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RESEARCH ARTICLE

Polyphenols extraction from sorghum grains using ultrasound, microwave and green solvents

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Abstract

Sorghum with rich-tannin grain is a crop used where birds are a pest at harvest time. However, this commodity is undervalued because its high levels of polyphenol cannot be used to feed monogastric animals. This study was carried out with the following objectives: To determine the best conditions for extraction of polyphenols from sorghum grains using ultrasound, microwave and to use water and ethanol as environmentally friendly solvents. The most abundant polyphenols extracted from sorghum grains were determined using HPLC-mass spectrometry analysis and chemical characterization of extracts from sorghum grains by FTIR-ATR. In addition, the mineral composition of the extracts was determined and color, alkaloids, pH and solubility were determined as part of the characterization. Polyphenols extraction was performed using 3 combinations of mass/volume (1:8, 1:12 and 1:16) and 3 aqueous ethanol (0 %, 30% and 70 %) with 9 treatments. These treatments were placed for 20 min in an ultrasound bath, after which samples were exposed to microwave for 5 min. The obtained phytochemical compounds were characterized by FTIR-(ATR) and HPLC/ESI/MS. Results showed that when HPLC/ESI/MS analyses were used, 38 polyphenol compounds were detected. This suggests that tannin-rich sorghum grains could be a source of diverse polyphenols that are linked to interesting biological activities.

Keywords: ethanol; FTIR-(ATR); HPLC mass; phytochemicals compounds; *Sorghum bicolor* (L.); water

Introduction

Sorghum has diverse uses and applications ranging from alcoholic beverages to balanced animal feed; however, sorghum varieties with high tannin content affect the quality of animal feed, giving it a tiny market (1). Tannins are part of the group of polyphenols, which are secondary metabolites that have biological activity such as antimicrobial, antioxidant, photoprotective capacity, inhibition of tumor growth, activation of hepatic detoxification systems and blockage of metabolic pathways that can cause carcinogenesis, etc. (2,3). Besides, polyphenols have antiviral, anticancer, anti-inflammatory, antidiabetic, insecticidal and herbicidal functions (4). On the contrary, a high content of tannins in sorghum grain affects the flavors and nutritional value of foods for monogastric animals and in birds, it causes astringency and therefore repellency (5). These tannins in the grain are in hydrolysable or condensed form ; sorghum synthesizes significant amounts of condensed tannins, positively associated with agronomic attributes but negatively associated with nutritional quality (6,7). Condensed tannins, such as those found in sorghum, are the result of the polymerization of flavan-3-ol units such as catechin, epicatechin or leukocyanidin linked each other by covalent bonds, or they can form interactions with other molecules and that due to their inability to be hydrolysed, they have been involved in various anti-nutritional activities, for example the sequestration of micronutrients, which makes digestion and absorption of nutrients impossible (8). On the other hand, hydrolysable tannins consist of 3 structures: esters of phenolic acids, gallic acid, 3, 4, 5-trihydroxybenzoic acid and ellagic acid (4′,5,5′, 6, 6′-hexahydroxydiphenic acid-2, 6, 2′, 6′-dilactone) with a linkage to a sugar (usually esterified glucose) and a polyalcohol; Its name refers to its easy ability to hydrolyse in the presence of acids (9).

Sorghum varieties that have grains with high polyphenol content are of special interest due to the various biological activities attributed to these compounds, which are why they have been named bioactive compounds (10). However, some of these compounds have been little studied. They can be extracted by different processes that do not alter

their composition and leave their various structures and characteristics intact.

Traditional ways of extracting bioactive compounds from plant sources use maceration, hydro distillation, Soxhlet, etc. (11). However, these techniques use high amounts of solvents and energy, long extraction times and low yields of extracted compounds (12). Some alternative extraction techniques are ultrasound and microwaves, which are environmentally friendly and use green solvents or a smaller amount of organic solvents (11). Ultrasound is based on sound source waves that vibrate up to 20000 cycles per second, creating microbubbles that undergo expansion and collapse processes (cavitation), altering the pressure and temperature of the liquid. Thus, compounds can be extracted more efficiently by the energy released and the efficiency (cost/benefit) it has (12). Meanwhile, in microwaves, through electric fields, they penetrate the polar molecules (mainly water, proteins, etc.), which, when trying to align and oscillate, create intramolecular frictions that cause heating.

Among the least polluting solvents are water and ethanol; a greater extraction of polyphenols is achieved in a mixture of ethanol/water as a solvent (13). Water has many advantages, including the absence of flammability and toxicity. While ethanol is a solvent with high affinity applied in extraction processes and can be obtained naturally from yeast fermentations and with various raw materials ranging from fruits, sugar cane, cereals, tubers, etc. (11). Based on this background, this study aimed to extract polyphenols from sorghum grains using ultrasound, microwaves and green solvents, for which the objective of this research was to evaluate the mass/volume relationships and types of solvents for the extraction of polyphenols from sorghum grains using ultrasound and microwaves and physiochemically characterize the extracts obtained. A brief and clear description of the purpose of the investigation, relating to the previous research and essential arguments, should be mentioned.

Materials and Methods

Vegetal material

The sorghum grain, variety BRS-72 from Monsanto, was obtained from a commercial crop established in the City of Tecoman, Colima, Mexico, which is located at latitude 18°54′37″ N, longitude 103°52′22″O and 32 min above sea level. First, the grain was cleaned, weighed and dried in an oven at 60 °C for 48 hr. Following the drying phase, the sample was finely ground using a multipurpose mill (TECNAL, TE-631/4). The sample was then stored in amber glass bottles to avoid direct light.

Mineral composition

The pulverized samples underwent analysis using a PANalytical Empyrean brand of equipment. These samples were analysed without any prior treatment. The study was conducted under specific conditions, comprising a 10 min run of Cu Kα radiation at 40 kV and 30 mA with 2θ.

Ultrasound-assisted extraction

Ultrasound-assisted solid-liquid extraction was performed using a Bransson 5500 ultrasonic bath with a volume of 5 L operating at 40 kHz frequency. In this study, 5 mass/volume (m/v) combinations were prepared, using water and ethanol in different proportions (as detailed in Table 1). Each sample was contained within a 50 mL Falcon tube and subjected to sonication for a duration of 20 min at room temperature. The samples were then covered from direct light and transferred to the microwave equipment. After sonication, the samples were shielded from direct light and transferred to the microwave equipment for further processing.

Microwave-assisted extraction

For the microwave-assisted extraction, a microwave equipment (Mars 6 CEM brand) with 50 mL reactors with double caps, adapted to a carousel, was used. The extraction was performed for 5 min at 70 °C, with a power of 800 W. Afterwards, the samples were filtered and subsequently placed in amber bottles to avoid their interaction with light.

Characterization

Color determination

The color measurement of the filtered extracts was carried out using a Colorimeter (WR-10QC model, AHCC brand, measurement range L: 0-100), using glass vials, with 70 % ethanol outside the vial.

Quantifying hydrolyzable polyphenols

The Folin-Ciocalteu methodology was used, with modifications. A gallic acid calibration curve was prepared at 0, 100, 200, 300, 400 and 500 ppm (14). Then, 400 µL of the sample was placed in an Eppendorf tube and 400 µL of Folin-Ciocalteu reagent was added. The sample was shaken and allowed to stand for 5 min. Then, 400 µL of 0.01 M sodium carbonate was added, mixed and allowed to stand for 5 min. After that, 2.5 mL of distilled water was added and a reading was taken at 790 nm using a Biomate brand UV-VIS Spectrophotometer.

Quantification of condensed polyphenols

This analysis used the HCl-Butanol technique with modifications (14). First, a calibration curve was performed using catechin at 0, 100, 200, 300 and 400 ppm. Then, 500 µL of a sample (250 µL of the extract and 250 µL of distilled water) were placed in a large test tube and 3 mL of HCl-Butanol solution (1:9) and 0.1 mL of the ferric reagent were placed and wave. Afterward, it was heated for 1 hr in a bath at 100 °C and the tubes were allowed to cool; finally, a reading

Table 1. Mass/volume proportion and solvents used in extracting polyphenols from sorghum grain

Treatment	Proportion (m/v)	Solvents
1	1:16	Ethanol 70 %
2	1:8	Ethanol 70 %
3	1:16	Distilled water100 %
4	1:8	Distilled water 100 %
5	1:12	Ethanol 30 %

was taken at 460 nm using a Biomate brand UV-VIS Spectrophotometer.

Determination of alkaloids

100 µL of sample dissolved in 10 % ethanol was placed and 20 µL of Wagner's reagent was added with 20 µL of concentrated hydrochloric acid. The sample was then allowed to stand for 10 to 15 min and a positive sample was determined if a brown precipitate was observed (15).

Determination of triterpenes

In this analysis, the qualitative test proposed by Liebermann-Burchad was followed (16). The reagent reacts with the double bonds of the conjugated rings of the molecules and they precipitate. First, 100 µL of the sample and 20 µL of anhydrous acetic acid and chloroform (1:1) were shaken in a test tube. After 1 hr, the change was recorded; the sample was considered positive if it was blue.

Separation and purification of polyphenols

Polyphenols were separated and purified by ion column chromatography using Amberlite XAD-16N as a filler, which was activated with methanol and washed with water. A cotton plug was placed on the chromatography column (borosilicate glass, 80 mm diameter, 100 mm length) and added water with Amberlite. The key to the column was opened for the Amberlite to be packed. On the other hand, each of the samples was centrifuged at 500 rpm for 10 min, the sample was placed in the column and distilled water was added until the solution extracted from the column was colorless. Subsequently, absolute reagent grade ethanol was added until a solution with an ethanol odor and intense color was obtained, which was taken as the ethanolic fraction, which was recovered and placed in glass trays to evaporate the liquid phase in an oven at 60 °C for 24 - 48 hr, in this way obtaining the dry extracts, in the form of crystals.

Solubility and pH tests

1 - 2 mg of each dry extract was placed in 7 test tubes previously washed with distilled water to rule out the presence of surfactants. Then 5 mL of the following solvents were added: water, methanol (CTR scientific brand, purity 99.8 %), ethanol (Quifersa, 96 %), ethyl acetate (Jalmek, 99.5 %), methylene chloride, ether and hexane (Baker Analyzed, 99.5 %, 98 % and 99 % respectively). Once the sample was dissolved, the pH was evaluated.

FTIR-ATR analysis

For FT-IR characterization, a Perkin-Elmer Spectrum 65 model spectrophotometer was used. The samples were analyzed without any type of treatment before analysis, working in a range of 4500 - 580 cm^{-1}, with 2 cm^{-1} of resolution, with 32 scans.

Identification of polyphenols by HPLC-MS

The identification of polyphenolic compounds in sorghum grain was carried out using mass-coupled HPLC equipment, using a Varian Prostar brand equipment, model 330 with a UV -visible diode array detector and coupled to a Varian brand mass detector (Model 500-MS). A flow rate of 0.2 mL/min, a C18 reverse phase column and a mass detection limit of 100 - 2000 m/z were used.

Results and Discussion

Color

Table 2 shows the results observed in the colorimetric analysis for the variables L, A, B, C and H. In the sorghum grain extracts, slight variations were observed in the parameters L, A, B and C and more variables for the parameter H, which corresponds to the Hue index. Using the language of L-A-B coordinates of the color ranges and how oriented towards black it is (C), the bio composites of the sorghum grain are not affected by the microwave and ultrasound methodology. According to the increase in the percentage of ethanol used in the extractions, there is a trend of elevation in C, which may denote a greater extraction of bioactive compounds. Color can be related to the variation of pigments, which are called polyphenolic compounds such as anthocyanins and carotenoids (17). The fact that these compounds are accentuated in products represents increased parameters such as the C parameter and/or the Hue index. Some authors specify the causes of color variations in products, due to the accumulation of specific carotenoids such as β-carotene, xanthophylls, β-cryptoxanthin, zeaxanthin, violaxanthin and capsanthin (18). The parameter L is found similar in all treatments. However, there are no significant differences between them, compared to the other parameters, the brightness is found increased, which attribute the change in this parameter to a lower total polyphenol content, but to a greater diversity of polyphenols, the parameter was associated with the presence of chlorogenic acid and/or catechins, which in turn can be highlighted, that treatment 1 and 2 are found towards the value of redness (a) and that they are also in the treatments in which compounds such as Epigallocatechin and Gallocatechin were identified (19). The variations in parameter b are linked to the presence of anthocyanins and flavonols, which coincide with some compounds derived from these, especially in treatment 2 (20). The intensity of the

Table 2. Colorimetric analysis of ground sorghum samples with three replicates for each treatment

Treatment	M: V EtOH/Water	L	A	B	C	H
1	1:16 70/30	32.3 ± 0.7	15.9 ± 0.1	13.9 ± 0.3	21.2 ± 0.2	41.1 ± 0.4
2	1:8 70/30	28.1 ± 0.9	16.9 ± 0.7	10.3 ± 0.3	19.8 ± 0.7	31.2 ± 1.0
3	1:8 0/100	40.0 ± 0.7	2.3 ± 0.2	1.8 ± 0.3	3.0 ± 0.0	38.2 ± 7.0
4	1:16 1/100	37.1 ± 0.7	3.2 ± 0.1	1.9 ± 0.5	3.7 ± 0.2	30.6 ± 7.0
5	1:12 30/70	32.1 ± 0.9	5.9 ± 0.1	5.1 ± 0.5	7.8 ± 0.4	40.8 ± 2.4

chroma color (C) was also found with higher values in treatments 1 and 2, which were ethanolic extracts. As already mentioned, the accumulation of pigments can vary according to the process used for extraction, among which light, temperature, solvent and pH are the most important (21).

Minerals

Fig. 1 shows the 5 major minerals present in sorghum, observing that potassium was the compound found in the highest proportion with 52 % of the total, followed by phosphorus, sulfur, silicon, calcium and chlorine with 20, 6, 5, 5 and 3 % respectively. The other 4 % corresponds to other minerals such as titanium, chromium, manganese, iron, cobalt, nickel, copper, gallium, bromine, iodine and molybdenum.

Sorghum is a good source of B complex vitamins such as thiamine, riboflavin and niacin and among the main minerals in sorghum are potassium, phosphorus, iron and to a lesser extent, calcium (22). By grinding the sorghum grain, flour can be obtained from which baking and confectionery products are made that do not contain gluten, which makes them suitable for consumption by celiacs (6).

Hydrolysable tannins

The amount of hydrolysable tannins due to different m/v ratios and solvents varied from 5.38 to 6.47 mg/g (Table 3), with the highest yields being observed with the m/v ratios and solvents (1:16, 70 % ethanol and 1:8, water 100 %). Hydrolysable tannins are those found in smaller quantities in the high-tannin sorghum grain, compared to the condensed ones, which are found around the grain proteins (23). Furthermore, the concentration of tannins differs according to the sorghum genotype, the pigmentation of the testa and the quantification method (24). Pentagaloyl glucose is one of the hydrolyzable tannins of sorghum that, in *in vivo* studies, was shown to prevent the growth of prostate and lung cancer cells and helped reduce tumors (25).

Condensed polyphenols

The largest amount (9.94 mg/g) of condensed tannins was obtained when the m/v ratio 1:8 was used, using water as a solvent. The B1_B2_ genes determine the presence of tannins in the seed coat. The pigmentation of the testa is due to the condensed tannins; for this reason, in sorghum grains considered low in tannins and without pigmented testa, small amounts of total polyphenols can be detected; other compounds, such as flavonoids, also affect the coloration of the grain. The chemical composition of sorghum grain has condensed tannins and only small amounts of other phenolic compounds (24). Sorghum is one of the cereals with the highest amount of condensed tannins (25). The presence of the enhancer determines the number of tannins (I_) and the spreader gene (S_), interacting with the genes B1_B2_, Tp_, tptp and other genes that control the thickness of the mesocarp, in the same way they modify the color of the grain, intensifying or decreasing the basic colors. When the dominant alleles exist, B1 and B2, the presence of tannins and pigmentation of the testa exists. Still, if one is in a homozygous recessive state, the grain does not have tannin coloration in the testa (26).

The cultivation of sorghum is profitable in tropical areas, however, one of the main pests is birds and one of the economic alternatives to control this pest is the planting of sorghum cultivars with high tannin content that are tolerant to birds. However, this also represents a disadvantage, since tannins form complexes with proteins, making them unusable by monogastric animals and therefore tannins are considered anti-nutritional compounds. When condensed tannins exceed 0.3 % of dry matter, they bind to proteins and precipitate, reducing the protein content, affecting the digestibility of the grain. Likewise, they inhibit the action of amylase by 10 - 30 % (27). Consequently, it affects their feeding efficiency and they can immobilize 12 times their size (23). On the other hand, these compounds have high potential to be used in the pharmaceutical and food industries due to their antimicrobial, antioxidant and anticancer properties.

Determination of alkaloids

All samples of sorghum grain extracts were negative for these compounds that give the plant resistance to herbivorous animals and insects (28). The absence of alkaloids determines a positive aspect regarding the safety of the consumption of sorghum grain and foods derived from it, since the level of single safe intake (ARfD) of alkaloids, established by the European food safety Agency (EFSA), should not be exceeded (29). If the presence of alkaloids is detected below the

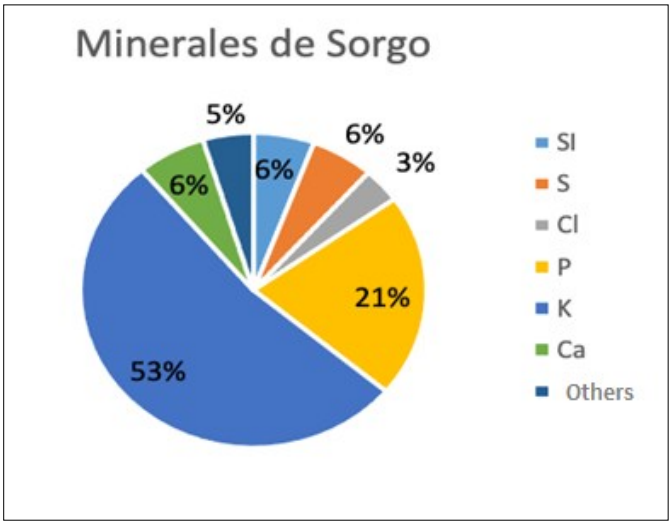

Fig. 1. Elements with majority percentages found in sorghum samples.

Table 3. The content of hydrolysable polyphenols and condensates of sorghum grain, extracted using ultrasound and microwaves

Ratio and solvent (m/v) and (%)	Hydrolysable tannins mg/g	Condensed tannins mg/g
1:16, ethanol 70 %	6.42 ± 0.27	8.26 ± 4.40
1:8, ethanol 70 %	6.17 ± 0.73	7.07 ± 1.80
1:16, water 100 %	5.57 ± 0.07	5.88 ± 0.37
1:8, water 100 %	6.47 ± 2.23	9.94 ± 1.62
1:12, ethanol 30 %	5.38 ± 1.77	4.55 ± 0.93

regulated limits, it is established that sorghum as silage is safe for consumption as food (30).

Determination of terpenes

The Lieberman-Burchard test is based on the change in color; if it is red, it indicates the presence of triterpenes and if it is blue, it is positive for terpenes (16). Triterpenes (Table 4) were detected in the sorghum grain samples extracted with the m/v ratios 1:16, 70 % ethanol and 1:12, 30 % ethanol. While terpenes were only detected in a sample of sorghum grain (1:8 m/v ratio, 70 % ethanol), that is, in the least diluted sample and when the greatest amount of ethanol was used (70 %).

Solubility tests

In this test, it was considered soluble (+) if it changed color, if it changed to a weak color, or a color was noticed. However, crystals were still present; it was marked as partially soluble (+/-) and if they did not dissolve, they were marked as insoluble (-). Their solubility that made it possible to decide which solvents would be used for future analyses. The dry extract samples were soluble in ether, ethanol and methanol (Table 5). Polyphenols are extracted with polar organic solvents, generally combined with water; the use of a particular solvent depends on the target polyphenols and the affinity of the group of compounds in the plant to be extracted (31, 32). Among the most studied solvents have been water, acetone, methanol and ethanol, due to the difference in their polarity, however in the present analysis the solvents methanol and water were chosen, since in addition to allowing the solubility of the compounds of interest, they do not leave residue in the extract and in this way are more environmentally friendly. Water in the extraction solvent mixture increases the solubility by weakening the hydrogen bonds; on the contrary, without the mixture with water, the absolute organic solvents produce a strengthening of the hydrogen bonds between the polyphenolic compounds and the proteins they present (33).

Table 4. Determination of terpenes/triterpenes using the Lieberman-Burchard test. (0) = no change, (-) = no precipitation, (+) = precipitation

Treatment	Sudden change	Terpenes (Blue)	Triterpenes (Red)
1:16, ethanol 70 %	-	-	+
1:8, ethanol 70 %	-	+	-
1:16, water 100 %	0	-	-
1:8, water 100 %	0	-	-
1:12, ethanol 30 %	-	-	+

Relative pH

The pH value of the solutions with the dry extract varied between pH 6 - 8 (Table 6). In most cases, the pH value did not change, due to the stability of functional groups that the extracts present in the different solvents. The extracted compounds are stable, even when present in the crude oil extraction. Extreme acidic and alkaline pH values can cause cis/trans isomerization of certain double bonds, regroupings and deesterifications (34).

FTIR-ATR Infrared Spectroscopy

Fig. 2 shows the FTIR-(ATR) spectrum, a) of sorghum without treatment, b) 1:8, water 100 %, c) 1:12, ethanol 30 %, d) 1:16, ethanol 70 %, e) 1:16, water 100 %. Evidence of the signal between 3320 - 3280 cm^{-1}, corresponding to the (-OH) bond, of the different phenols present in the extracts obtained, the presence of bands at 3280 cm^{-1}, corresponding to NH bonds, is also highlighted. The presence of characteristic bands (-C-H) of methyl, at 2900 cm^{-1}, unlike the spectra b) and e) in which this band is not present, other bands that were evident were at approximately 1050 cm^{-1}, corresponding to the single bond to oxygen (-C-O). Treatment 2 (1:8 m/v, 70 % ethanol) was not included in the analysis because it formed a resin that made this type of analysis difficult.

These analyses have been used for identification in polyphenolic extracts and essential oils (35). In the case of sorghum, necessary signals are shown within phenolic groups due to methyl groups, single and double bonds with oxygen and possible carboxylic acids. This agrees with many of the functional groups of the molecules identified in the HPLC-Mass analysis. Since some treatments were obtained with a percentage of ethanol in their extraction, it can be attributed the presence of vibrations of stretching of -OH groups, given in ranges of 3200 - 3687 cm^{-1} , however this signal is detected in all the treatments, which suggests that the signals correspond to the hydroxyl groups of the phenolic compounds (36, 37). Other authors relate these signals to aromatic rings, flavonoids and amino acids, due to the NH vibrations (3280 cm^{-1}), as well as to the 3350 and 3180 cm^{-1} stretches as secondary amines (38, 39). In addition, the signals at 2900 cm^{-1} are detected as methyls and some authors relate these stretching vibrations to the lipid range (40). The vibrations detected at 1015 corresponding to the C-O bonds, also attributed to the ester groups, are very present

Table 5. Solubility of dry extracts of sorghum grain polyphenols with high tannin content

Treatment	Water	Ether	Hexane	Methanol	Ethanol	Ethyl acetate	Chloride methyl
1:16, ethanol 70 %	-	+/-	+	+	+/-	+	+
1:8, ethanol 70 %	+/-	+/-	+/-	+	+/-	-	+/-
1:16, water 100 %	-	+	-	+/-	+/-	-	-
1:8, water100 %	+	+/-	-	-	+/-	+/-	-
1:12, ethanol 30 %	+/-	+/-	-	+	+/-	+/-	+/-

Table 6. pH values of sorghum grain extracts. T= treatment

T	Water	Ether	Hexane	Methanol	Ethanol	Ethyl acetate	Chloride methyl
1	7	7	6	8	6	6	7
2	6	6	7	6	6	6	6
3	5	6	6	6	6	6	6
4	7	6	6	7	6	6	6
5	6	6	7	6	6	7	6

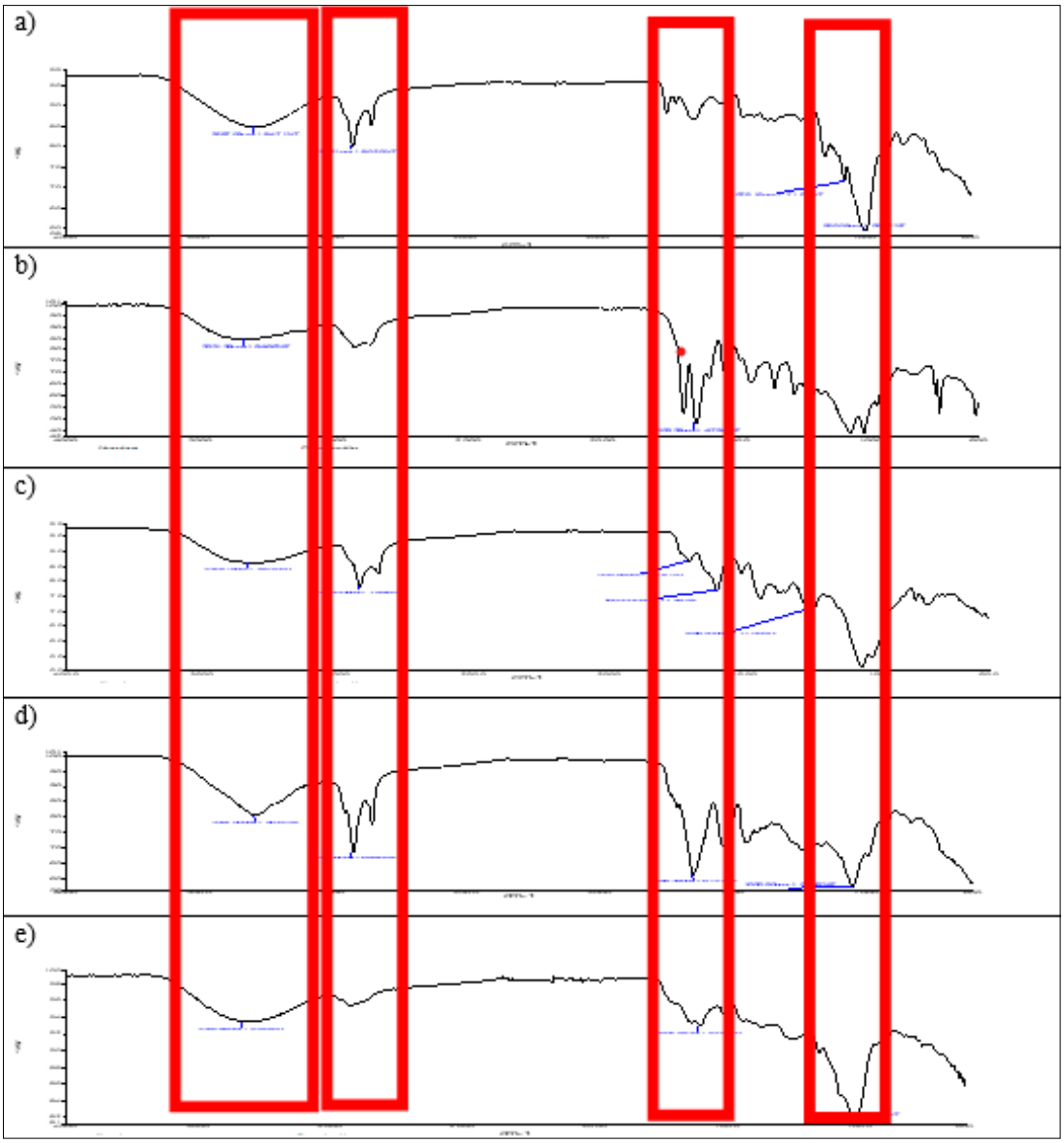

Fig. 2. FTIR-ATR spectra of the sorghum grain extract sample. M/v and solvent ratios: a) without treatment, b) 1:8, 100 % water, c) 1:12, 30 % ethanol, d) 1:16, 70 % ethanol, e) 1:16, 100 % water.

in polyphenols, mainly hydrolysable tannins such as gallic acid, cinnamic acids and benzoic acids (41).

Identification of the extracted polyphenolic compounds

The identification of the polyphenolic compounds of each of the sorghum grain extracts was carried out by high-performance liquid chromatography (HPLC), a method used by various authors , since it allows the identification of a great diversity of components present in plant tissues that are produced as a defense against animals, fungi and parasitic. Many of these compounds have microorganism-inhibiting capabilities. In addition, these polyphenols are used for different food industry processes, mainly as antioxidants to reduce the oxidation rate of products and therefore prolong their useful life and in pharmaceuticals due to their antioxidant capacity, neutralize free radicals and help prevent cardiovascular, cerebrovascular diseases and cancer (42 - 44). In this study, a total of 29 polyphenolic compounds were detected (Table 7). Treatment 1 (1:16 m/v, 70 % ethanol) was not included because the sample was insufficient for this analysis. With the m/v ratio 1:12 ethanol 30 %, it was possible to obtain the largest and most diverse number of polyphenolic compounds. Several authors state that HPLC analysis is possible to detect acid-based polyphenols (42), epicatechins and catechins, as was seen in this study (42, 45). Related to the yield in quantity and family of compounds, low frequency ultrasound technologies allowed higher yields in the extraction of phytochemical compounds, since through mechanical waves and non-thermal energy, it causes a physicochemical change in the solvent matrix and consequently the release of these compounds. In contrast, the microwave, using electromagnetic radiation reduces processing time and cost (46).

The compounds that are present in the majority of the different ratios were: (+)-Gallocatechin 3,7-Dimethylquercetin, p-Coumaroyl glucose and 7,4'-Dihydroxyflavone, which belong to families of compounds such as flavonoids and anthocyanins that have been used for its effect on cardiovascular health, for its antimicrobial, antioxidant and cytoprotective activity, even for attracting pollinators and preventing photo-oxidative damage in plants (42, 47, 48). In addition to the mentioned properties, various pharmacological and medical activities have been attributed to phenolic compounds. Among these, its

Table 7. Polyphenolic compounds identified in sorghum grain extracts obtained with ultrasound and microwaves. RT = Retention time

Mass (g/mol)	RT (min)	Compound	T-m/v			
			2-1:8	3-1:16	4-1:8	5-1:12
108.8	15.079	Catechol			*	
268.8	44.509	7,3',4'-Trihydroxyflavone	*			
304.8	23.776	(-)-Epigallocatechin	*			
311	51.23	Caffeoyl tartaric acid		*	*	
325.1	56.713	p-Coumaroyl glucose	*	*		
330.7	3.536	Galloyl glucose		*		
336.8	27.774	3-p-Coumaroylquinic acid			*	
338.9	53.509	Esculin			*	
340.9	3.11	Caffeoyl glucose	*			*
352.5	2.685	1-Caffeoylquinic acid			*	
352.7	3.638	(+)-Gallocatechin	*	*	*	*
540.1	55.293	Oleuropein (possibility)	*			
564.1	54.302	Pelargonidin 3-O-sambubioside	*			
566.1	55.731	Phloretin 2'-O-xylosyl-glucoside	*			
366.8	29.742	3-Feruloylquinic acid			*	
377.0	2.856	3,4-DHPEA-EA	*			
623	14.236	Isorhamnetin 3-O-glucoside 7-O-rhamnoside				*
288.8	23.772	(+)-Catechin				*
252.8	26.609	7,4'-Dihydroxyflavone	*			*
882.9	27.998	Prodelphinidin trimer C-GC-C				*
371	31.523	Sinensetin				*
302.8	33.1	Dihydroquercetin				*
704.9	38.279	(-)-Epicatechin-(2a-7) (4a-8)-epicatechin 3-O-galactoside				*
284.8	40.885	Luteolin				*
268.8	44.336	Apigenin				*
329	46.742	3,7-Dimethylquercetine		*	*	*
295	55.574	p-Coumaroyl tartaric acid				*
279	56.487	p-Coumaroyl malic acid				*
325	58.67	p-Coumaroyl glucose				*
		Total	10	5	8	15
		# of different compounds	6	1	5	11

vasodilatory, anticarcinogenic, anti-inflammatory, immune response stimulators, antiallergy, estrogenic effects and inhibitors of phospholipase A2, cyclooxygenase, lipoxygenase, glutathione reductase and xanthine oxidase stand out (49). They have recently been studied for their nutraceutical potential against coronavirus (50). Low-frequency ultrasound technologies allowed higher yields in the extraction of phytochemical compounds, since through mechanical waves and non-thermal energy, they cause a physicochemical change in the solvent matrix and consequently the release of these compounds, while microwaves, through electromagnetic radiation, reduce processing time and cost.

Conclusion

The highest extraction of phytochemical compounds from sorghum grain was achieved using ultrasound and microwaves and with the m/v ratio 1:12 ethanol 30 %, with 15 polyphenols, of which 11 were not detected in other extracts. The extracts were physicochemically characterized by color, solubility, pH, hydrolysable tannins and condensates. According to the mass-coupled HPLC analysis, the polyphenols found in most of the extracts were: (+)-Gallocatechin and 3,7-Dimethylquercetin. Using infrared analysis by Fourier transforms, it was possible to determine the phenolic groups by methyl groups, single and double bonds with oxygen and possible carboxylic acids. Given this and because the present study focused on the characterization of the extracts obtained by different ways (m/v concentration, solvent), it is established as a precedent for the subsequent determination of biological activities, using sorghum as a primary source, thus providing an added value to a raw material, which on the contrary has been undervalued as food. Still, it can be of great use in the biological field. Thus, it is important to highlight the biological importance of the extraction of a wide range of phytochemicals derived from sorghum, because they effectively treat non-communicable diseases. Nutritionally, it is essential for its starch and dietary fiber content, in addition to protein and for being a gluten-free cereal.

In addition, the aforementioned polyphenolic compounds were obtained through eco-friendly processes in terms of energy and time savings and using solvents with less impact on the environment.

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Authors' contributions

CGRG drafted the manuscript and participated in experimental analysis. SGA performed characterization studies by FTIR. GGGM participated in all experimental analyses. AVJA carried out phytochemical identification by HPLC. FGAC supervised all experiments and performed the statistical analysis. LBCM supervised all experiments and participated in mineral quantification. RHR conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

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During the preparation of this work, the author(s) used OpenAI/chatGPT to correct English language style. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the publication's content.

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