric flask.

Analysis of seed extracts by GC-MS

Mass spectra were determined by injection of 1 mL of a methanolic solution of the solid (1 mg mL⁻¹) into a GC-MS. The analysis was performed by comparison with the NIST library spectra 05, 21, 27, 107 and 147 using the same Shimadzu equipment and accessories described previously and the same analysis conditions. The fatty acids from the oil of the studied seeds were methylated according to the International Union of Pure and Applied Chemistry (IUPAC) 2301 method¹⁸ with some modifications, using the following procedure: approximately 100 mg of oil extracted from the hexane fraction was weighed in Eppendorf microcentrifuge tubes. Next, 2 mL of hexane were added followed by 0.2 mL of methanolic KOH solution (2 mol L⁻¹). The microtube was placed onto a vortex mixer for 60 s, and 2 mL of a saturated solution of NaCl were added. Subsequently, 1 mL of the organic phase was injected into the gas chromatograph, and the components were identified by comparing their retention times with those of the methyl ester standards (C18 to C20, Supelco). Quantification of the esters was performed by area normalization, and the determinations were performed in triplicate beginning at the lipid extraction stage. Compounds not identified in this way were compared to the NIST library of the chromatograph (spectra 05, 21, 27, 107 and 147).

Results and Discussion

Moisture content of the ground seeds and coal production yield

The moisture content of the ground seeds and average coal yield were 20.1 ± 0.17 and 66.7 ± 1.5%, respectively, for umburana seeds and 9.75 ± 0.23 and 41.9 ± 2.2%, respectively, for umbu seeds.

Phytochemical tests

Phytochemical tests with Ac, Ac-C, St, and St-C showed negative results for saponins because the foam formed in the solutions after stirring quickly disintegrated.

Tests for tannins were positive for the umburana samples (Ac and Ac-C) and umbu seeds (St), and a green color and precipitate formation were observed in the extracts, indicating the presence of condensed tannins.

In search of organic acids, the use of Pascová reactive yielded a blue color only with the extract of *in natura* umbu seeds (St). However, fatty acids were found in both seed extracts when analyzed by GC-MS, which can be explained by the higher limit of detection of the applied phytochemical tests.

For alkaloids, the results were considered positive in the samples of umburana (Ac and Ac-C) because of haze/precipitate formation with Dragendorff, Mayer and Wagner reagents. The presence of alkaloids and tannins in the umburana seeds can justify the popular use of these seeds as insect repellents, as these compounds are toxic and used by plants in defense mechanisms.¹⁹

The development of blue fluorescence was observed in umburana samples (Ac and Ac-C) and umbu samples (St) when they were exposed to UV light, indicating the presence of coumarins. The use of umburana seeds as flavoring agents and in the preparation of a fine powder used in rural areas to induce sneezing in the treatment of nasal congestion is due to the presence of coumarins. However, based on the hepatotoxicity of coumarin and its carcinogenicity (as observed in mice), in 1994, the European Commission recommended²⁰ the reduction of the maximum coumarin content in foods and beverages to 0.5 mg kg⁻¹ (maximum value detectable at the time). The addition of coumarin in foods is now banned in many countries.²¹

The test for flavonoids in *in natura* umburana (Ac) showed positive results, with pink color development (Shinoda reaction) and yellow color development when

the sample was brought to pH 11, indicating the presence of flavones, flavonols and xanthenes. Additionally, there was positivity for catechins (catechin tannin) as indicated by the yellow/brown color observed upon heating the solution after acidification to pH 3. The *in natura* umbu sample (St) also showed positive results for flavonoids (flavones, flavonols and xanthenes) besides red coloration after heating the solution to pH 3, indicating the presence of leucoanthocyanidins. The presence of flavonoids in these seed materials is relevant due to the interesting properties of these compounds, such as their antitumor, anti-inflammatory, antiviral, and antioxidant activities.¹⁷

An analysis of terpenes indicated positive results only for *in natura* umburana (Ac) through the emergence of a stable, slightly yellowish color in the solution with the Liebermann-Burchard reaction.

The test for steroids showed positive results with the Salkowski reaction for umburana and umbu, both *in natura*.

Table 2 shows the mean extraction yields using different solvents for *in natura* umburana seeds, and as coal (Ac and Ac-C) and Table 3 shows the yields for *in natura* umbu seeds, and as coal (St and St-C), including the results related to the solvents used.

The data in Tables 2 and 3 show that, for all samples, the hexane extract had the greatest mass when compared to the other extracts and the yield of the coal sample extracts was smaller than that of seeds *in natura*. One exception was the hexane coal extract of the umburana seed, the extract of which had a greater mass. This can be explained by the elimination of moisture during heating to obtain coal, which favors the extraction of compounds that are generally found in the hexane fraction.

Table 2. Extraction yields from *in natura* umburana seed (Ac) and from its respective coal (Ac-C)

Solvent	Extraction / g	Extraction / %	Extraction / g	Extraction / %
Hexane	Ac1		Ac-C1	
	$3.4 \pm 7.2 \times 10^{-3}$	68	$3.5 \pm 4.0 \times 10^{-3}$	82
Dichloromethane	Ac2		Ac-C2	
	$0.56 \pm 7.5 \times 10^{-3}$	11	$0.36 \pm 7.6 \times 10^{-3}$	8.4
Ethyl acetate	Ac3		Ac-C3	
	$0.13 \pm 9.3 \times 10^{-3}$	2.5	$0.043 \pm 3.9 \times 10^{-3}$	1.0
Ethanol	Ac4		Ac-C4	
	$0.90 \pm 4.7 \times 10^{-3}$	18	$0.38 \pm 2.2 \times 10^{-3}$	8.9
	Ac		Ac-C	
Total extracted mass / g	$5.0 \pm 6.2 \times 10^{-3}$	100	$4.3 \pm 9.2 \times 10^{-3}$	100
Extraction yield / %	$33 \pm 4.0 \times 10^{-2}$	–	$29 \pm 6.0 \times 10^{-3}$	–

Ac: *in natura* umburana seed; Ac-C: umburana coal.

Table 3. Extraction yields from *in natura* umbu seeds (St) and from its respective coal (St-C)

Solvent	Extraction / g	Extraction / %	Extraction / g	Extraction / %
Hexane	St1		St-C1	
	$0.24 \pm 6.2 \times 10^{-3}$	53	$0.074 \pm 3.3 \times 10^{-3}$	39
Dichloromethane	St2		St-C2	
	$0.059 \pm 7.8 \times 10^{-3}$	12	$0.041 \pm 91.4 \times 10^{-3}$	20
Ethyl acetate	St3		St-C3	
	$0.026 \pm 3.0 \times 10^{-3}$	5.7	$0.033 \pm 2.2 \times 10^{-3}$	16
Ethanol	St4		St-C4	
	$0.13 \pm 4.9 \times 10^{-3}$	29	$0.044 \pm 4.0 \times 10^{-3}$	28
	St		St-C	
Total extracted mass / g	$0.46 \pm 5.4 \times 10^{-3}$	100	$0.19 \pm 7.6 \times 10^{-3}$	100
Extraction yield / %	$3.1 \pm 4.0 \times 10^{-2}$	–	$1.3 \pm 5.0 \times 10^{-2}$	–

St: *in natura* umbu seed; St-C: umbu coal.

Identification of the solid (white crystals) formed during storage of the milled seed of umburana

In a preliminary analysis, it was observed that the solid volatilized from the milled umburana seed had characteristics of coumarins (i.e., the white crystal form and odor of vanilla). In an attempt to identify the material, it was analyzed by TLC using a hexane/ethyl acetate (6:4) eluent system and identification with solid iodine, which appears in images only as a stain, indicative of a single substance constituting the solid in question.

Solubility and melting point tests were also performed with the solid. Low solubility was observed in water, and high solubility was observed in ether, chloroform and ethanol. Using the Microquímica MQAPF 301 apparatus, the average melting point of the solid was determined to be 69.9 ± 0.1 °C. These results indicate that the solid could be coumarin (1,2-benzopyrone), which is described as a white crystal at room temperature with an aroma of vanilla, a melting point between 68 and 70 °C, high solubility in ethanol, chloroform and ethyl ether and slight solubility in water.²²

Infrared and mass spectra were obtained to confirm the identity of the material. Figure 3A shows the IR spectrum of the solid produced during the storage of ground umburana seeds. Following the analysis presented by Lopes and Fascio,²² the IR spectrum of the studied solid shows the presence of the following characteristic signals: (a) in the wavefrequency of 1706 cm^{-1} , a carbonyl of a conjugate ester; (b) in 1600 and approximately 1450 cm^{-1} , 2 signals indicating a C=C aromatic bond; (c) in the range from 1300 to 1000 cm^{-1} , 3 signals indicating an ester C–O (aromatic); and (d) in 770 and 735 cm^{-1} , signals indicating benzene disubstituted in the 1,2-positions. It may also be

noted that this spectrum is very similar to that of coumarin (1,2-benzopyrone) shown in Figure 3B.

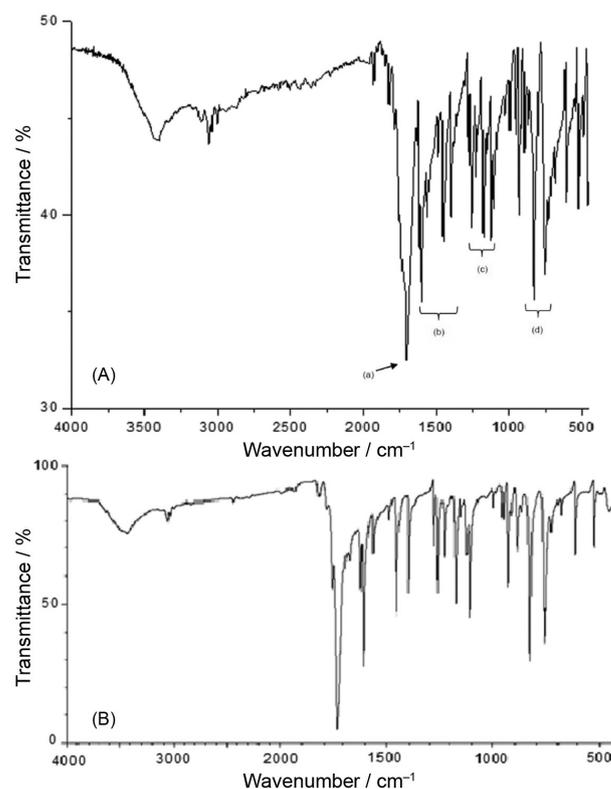


Figure 3. Infrared spectrum of (A) the solid produced during the storage of umburana ground seeds (this work); and (B) coumarin (1,2-benzopyrone, Advanced Industrial Science and Technology (AIST)).²³

The similarity of the mass spectrum when compared to the spectrum of the coumarin standard was 97%, confirming that the material was indeed coumarin. Canuto *et al.*²⁴ and Rossi¹³ also identified this compound in the bark and xilopods of the umburana tree.

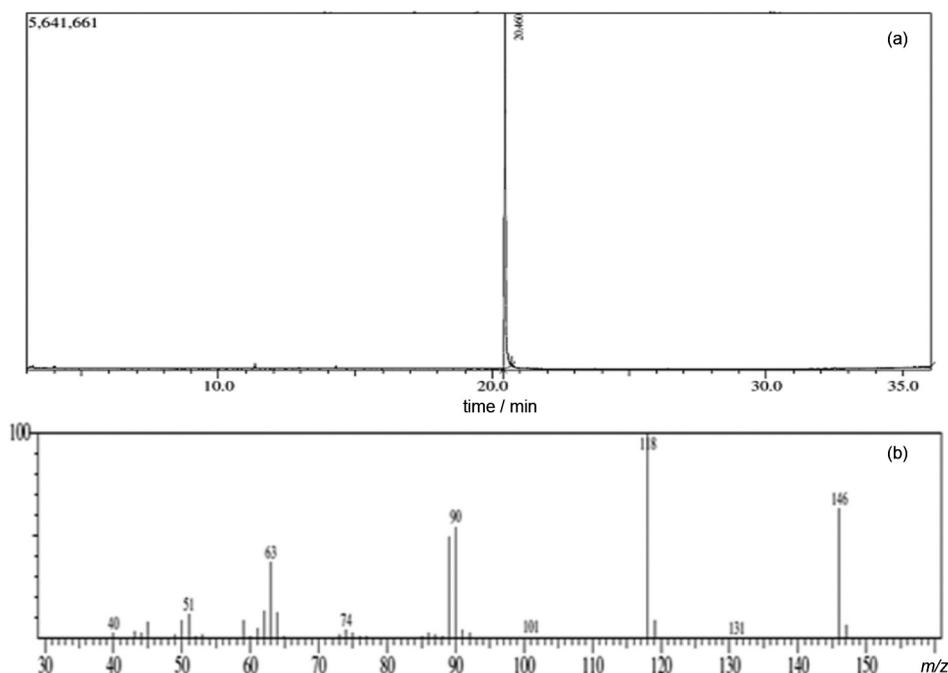


Figure 4. (a) Chromatogram and (b) corresponding mass spectrum of the solid compound produced during the storage of ground umburana seeds, identified as coumarin.

Figure 4 presents the chromatogram (Figure 4a) and the mass spectrum (Figure 4b) of the solid produced during the storage of ground umburana seeds. A strong signal is observed at a retention time of 20.46 min.

Analyses of the constituents of the seeds by HPLC

The UV absorption profile was determined using standards of each compound, and the wavelength (λ) was optimized for measurement by HPLC-UV. The absorption bands were evaluated individually and subjected to UV light scanning in the wavelength range of 200-400 nm. In this range, spectra of gallic acid, *L*-ascorbic acid and coumarin were produced. Then, the sweep spectra of each compound were superimposed, aiming to determine the optimum wavelength for measurement by HPLC-UV, where the intensity and selectivity were adequate.

The chromatograms of the extracts of each sample showed that the following were present in the *in natura* umburana seeds: *L*-ascorbic acid and coumarin in the DCM and ethyl acetate extracts and gallic acid in the ethanolic extract. The coal showed the same profile, with the exception of coumarin, which was also present in the ethanol extract. In the *in natura* umbu seeds, the presence of *L*-ascorbic acid in DCM and in ethyl acetate extracts was observed. Gallic acid was present in all extracts, and coumarin was only present in the DCM extract. However, in their corresponding coals, only *L*-ascorbic acid was observed in all extracts. The presence of coumarin in

in natura umburana seeds was expected because the standard used was isolated from the seed itself. Moreover, Rossi¹³ reported the presence of this substance in those seeds. The positive result found in the *in natura* umbu seed was confirmed by addition of a coumarin standard to the DCM extract, which led to an increase in the peak area without the appearance of new signals.

Comparing these results with those found in the chemical tests, there was a concordance for coumarin in all analyses, with positive results for the *in natura* umburana seeds, the corresponding umburana coal and *in natura* umbu seed. The same result was also observed for gallic acid when compared to the results found in the phytochemical tests: positive for the *in natura* umburana seed, the umburana coal and the *in natura* umbu seed. The presence of gallic acid was confirmed by standard addition to the three extracts (ethanolic extracts of the *in natura* umburana seed, umburana coal and *in natura* umbu seed), which produced peaks at retention times similar to those of the standard.

The presence of *L*-ascorbic acid was confirmed in the two seed coals and was monitored at 245 nm by standard addition to the ethyl acetate extract. In this solvent, preliminary positive results were observed in all samples (*in natura* and as a coal).

Fatty acids in the seeds

Initially, the esterification reaction was studied in the hexane extracts. The fatty acids were identified by

comparing the retention times of their methyl esters in the samples and standards using GC-MS. Chromatograms were generated, and mass spectra contained ions typical of the methyl ester equivalents to the fatty acids. The acids not identified in this way were compared to the library spectra.

Table 4 shows the retention times (t_R) of methyl esters analyzed by GC-MS in Ac, Ac-C, and in St and St-C.

The data in Table 4 shows that the umburana seeds contained ten fatty acids: one unsaturated fatty acid (oleic acid), eight saturated fatty acids, and one unidentified fatty acid. Ac-C contained myristic acid and the same acids found in Ac. In umbu, it was noted that St-C showed almost the same fatty acid composition as St; however, linoleic acid was not found.

The presence of linoleic acid is quite relevant because according to Mendes *et al.*,²⁵ both the n-3 and the n-6 unsaturated fatty acids can reduce low density lipoprotein (LDL) levels (i.e., the bad cholesterol).

The results for umbu seeds are similar to those reported by Borges *et al.*,¹⁰ who also found the following saturated acids in this seed: palmitic acid and stearic acid (both in the majority) with oleic acid as the predominant unsaturated acid. In the case of umburana, previously published studies only revealed the fatty acid composition of the stem bark and xilopods.

Table 5 lists the fatty acids present in Ac, Ac-C, St and St-C determined as a function of peak area in the total ion chromatograms from their equivalent esters.

The data presented in Table 5 show that in the two types of seeds, palmitic acid is the most abundant saturated fatty

acid, followed by stearic acid. It was also observed that oleic acid is the only unsaturated acid in the umburana seed, accompanied by linoleic acid in the umbu seed and majority in both seeds. It was also observed that the oleic acid percentage decreased by 14% from the *in natura* umburana seed to its coal, which was expected due to the temperature required to obtain the coal, which favors the breakdown of unsaturated bonds as well as the carbon chain itself. This temperature effect may also explain the slight difference between the percentage of these acids in the *in natura* umbu seed and in its coal, where an increase in acid concentration should occur due to decomposition of the linoleic acid existing in the *in natura* seed used to prepare the coal. This thereby compensates for its loss by thermal decomposition in the *in natura* seed.

Conclusions

Regarding the study of umburana seeds, the average yield of coal from ground umburana seeds was higher than that obtained with ground umbu seeds, (66.7 ± 1.5 and $41.9 \pm 2.2\%$, respectively). In the *in natura* umburana seeds, the following secondary metabolites were detected: tannins, alkaloids, coumarins, flavonoids, triterpenes and steroids; the first three were also present in the coal. Also found in this *in natura* seed were 10 fatty acids; eight saturated acids (palmitic, margaric, oleic, stearic, *n*-nonadecanoic, arachidonic, *n*-heneicosanoic and lignoceric); one unsaturated fatty acid (oleic acid), which was the major species (61.7%); and another unidentified fatty acid. The

Table 4. Retention times (t_R) of methyl esters analyzed by GC-MS in *in natura* umburana seeds (Ac), umburana coal (Ac-C), *in natura* umbu seeds (St) and umbu coal (St-C)

Fatty acid	Corresponding ester (IUPAC name)	Molecular formula	t_R / min	Presence (+)			
				Ac	Ac-C	St	St-C
Myristic	tetradecanoic	C ₁₅ H ₃₀ O ₂	24.58	–	+	+	+
Palmitic	hexadecanoic	C ₁₇ H ₃₄ O ₂	26.91	+	+	+	+
Margaric	heptadecanoic	C ₁₈ H ₃₆ O ₂	27.96	+	+	–	–
Linoleic ^a	<i>cis,cis</i> -9,12-octadecadienoic	C ₁₉ H ₃₄ O ₂	28.69	–	–	+	+
Oleic ^a	<i>cis</i> -9-octadecenoic	C ₁₉ H ₃₆ O ₂	28.74	+	+	+	+
Elaidic ^a	<i>trans</i> -9-octadecenoic	C ₁₉ H ₃₆ O ₂	28.79	–	–	–	–
Estearic ^a	octadecanoic	C ₁₉ H ₃₈ O ₂	28.96	+	+	+	+
<i>n</i> -Nonadecilic	nonadecanoic	C ₂₀ H ₄₀ O ₂	29.90	+	+	+	+
N. I.	–	–	30.61	+	+	–	–
Arachidonic ^a	eicosanoic	C ₂₁ H ₄₂ O ₂	30.81	+	+	+	+
<i>n</i> -Heneicosoic	heneicosanoic	C ₂₂ H ₄₄ O ₂	31.67	+	+	+	+
Behenic	docosanoic	C ₂₃ H ₄₆ O ₂	32.50	+	+	+	+
Lignoceric	tetracosanoic	C ₂₄ H ₄₈ O ₂	34.05	+	+	–	–

^aUsed as standards. t_R : Retention time; Ac: *in natura* umburana seed; Ac-C: umburana coal; St: *in natura* umbu seed; St-C: umbu coal; N. I.: not identified.

Table 5. Fatty acid compositions of *in natura* umburana seeds (Ac), umburana coal (Ac-C), *in natura* umbu seeds (St) and umbu coal (St-C) calculated according to the areas of the chromatographic peaks.

Fatty acid		Composition / %			
Name	Symbol	Ac	Ac-C	St	St-C
Saturated					
Myristic	C14:0	–	0.19	0.18	0.28
Palmitic	C16:0	16	17	20	24
Margraic	C17:0	0.62	3.4	–	–
Stearic	C18:0	10	15	15	19
<i>n</i> -Nonadecylic	C19:0	3.4	7.6	1.2	5.7
Araquidic	C20:0	41	5.1	1.4	5.3
<i>n</i> -Heneicosoic	C21:0	0.68	1.3	0.33	1.3
Behenic	C22:0	1.3	0.88	0.14	0.58
Lignoceric	C23:0	0.23	0.15	–	–
Total saturated acids		37	51	39	56
Unsaturated					
Oleic	C18:1 (n-9)	62	48	43	44
Linoleic	C18:2 (n-6)	–	–	18	–
Not identified	N. I.	1.6	0.54	–	–
Total unsaturated acids		63.6	48.54	61	44

Ac: *in natura* umburana seed; Ac-C: umburana coal; St: *in natura* umbu seed; St-C: umbu coal; N. I.: not identified.

coal of this seed contained the same fatty acids in addition to myristic acid.

By testing the solubility, melting point, IR and GC-MS, it was confirmed that the solid that had crystallized during the storage of umburana ground seeds was coumarin.

HPLC analysis of umburana seed extracts also confirmed the presence of coumarin and revealed for the first time the presence of *L*-ascorbic acid and gallic acid in the *in natura* seed as well as in the coal. The results of this phytochemical study show that these biological materials are unsuitable for use in the desalination device due to the presence of alkaloids and coumarin, which are toxic substances that are partially soluble in water. However, due to the ease of obtaining coumarin from the umburana ground seeds, although regarded as a toxic substance in foods, their use after extraction may be viable in cosmetics and cleaning products or as starting material for drug synthesis, among other uses.

Regarding the study of umbu, its *in natura* seeds contained seven saturated fatty acids (myristic, palmitic, stearic, *n*-nonadecylic, arachidic, behenic and *n*-heneicosoic) and two unsaturated acids (oleic (majority, 43%) and linoleic). The respective coal had the same composition of saturated fatty acids as its *in natura* seed, but contained only one unsaturated fatty acid (oleic acid). These seeds, which are rich in fatty acids, could be used in other applications,

especially due to the presence of linoleic acid, which is considered beneficial to health. In the *in natura* umbu seed, tannins, organic acids, coumarins, flavonoids and steroids were detected. None of the tested metabolites were found in its coal, and this result is promising for its use in a desalination device.

Using HPLC, several compounds were found in the extracts of the *in natura* umbu seed, including coumarin, *L*-ascorbic acid and gallic acid; this is the first report of these substances in this seed. In umbu coal, only *L*-ascorbic acid was observed, which reinforces that this material is promising for use in a desalination device. *L*-Ascorbic acid is a water-soluble substance, prevents diseases and is extremely important in fighting free radicals.

Acknowledgments

Program Cisternas-BRA-007-B, Instituto Ambiental Brasil Sustentável-IABS, Agência Espanhola de Cooperação Internacional para o Desenvolvimento and CNPq are acknowledged for financial support.

References

- Oliveira, C. N.; Campos, V. P.; Medeiros, Y. D. P.; *Quim. Nova* **2010**, *33*, 1059.

2. Buross, O. K.; *The ABC's of Desalting*, 2nd ed.; International Desalination Association: Topsfield, 2000.
3. Menezes, J. S.; Campos, V. P.; Costa, T. A. C.; *Desalination* **2011**, *281*, 271.
4. Menezes, J. S.; Campos, V. P.; Costa, T. A. C.; *Quim. Nova* **2012**, *35*, 379.
5. Tabosa, I. M.; Souza, J. C. A.; Graça, D. L.; Filho, J. M. B.; Almeida, R. N.; Riet-Correa, F.; *Vet. Hum. Toxicol.* **2000**, *42*, 155.
6. Washburn, K. E.; Breshears, M. A.; Ritchfv, J. W.; Morgan, S. E.; Streeter, R. N.; *J. Am. Vet. Med. Assoc.* **2002**, *22*, 1837.
7. Baca, S. F.; Vallenias, A.; Novoa, C.; Ochoa, J.; Cueva, S.; *Rev. Fac. Med. Vet. Univ. Nac. Mayor San Marcos* **1967**, *18-20*, 131.
8. Figueiredo, L. J. C.; Ferreira, M. M.; Antas, I.; Távora, J. P. F.; Simões, S. D.; *Arq. Esc. Med. Vet. Univ. Fed. Bahia* **1996**, *18*, 174.
9. Kitts, D. D.; *Trends Food Sci. Technol.* **1997**, *8*, 198.
10. Borges, S. V.; Maia, M. C. A.; Gomes, R. C. M.; Cavalcante, N. B.; *Quim. Nova* **2007**, *30*, 49.
11. Leal, L. K. A. M.; Nechio, M.; Silveira, E. R.; Canuto, K. M.; Fontenele, R. A.; Viana, G. S. B.; *Phytother. Res.* **2003**, *17*, 335.
12. Canuto, K. M.; Silveira, E. R.; *Quim. Nova* **2006**, *29*, 1241.
13. Rossi, T.; *Amburana cearensis (Freire Allemão)*; Instituto de Pesquisas e Estudos Florestais: Piracicaba, 2008; <http://www.ipef.br/identificacao/amburana.cearensis.asp> accessed in January 2016.
14. Costa, A. F.; *Farmacognosia*, 4^a ed.; Fundação Calouste Gulbenkian: Lisboa, 1994.
15. Barbosa, W. L. R.; Quignard, E.; Tavares, I. C. C.; Pinto, L. N.; Oliveira, F. Q.; Oliveira, R. M.; *Manual para Análise Fitoquímica e Cromatográfica de Extratos Vegetais*; Revista Científica da UFPA: Belém, 2001; http://www2.ufpa.br/rcientifica/didaticos_cientificos/pdf_textos/abord_fitoquimica.pdf, accessed in February 2016.
16. Matos, F. J.; *Introdução à Fitoquímica Experimental*, 3^a ed.; Universidade Federal do Ceará: Fortaleza, 2009.
17. Simões, C. M. O.; Schenkel, E. P.; Gosmann, G.; Mello, J. C. P.; Mentz, L. A.; Petrovick, P. R.; *Farmacognosia: da Planta ao Medicamento*, 5^a ed.; Universidade Federal do Rio Grande do Sul: Porto Alegre, 2004.
18. International Union of Pure and Applied Chemistry (IUPAC) method 2301: *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th ed.; Blackwell Scientific Publications: New York, 1987.
19. Cohen, A. J.; *Food Chem. Toxicol.* **1979**, *17*, 277.
20. Comissão Europeia (CE); *Ciências e Técnicas de Alimentação Humana. Relatórios do Comitê Científico da Alimentação Humana*, 36^a ed.; CE - Direção Geral da Indústria: Lisboa, 1997.
21. <http://www.cropwatch.org/Coumarin%20-%20the%20real%20story%20update%20feb%202009.pdf> accessed in January 2016.
22. Lopes, W. A.; Fascio, M.; *Quim. Nova* **2004**, *27*, 670.
23. http://sdfs.riodb.aist.go.jp/sdfs/cgi-bin/direct_frame_top.cgi accessed in January 2016.
24. Canuto, K. M.; Silveira, E. R.; Bezerra, A. M. E.; *Quim. Nova* **2010**, *33*, 662.
25. Mendes, A. C. R.; Albino, E.; David, P. B. S.; Neto, A. C.; *Hig. Alim.* **1998**, *12*, 11.

Submitted: September 29, 2015
Published online: February 16, 2016