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COMPARATIVE ANTIOXIDANT POTENTIAL OF AQUEOUS AND ACETONE / WATER EXTRACTS OF GRAINS AND BRANS OF GRANIFEROUS SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH] HYBRID GUANIPA 71 *IN VITRO*

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ABSTRACT: Sorghum is an important food source with substantial nutritional value. An *in vitro* antioxidant evaluation of different extractions of graniferous sorghum [*Sorghum bicolor* (L.) Moench] hybrid Guanipa 71 was proposed here. Acetone / water extracts of both grains and brans revealed strong antioxidant properties by reducing Fe^{3+} (22.9% and 42.3%) and chelating Fe^{2+} (47.3% and 32.4%), respectively. Moreover, both extracts were found to inhibit the $DPPH^{\bullet}$ radical, exhibiting an IC_{50} (µg/mL) of 31.34 and 36.48 respectively, as well as, to reduce Molybdate (VI) through the relative antioxidant capacity assay (IC_{50} of 330and 227.6µg/mL, respectively). They also inhibited the oxidative degradation of 2-deoxyribose (2-DR) provoked by the Fenton reaction by 29.60% and 17.77%, respectively. Furthermore, aqueous extracts of grains and brans chelated Fe^{+2} by 65.09% and 59.12% and inhibited the degradation of (2-DR) in the Fenton reaction by 17.18% and 10.67%, respectively. Overall, the results showed that the acetone / water extraction from the brans of sorghum showed the best antioxidant effects, *via* electron transfer, radical scavenging and metal chelating activity. The antioxidant property may be attributed to the appreciable content of flavonoids and phenolic acids in the extract, including catechin, rutin, quercitrin, quercetin, luteolin and chlorogenic, caffeic, gallic and ellagic acids.

INTRODUCTION: Sorghum [*Sorghum bicolor* (L.) Moench] is a plant that is native of sub-Saharan Africa [1]. It has a wide variety of use, highlighting its largely use in the manufacture of feed rations [2]. Although, it is widely used in animal feed [3].

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It is however considered a basic food for millions of people in the world, supplying close of 70% of the daily caloric intake [1, 2]. Interestingly, some studies have shown that sorghum is a rich source of phenolic compounds and possess higher antioxidant activity compared to other cereals such as oats, rice and wheat [4].

Report by Fardet *et al*., 2008 [5] revealed that a diet rich in sorghum could prevent diseases in which oxidative stress has been implicated in their etiology, due to the content of polyphenols, tannins and anthocyanins.

Previous *in vitro* studies on the pharmacological properties of sorghum include cholesterol reduction, antioxidant, anti-inflammatory and anti-carcinogenic activities [6, 7]. These properties have been attributed to the presence of phenolic compounds in sorghum cultivars. These include hydrobenzoic and hydroxynamic acids derivatives and flavonoids that mainly encompass anthocyanins and tannins [1]. Such compounds possess high *in vitro* and *in vivo* antioxidant activity and may significantly contribute to the health benefits associated with whole grain consumption [8].

A variety of studies has investigated the impact of this cereal on metabolic parameters in feeding animals [9].

However, there are very few studies investigating the *in vitro* and *in vivo* antioxidant potential of crude extracts of sorghum grains. Likewise, there is no data in the literature evaluating the antioxidant potential of brans extracts from sorghum, which makes its research very necessary, since sorghum is widely used in animal feed in Venezuela, and because its nutritional value is known similarly to traditional cereals. In addition, the study of the antioxidant activity of the sorghum brans could direct an appropriate and rational use to this part of the cereal that is generally discarded.

Considering these aspects, this study was conducted to carry out aqueous and acetone / water extractions of grains and brans from sorghum commonly used in Venezuela (hybrid Guanipa 71), in order to identify the phytochemical components present in the extracts, and to investigate the antioxidant potential through the *in vitro* assays: free radical scavenging capacity, Iron (Fe^{+2}) chelating capacity, Iron (Fe^{+3}) reducing antioxidant power, and relative antioxidant capacity (RAC).

MATERIAL AND METHODS:

Reagents: All chemicals used were of analytical grade. Methanol, formic, caffeic, chlorogenic, gallic and ellagic acids were purchased from Merck (Darmstadt, Germany). Catechin, quercetin, quercitrin, rutin, luteolin, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and 1, 10 o-phenanthroline (ophe) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2-Deoxy-D-ribose (2-DR) was purchased from Sigma Aldrich Co. (Steinheim, Germany). Ammonium molybdate was purchased from Labsynth LTDA (São Paulo, Brazil).

Plant Material and Tannins Identification: The plant material (graniferous sorghum Guanipa 71) was collected in the city of Turén (09º 20 '03" North latitude and 69º 07' 21" West longitude) from the Portuguesa state - Venezuela, located at a height of 275 meters above the sea level, with an annual average of 27 ºC, 1395 mm of rainfall, 1975 mm of evaporation and 4.3 dry months. A sample of sorghum with brans **Fig. 1A** was subjected to a simple test for the identification of condensed tannins, called "bleach test with chlorine [10]. This test consisted of mixing the grains from sorghum with KOH and NaOCl (2.0 - 2.5% w/w) in active chlorine. The mixture was heated in a water bath at 60 °C for 10 min, with occasional mixing. At the end of the procedure, the grains containing condensed tannins acquired a dark brown coloration. The plant materials used for carrying out the present investigation are shown in **Fig. 1**.

Preparation of Grains and Brans Extracts from Sorghum: Mechanical separation and grinding of small samples of sorghum grains **Fig. 1B** and brans **Fig. 1C** were carried out to perform two different micro extractions: aqueous extraction and a mixture containing 7:3 acetone : water [11, 12]. The acetone / water mixture is considered to be more effective than alcoholic solvents, since it inhibits the tannins-proteins interaction and has the advantage of extracting hydrophilic components, such as sugars and phenolic compounds [9]. Whole grains and brans of sorghum Guanipa 71 were mechanically separated. Both grains and brans were then ground using a Marconi 630/1 grinder into powdery form.

The extractions of ground samples were then carried out with distilled water or 7:3 acetone: water mixture for 96 h. The plant residues were subsequently separated, re-extracted with the respective solvents for 24 hours and the supernatants were filtered with 205 µm filter paper. The resulting volumes were concentrated and heated at 50 °C (aqueous extracts) or reduced by acetone evaporation at 61 °C using a R-3 BUCHI Rotavaporator (acetone / water extracts). The concentrated extracts were lyophilized using an Enterprise II Terroni lyophilizer.

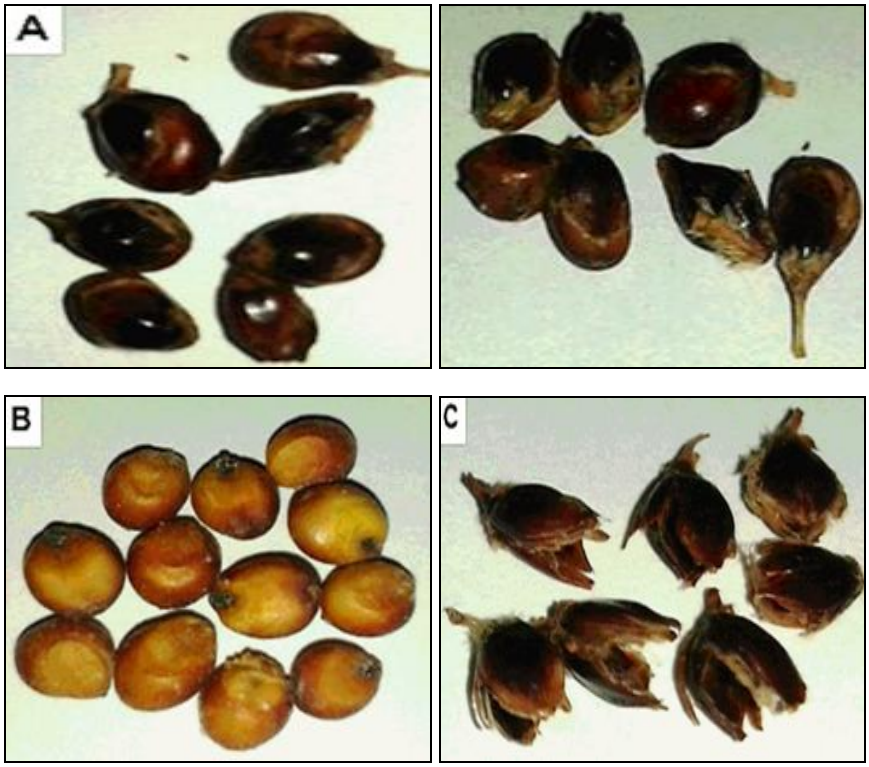


FIG. 1: SAMPLES OF SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH] HYBRID GUANIPA 7 A) GRAINS OF SORGHUM WITH BRANS. B) GRAINS OF SORGHUM WITHOUT BRANS. C) BRANS FROM GRAINS OF SORGHUM

Phytochemical Tests for the Identification of Flavonoids, Tannins and Polyphenols: The whole sorghum grain was ground using a CEMOTEC 1090 grinder (Foss Tecator, Hoganäs, Sweden). All the ground samples were passed through a 500µm sieve. An aliquot of each micro extract was placed in test tubes to perform four (4) phytochemical tests to determine the presence / absence of flavonoids, tannins and polyphenols [10 -12].

Reaction with Concentrated H_2SO_4: The micro extracts mixtures were observed for the appearance of reddish color as indicative of flavonoids content.

Reaction with 2% $FeCl_3$: Green, yellow-brown and violet colors were indicative of positive test for flavonoids.

Reaction with Concentrated HCl / Δ: The presence of anthocyanins (red flavilium salt) in sorghum extracts were detected by the conversion of the flavan-3, 4-diols (catechins) after the treatment with mineral acid (HCl) and heating, which causes dehydration followed by oxidation by oxygen from the air.

Reaction with 2% $FeCl_3$: The presence of hydrolysable tannins was confirmed by the appearance of a blue color; condensed tannins by the green color; and polyphenols by the colors that varied from blue to red.

Detection and Quantification of Compounds by HPLC-DAD: High performance liquid chromatography with a photodiode array detector (HPLC-DAD) was performed with the HPLC system (Shimadzu, Kyoto, Japan), Prominence Auto Sampler (SIL-20A), equipped with Shimadzu LC-20AT reciprocating pumps connected to the degasser DGU 20A5 with integrator CBM 20A, UV-VIS detector DAD (diode) SPD-M20A and Software LC solution 1.22 SP1. The chromatographic analyzes of reverse phase were performed under gradient conditions with a C18 (4.6 mm × 150 mm) column filled with particles of 5 µm diameter. The mobile phase consisted of water containing 1% formic acid and methanol, following the method described by Pereira *et al*., 2014 [13], with minor modifications. The extracts (5 mg/mL) and the mobile phase were filtered with a 0.45 µm membrane filter (Millipore) and degassed by ultrasonic bath. The quantification was carried out by integration of the peaks using the external standard method, at 270 nm for gallic acid, 280 nm for catechin, 327 nm for chlorogenic, caffeic,

ellagic acids, and 365 nm for quercetin, quercitrin, luteolin, and rutin. Standard solutions were prepared in the mobile phase in a range of 0.030 - 0.250 mg/mL for catechin, quercetin, quercitrin, luteolin, rutin; and 0.020 - 0.200 mg/mL for ellagic, gallic, caffeic and chlorogenic acids. Chromatographic peaks were confirmed by comparing their retention time (Rt) with those of reference standards and by DAD spectra (200 - 500) nm. All chromatography operations were carried out at ambient temperature and in triplicate.

***In vitro* Antioxidant Assays:**

DPPH Free Radical Scavenger Activity: The free radical scavenger activity of aqueous and acetone/water extracts was determined by the photocolorimetric method of $DPPH^{\bullet}$ (1, 1, diphenyl-2-picrylhydrazyl), according to Choi *et al*., 2004 [14], using ascorbic acid as a standard antioxidant. The inhibition of $DPPH^{\bullet}$ by the extracts was accompanied by the disappearance of the $DPPH^{\bullet}$ absorption. The degree of color change is proportional to the concentration and potency of the antioxidants present in the extracts. The final concentrations of ascorbic acid and sorghum extracts were 0.005, 0.01, 0.025, 0.05, 0.1 and 0.5 mg/mL. Absorbance measurements were performed in a (λ) of 518 nm on a UV-Visible TR Reader Chimadzu spectrophotometer, after 30 min of incubation at room temperature in the dark. Percentages (%) of $DPPH^{\bullet}$ radical inhibition were calculated using the following equation:

$$\% \text{ of DPPH inhibition} = [(Abs._{Control} - (Abs._{Extract} - Abs._{Blank})] \times 100 \% / Abs._{Control}$$

Where, $Abs._{Control}$ is the absorbance of 0.3 mM $DPPH^{\bullet}$ solution; $Abs._{Extract}$ is the absorbance of the extracts containing $DPPH^{\bullet}$; $Abs._{Blank}$ is the absorbance of the extracts without $DPPH^{\bullet}$.

Relative Antioxidant Capacity Assay: The relative antioxidant capacity (RAC) of each extract was evaluated using the phosphomolybdenum reagent method, based on the reduction of Mo (VI) to Mo (V) by the extracts with subsequent formation of the phosphate / Mo (V) green complex in acid pH and high temperature. Briefly, final concentrations of 0.025, 0.05, 0.1 and 0.25 mg/mL of aqueous and acetone/water extracts were heated for 90 minutes at 95 °C in the presence of 4 mM ammonium molybdate, 0.6 M sulfuric acid and 28 mM sodium phosphate buffer, pH 7.4. Afterwards, the absorbance was obtained spectrophotometrically at 695 nm. As reference, a solution of rutin was used in the concentration of 0.25 mg/mL, representing 100% of the antioxidant activity. For calculating the relative antioxidant activity, the following formula was used according to Prieto *et al*., 1999 [15].

$$\text{Relative Antioxidant Capacity of Rutin (\%)} = (Abs._{Extract} - Abs._{Blank\ extract}) \times 100\% / (Abs._{Control\ rutin} - Abs._{Blank\ rutin})$$

Where, $Abs._{Extract}$ is the absorbance of the extracts containing the molybdate reagent; $Abs._{Blank\ extract}$ is the absorbance of the extracts without the molybdate reagent; $Abs._{Control\ rutin}$ is the absorbance of rutin containing the molybdate reagent; $Abs._{Blank\ rutin}$ is the absorbance of rutin without the molybdate reagent.

Fe^{+2} Ion Chelating Activity and Fe^{+3} Ion Reducing Antioxidant Power: The ability of the extracts to chelate Fe^{2+} or reduce Fe^{3+} was determined by the 1, 10 o-phenanthroline (o-phe) test, according to the method of Minotti and Aust 1987 [16], with some modifications. The assay was based on the reaction of free Fe^{2+} with (o-phe) to form an orange-red complex ferroin [$(ph)3Fe^{+2}$]. The reaction was performed in 96 well plates in the presence of 0.1M Tris-HCl pH 7.4 and 300 μM o-phe. 0.5 and 0.1 mg/mL of the extracts were added separately with 100 μM Fe^{2+} ion ($FeSO_4$) and/or 100 μM Fe^{3+} ion ($FeCl_3$). Absorbances were measured at 0, 2.5, 5, 10, 20, 30, 45, 60, 75, 90 min on a UV / Visible TR Reader Chimadzu spectrophotometer at λ 510 nm. To evaluate the stability of the formed chelates, 0.005 M ascorbic acid was added in each reaction system at 90 min and the absorbance was measured one hour later. The percentages of chelating and reducing activity of each extract were expressed as the percentage compared to the respective controls, using the following equations:

$$\text{Chelation activity of } Fe^{+2} \text{ (\%) t(min)} = (Abs._{Control\ Fe^{+2}} - Abs._{Extract\ Fe^{+2}}) \times 100\% / Abs._{Control\ Fe^{+2}}$$

$$\text{Reducing power of } Fe^{+3} \text{ (\%) t(min)} = (Abs._{Extract\ Fe^{+3}} - Abs._{Control\ Fe^{+3}}) \times 100\% / Abs._{Control\ Fe^{+2}}$$

Where, $Abs._{Control\ Fe^{+2}}$ is the absorbance of o-phe containing Fe^{+2} without extracts, $Abs._{Extract\ Fe^{+2}}$ is the absorbance of the extracts containing o-phe and Fe^{+2}, $Abs._{Control\ Fe^{+3}}$ is the absorbance of o-phe containing Fe+3 without extracts, $Abs._{Extract\ Fe^{+3}}$ is the absorbance of the extracts containing o-phe and Fe^{+3}.

Oxidative Degradation of 2-Desoxy-D-ribose (2-DR) Assay: *In vitro* inhibition of 2-DR degradation was determined according to the methodology of Gutteridge 1981 [17], based on the quantification of malondialdehyde (MDA), the main product formed from the attack of the hydroxyl radical to 2-DR. The MDA formed from the degradation of 2-DR (3 mM) was detected and quantified spectrophotometrically in acid medium after the addition of thiobarbituric acid (TBA) which, after heating, forms a chromophore (MDA-TBA2) with absorption peak at 532 nm and molar absorptivity coefficient (ε) of 14,900 $M^{-1}cm^{-1}$ [17].

According to the methodology, the hydroxyl radical was generated in separated systems by the following pro-oxidant conditions: i) 1 mM H_2O_2, ii) 0.05 mM $FeSO_4$ and iii) 1 mM H_2O_2 + 0.05 mM $FeSO_4$ in the presence of 3 mM 2-DR and 50 mM potassium phosphate buffer (pH 7.4) pre-incubated for 20 min at room temperature. Afterwards, the extracts at final concentrations of 0.025, 0.05, 0.1 and 0.25 mg/mL were added to the system for 60 min. Reactions were stopped by the addition of 2.8% trichloroacetic acid (TCA) and 0.8% TBA. The reaction medium was then heated for 20 min at 100 °C and the absorbance determined spectrophotometrically at 532 nm. The results were expressed as percentage of protection against oxidative degradation following the equation of Mauricio 2006 [18].

$$\text{2-DR protection (\%)} = [(Abs._{Control} - (Abs._{Extract} - Abs._{Blank})] \times 100\% / Abs._{Control}$$

Where, $Abs._{Control}$ is the absorbance of 2-DR containing the pro-oxidant without extracts, $Abs._{Extract}$ is the absorbance of 2-DR containing pro-oxidant and extracts, and $Abs._{Blank}$ is the absorbance of the pro-oxidant containing extracts and without 2-DR.

Antioxidant Mechanism of Sorghum Extracts by the 2-DR Oxidative Degradation Test: Varying the concentration of 2-DR (3.0, 3.5 and 4.0 mM) we determined whether the mechanism of antioxidant action caused by the extracts was due metal chelator (Fe^{+2}) or hydroxyl radical scavenger [18]. In this test, the antioxidant is considered of the chelating type when the percentage of protection remains constant as the 2-DR concentration increases, therefore, it becomes complexed to the metal and inhibits the formation of the hydroxyl radical ($OH^{\bullet}$).

When the percentage of protection decreases as the concentration of the 2-DR increases, the antioxidant is of the scavenger type, since it is competing with 2-DR for the hydroxyl radical [18]. Accordingly, the oxidative degradation of 2-DR was generated by exposure to 1 mM H_2O_2 + 0.05 mM $FeSO_4$. The protection of 2-DR at three different concentrations (3.0 mM, 3.5 mM and 4.0 mM) was calculated following the equation of Mauricio 2006 [18].

$$\text{2-DR protection (\%)} = [(Abs.\ (H_2O_2 + Fe^{+2})_{Control} - (Abs._{Extract} - Abs._{Blank})] \times 100\% / Abs.\ (H_2O_2 + Fe^{+2})_{Control}$$

Statistical Analyzes: The results were expressed as mean ± S.E.M. Statistical analyzes were performed using a two-way (ANOVA), followed by Turkey's multiple comparisons test using the GraphPad Prism 6.0 program. Differences were considered significant when $p < 0.05$.

RESULTS:

Extractions Yield and Qualitative Analysis of Sorghum: The presence of condensed tannins was detected in samples of whole sorghum. As shown in **Table 1**, the presence of flavonoids in the extracts of both grains and brans was observed. Among these flavonoids, catechin was found to be present in all the extracts. The presence of polyphenols, condensed and hydrolyzable tannins from grains and brans was detected in acetone / water extracts, however, they were absent in aqueous extracts. A higher percentage yield was obtained in the extractions carried out using 7:3 acetone / water mixture, confirming this mixture as the best extraction option.

The results also revealed that the brans from sorghum subjected to both aqueous and acetone / water extractions had higher percentage yields in

comparison to the grains **Table 1**. The performance of the two types of extractions provides the basis for future studies in which other cultivars of sorghum will be used.

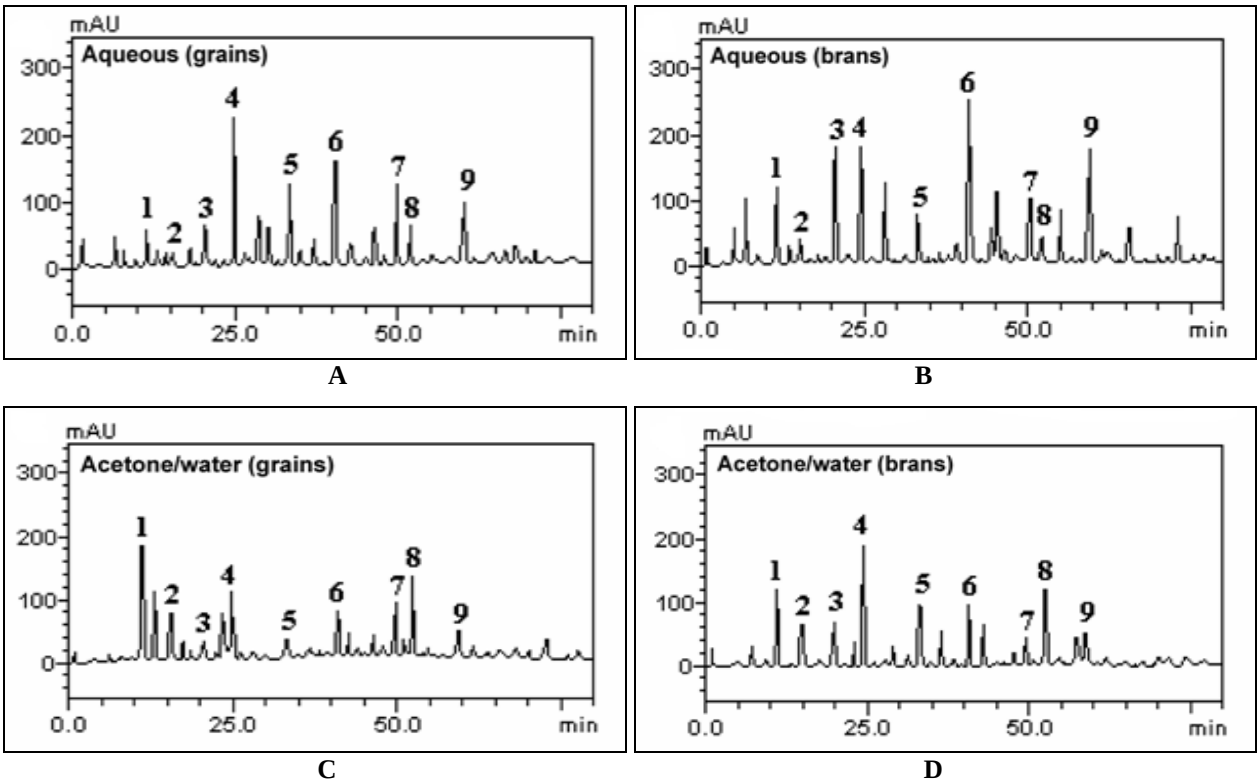
TABLE 1: PERCENTAGES OF EXTRACTIONS YIELD (%) AND IDENTIFICATION OF POLYPHENOLS, FLAVONOIDS AND TANNINS FROM AQUEOUS AND ACETONE / WATER EXTRACTS OF SORGHUM

	Acetone / Water extraction			Aqueous extraction		
Sample	**Whole material m(g)**	**m(g) extract**	**% (m/m) yield**	**Whole material m(g)**	**m(g) extract**	**% (m/m) yield**
Grain	285	11.05	3.87	185	4.92	2.66
brans	130	7.94	6.1	125.5	4.63	3.69

	Acetone / Water extraction		Aqueous extraction	
Reaction	**Grain**	**brans**	**Grain**	**brans**
H_2SO_4 conc. Flavonoids	+	+	+	+
2% $FeCl_3$ Flavonoids	+	+	+	+
HCl conc./ Δ (catequins)	+	+	+	+
2% $FeCl_3$ (polyphenols)	+	+	-	-
2% $FeCl_3$ (condensed tannins)	+	+	-	-
2% $FeCl_3$ (condensed tannins)	+	+	-	-

Positive test: (+); Negative test: (-)

HPLC-DAD Analysis: The HPLC performance revealed the presence of gallic acid (R_t = 12.35 min, peak 1), catechin (R_t = 15.07 min, peak 2), chlorogenic acid (R_t = 20.11 min, peak 3), caffeic acid (R_t = 24.81min peak 4), ellagic acid (R_t = 33.98, peak 5), rutin (R_t = 40.56 min, peak 6), quercitrin (R_t = 50.13 min, peak 7), quercetin (R_t = 52.17 min, peak 8), and luteolin (R_t = 60.12 min, peak 9) in grains and brans extracts from Sorghum **Fig. 2**. As can be seen in **Fig. 2E**, the aqueous extract of grains from sorghum showed a higher concentration of caffeic acid (29.14 mg/g ± 0.01) and rutin (21.5 8mg/g ± 0.03). In the aqueous extract of brans the main compounds were rutin (31.87 mg/g ± 0.02), caffeic acid (23.01 mg/g ± 0.03), chlorogenic acid (22.95 mg/g ± 0.01) and luteolin (22.67 mg/g ± 0.03). Regarding the acetone / water extractions, extracts of grains had a higher concentration of gallic acid (21.79 mg/g ± 0.01) and quercetin (16.27 mg/g ± 0.02). In the extracts of brans, the majority compounds were caffeic acid (23.05 mg/g ± 0.01), gallic acid (15.49 mg/g ± 0.03), and quercetin (15.3 mg/g ± 0.02). All values were expressed as the mean ± SD.



Compounds	Aqueous Grains mg/g	Aqueous brans mg/g	Acetone / Water (Grains)	Acetone / Water (brans)
Gallic acid	6.23 ± 0.01[a]	16.07 ± 0.02[a]	21.79 ± 0.01[a]	15.49 ± 0.03[a]
catequin	1.98 ± 0.03[b]	3.14 ± 0.01[b]	9.36 ± 0.01[b]	9.28 ± 0.02[b]
Chlorogenic acid	6.47 ± 0.02[c]	22.95 ± 0.01[c]	3.07 ± 0.02[c]	9.31 ± 0.01[b]
Caffeic acid	29.14 ± 0.01[d]	23.01 ± 0.03[e]	14.39 ± 0.01[d]	23.05 ± 0.01[c]
Ellagic acid	15.67 ± 0.01[e]	8.62 ± 0.01[d]	3.18 ± 0.03[c]	13.27 ± 0.03[d]
Rutin	21.58 ± 0.03[f]	31.87 ± 0.02[e]	9.12 ± 0.01[b]	13.29 ± 0.01[d]
Quercitrin	15.69 ± 0.01[e]	9.94 ± 0.03[f]	13.95 ± 0.01[d]	5.12 ± 0.01[e]
Quercetin	6.42 ± 0.01[a]	3.08 ± 0.01[b]	16.27 ± 0.02[e]	15.32 ± 0.02[a]
Luteolin	9.87 ± 0.02[g]	22.67 ± 0.03[c]	5.03 ± 0.03[f]	5.17 ± 0.01[e]

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FIG. 2: REPRESENTATIVE PROFILE OF HPLC OF GRANIFEROUS SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH] HYBRID GUANIPA 71. A) AQUEOUS EXTRACT OF SORGHUM GRAINS; B) AQUEOUS EXTRACT OF SORGHUM BRANS; C) ACETONE/WATER EXTRACT OF SORGHUM GRAINS AND D) ACETONE/WATER EXTRACT OF SORGHUM BRANS. GALIC ACID (PEAK 1), CATEQUIN (PEAK 2), CHLOROGENIC ACID (PEAK 3), CAFFEIC ACID (PEAK 4), ELAGIC ACID (PEAK 5), RUTIN (PEAK 6), QUERCITRIN (PEAK 7), QUERCETIN (PEAK 8) AND LUTEOLIN (PEAK 9). E) PHENOLIC COMPOSITION (mg/g) OF SORGHUM EXTRACTS

Results are expressed as mean ± standard deviation (SD) from three determinations. Means followed by different letters differ by the Tukey test at $p < 0.05$. The calibration curve for gallic acid: Y = 13574x + 1285.9 (r = 0.9998); chlorogenic acid: Y = 11983x + 1239.4 (r = 0.9996); caffeic acid: Y = 11792x + 1367.8 (r = 0.9999); catechin: Y = 12605x + 1249.6 (r = 0.9997); ellagic acid: Y = 12658x + 1273.1 (r = 0.9993); quercetin: Y = 13509x + 1198.9 (r = 0.9998); rutin: Y = 11954x + 1186.5 (r = 0.9994); luteolin: Y = 12408x + 1197.2 (r = 0.9999) and quercetin: Y = 13752x + 1276.8 (r = 0.9998).

DPPH• Free Radical Scavenger Activity: All extracts were found to present strong DPPH radical scavenger activity. The non-linear regression of the curves revealed that the aqueous extract of sorghum grains was the least effective (IC_{50} 430.3 µg/mL), followed by the aqueous extract of brans (IC_{50} 481 µg/mL). On the other hand, the acetone/water extract of brans exhibited a higher IC_{50} potential of 31.34 µg/mL, followed by acetone/water extract of grains (IC_{50} 36.48 µg/mL). Ascorbic acid achieved a greater sequestering activity (IC_{50} 5.52 µg/mL) **Fig. 3A**. Two-way ANOVA statistical analysis revealed that the scavenging activities of the acetone / water extracts were statistically superior in comparison to the aqueous extracts.

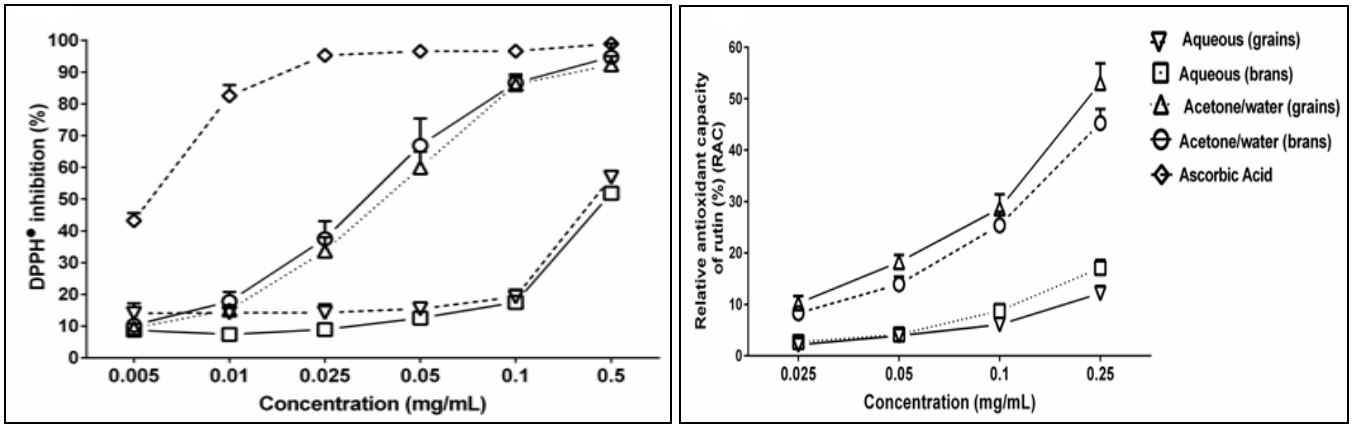
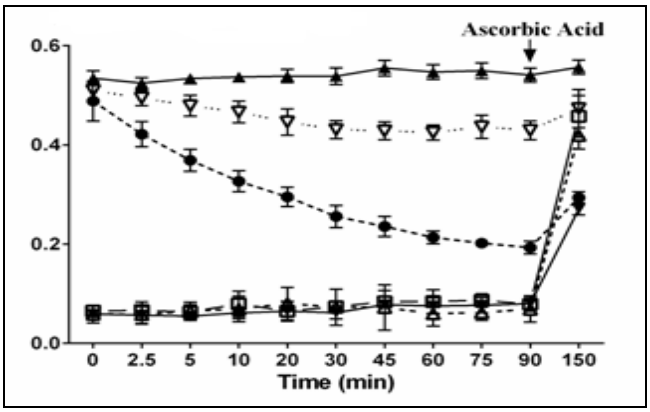


FIG. 3: A) FREE RADICAL SCAVENGING ACTIVITY OF SORGHUM EXTRACTS, MEASURED BY THE 1, 1-DIPHENYL-2 PICRYHYDRAZYL (DPPH•) FREE RADICAL ASSAY. ASCORBIC ACID WAS USED AS POSITIVE CONTROL (n = 6). B) EFFECT OF EXTRACTS FROM SORGHUM ON THE REDUCTION OF Mo (VI) TO Mo (V) (n = 11).Values are expressed as mean ± S.D; two-way ANOVA followed by Tukey's test. GraphPad Prism 6.0 was used.

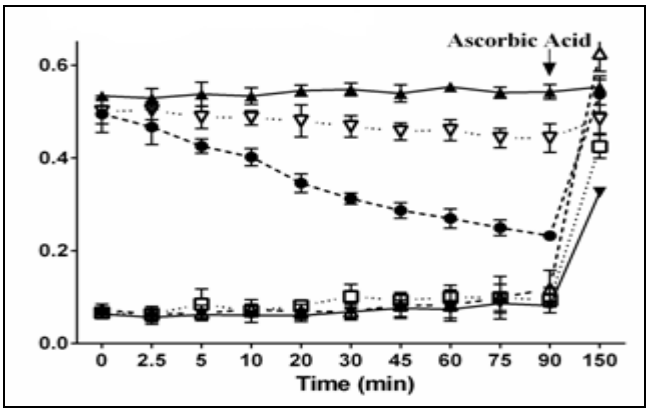
Relative Antioxidant Capacity (RAC): The phosphomolybdenum complexation method is a simple and inexpensive way to evaluate the RAC of a complex mixture of compounds, with the advantage of evaluating the antioxidant capacity of both lipophilic and hydrophilic components. The observation of the green phosphate / Mo (V) complex formation, indicative of Mo (VI) reduction, revealed that all the extracts exhibited substantial RAC **Fig. 3B**. Non-linear regression of the curves revealed that acetone/water extracts were more effective, exhibiting an IC_{50} of 227.6 µg/Ml (grains) and 303 µg/mL (brans). The aqueous extracts exhibited an IC_{50} of 1439 µg/mL

(brans) and 36.48 µg/mL (grains). Statistical analysis by two-way ANOVA, followed by the multiple comparison test, revealed that acetone / water extracts of sorghum grains and brans presented a higher RAC than the aqueous extracts.

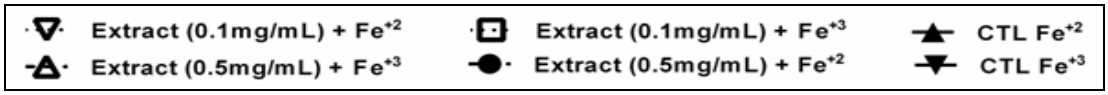
Fe^{+2} Ion Chelating Activity Assay and Fe^{+3} Ion Reducing Power: Statistical analysis by two-way ANOVA, followed by the Tukey's test, showed that all extracts were effective in chelating Fe^{+2}, showing greater potential at the concentration of 0.5 mg/mL. In comparative terms, it was observed that the chelating abilities of aqueous extracts of sorghum grains and brans (0.5 mg/mL) were kinetically greater when compared to acetone / water extracts **Fig. 4**. However, it was evidenced that acetone / water extracts formed thermodynamically more stable chelates, which was sustained by a lower absorbance of the ferroin chelate $(ph)_3 Fe^{+2}$ when ascorbic acid was added in the presence of such extracts, in relation to the Fe^{+2} controls at 150 min. This evidenced that the chelates formed between the Fe^{+2} ion and the compounds present in the acetone / water extracts had higher thermodynamic stabilities, and consequently higher stabilization energies than ferroin chelate. In addition, acetone / water extracts also exhibited higher reducing power in the o-phe assay.

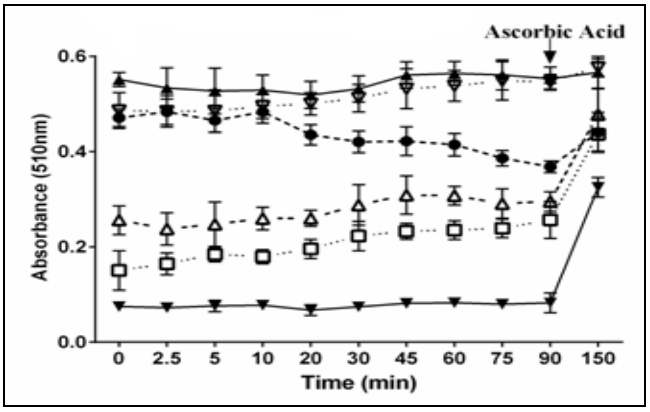


A) Aqueous (Grains)

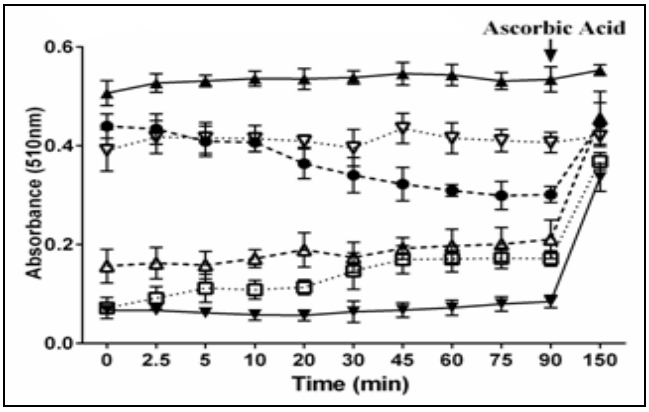


B) Aqueous (Brans)





C) Acetone / Water (Grains)



D) Acetone / Water (brans)

Sample (0.5 mg/ml)	(%) Fe^{+2} chelation / Time (min)								Ascorbic acid
	2.5	5	20	30	45	60	75	90	150
Aqueous (Grains)	22.876	33.021	46.409	54.325	58.524	62.267	63.966	65.091	48.998
Aqueous (Brans)	20.315	25.827	38.246	44.492	48.860	53.438	55.708	59.115	9.686
Acetone / Water (Grains)	12.989	17.231	22.984	26.794	28.326	29.404	32.416	32.402	26.697
Acetone / Water (brans)	22.267	30.935	37.635	39.839	45.551	46.124	46.918	47.336	28.049

E

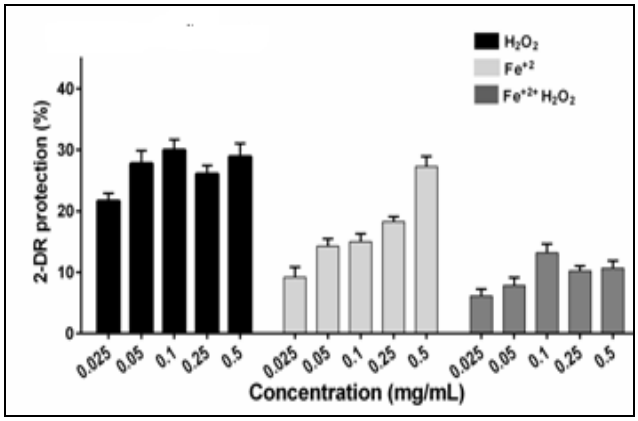
Sample (0.5 mg/ml)	(%) Fe^{+3} reduction / Time (min)								Ascorbic acid
	2.5	5	20	30	45	60	75	90	150
Aqueous (Grains)	-1.680	1.713	1.904	2.495	-0.586	-2.906	-2.519	-1.529	30.128
Aqueous (Brans)	3.439	3.973	3.454	3.875	4.910	7.770	7.262	8.803	43.566
Acetone / Water (Grains)	33.001	31.789	38.918	41.223	42.310	41.310	39.858	42.252	26.092
Acetone / Water (brans)	19.338	19.970	19.970	22.602	23.817	25.131	24.292	22.903	22.579

F

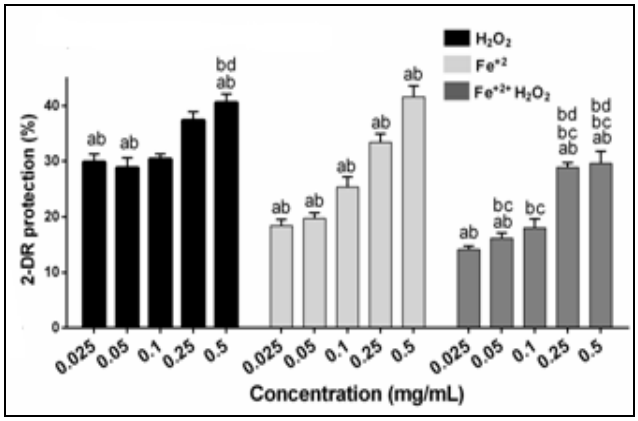
FIG. 4: EFFECT OF AQUEOUS AND ACETONE / WATER EXTRACTS OF SORGHUM GRAINS AND BRANS ON ABSORBANCE OF Fe^{+2}/O-PHENANTHROLINE AND Fe^{+3}/O-PHENANTHROLINE REACTION SYSTEMS

Values are expressed as means ± S.E.M. (n = 11). Two-way ANOVA, followed by the Tukey's multiple-comparison test. The GraphPad Prism 6.0 program was used.

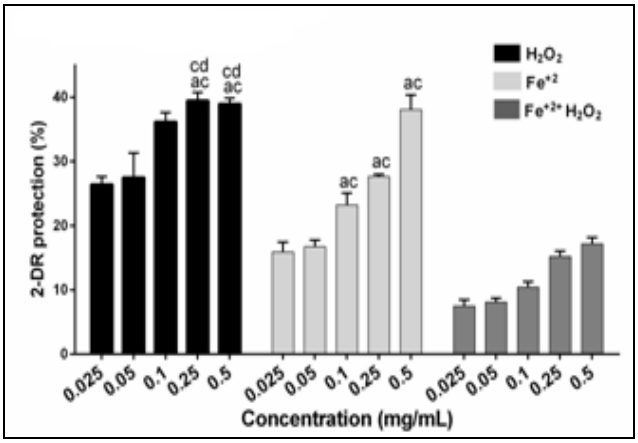
Oxidative Degradation of 2-Desoxy-D-ribose (2-DR): As can be seen in **Fig. 5**, the extracts were capable to exhibit significant protection against degradation of 2-DR generated by the different oxidative systems. Statistical analysis revealed that the concentrations of 0.25 and 0.5 mg/mL of the aqueous extract of sorghum grains and acetone / water extract of brans exhibited significantly higher protection in the H_2O_2 oxidative system. With respect to the oxidative degradation of 2-DR by the separated Fe^{+2} system, as well as, by the Fenton system (50 μM Fe^{+2} + 1 mM H_2O_2), acetone / water extracts were shown to be more effective. Overall, the protection afforded by the extracts to 2-DR was statistically lower in the Fenton system than in the separated Fe^{+2} and H_2O_2 oxidative systems, probably due to the higher concentration of $^{\bullet}OH$ formed by the direct reaction of Fe^{+2} and H_2O_2, When considering the overall statistical analysis of the results, it was observed that acetone / water extract of sorghum brans was more effective in inhibiting the degradation of 2-DR in all three oxidative systems **Fig. 5A - D**.



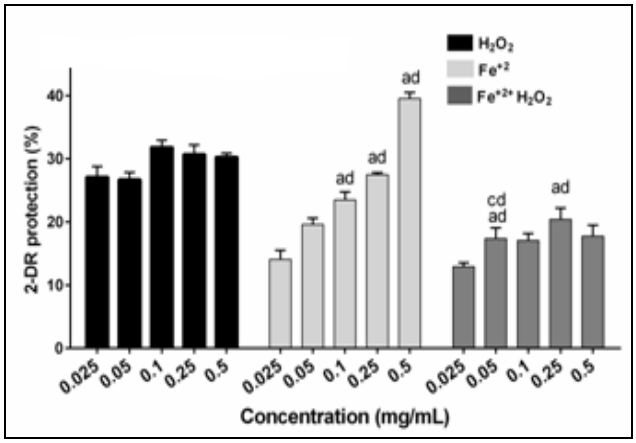
A) Aqueous (brans)



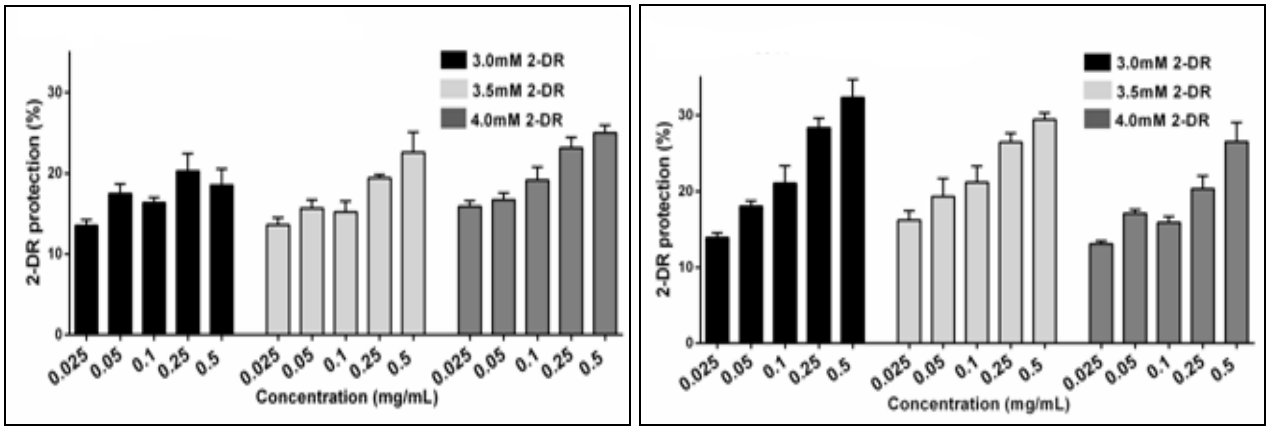
B) Acetone / Water (brans)



C) Aqueous (Grains)



D) Acetone / Water (Grains)



E) Acetone / Water (Grains) **F) Acetone / Water (brans)**

FIG. 5: A-D) PERCENTAGE OF 2-DR PROTECTION OF AQUEOUS AND ACETONE/WATER EXTRACTS OF SORGHUM GRAINS AND BRANS, USING THREE *IN VITRO* DEGRADATION SYSTEMS: 1 mm H_2O_2-EXT; 50mm Fe^{+2}-EXT; 50 mm Fe^{+2} + H_20_2 1MM-EXT (n = 8). E AND F) PERCENTAGE OF 2-DR PROTECTION BY ACETONE / WATER EXTRACTS OF SORGHUM GRAINS AND BRANS, IN THE OXIDATIVE DEGRADATION GENERATED BY THE *IN VITRO* FENTON REACTION (50 mm Fe^{+2} + 1 mm H_2O_2), VARYING 2-DR CONCENTRATIONS (3.0 mm, 3.5 mm, 4.0 mm (n = 4). Values are expressed as means ± S.E.M. [two-way ANOVA, followed by the Tukey's multiple-comparison test. The GraphPad Prism 6.0 program was used. Letters above the bars mean significant protection between the extracts compared (A, B, C and D), at the same oxidative system and same concentrations.

Antioxidant Mechanism of Sorghum Extracts: In order to determine whether the protection exhibited by the extracts in the Fenton system (Fe^{+2}/H_2O_2) involved the chelating power or the scavenger action, the test of 2-DR variation was performed. For this assay, the acetone / water extracts were considered, because they were the ones that exhibited higher percentages of protection against the Fenton reaction. Statistical analysis by two-way ANOVA showed that the protection of the 2-DR conferred by the extracts remained significantly constant by varying the concentrations of the 2-DR target molecule **Fig. 5E** and **F**. It is likely that the set of compounds present in the two extracts acted as a chelating-type antioxidant. The metal was complexed and the formation of the hydroxyl radical was consequently inhibited.

The summary of the IC_{50} values and the antioxidant activity percentages showed that acetone/water extracts exhibited higher antioxidant potentials than aqueous extracts **Table 2**. Overall, the order of antioxidant potential of the extracts are as follow: acetone/water (brans) > acetone/water (grains) > aqueous (grains) > aqueous (brans).

TABLE 2: SUMMARIZED EFFECTS OF THE SORGHUM EXTRACTS

Assay		Aqueous extracts		Acetone / Water extracts	
		Grains	**brans**	**Grains**	**brans**
IC_{50} DPPH (µg/ml)		430.3	481	36.48	31.34
IC_{50} RAC (µg/ml)		3010	1439	227.6	303
Ophe% chelation 90 min		65.09	59.12	32.40	47.34
Ophe% reduction 90 min		-1.53	8.8	42.25	22.91
2- DR protection (%)	H_2O_2	38.96	28.91	30.27	40.57
	Fe^{+2}	38.08	27.27	39.53	41.58
	$H_2O_2 + Fe^{+2}$	17.18	10.67	17.77	29.60

DISCUSSION: The relevance of studies supporting the use of food rich in phytochemicals as nutraceuticals aroused our curiosity on the identification of bioactive compounds present in grains and brans of sorghum hybrid Guanipa 71, a cereal extensively used worldwide. We also focused on the comparative investigation of the *in vitro* antioxidant potential conferred by the extracts. Firstly, we detected the presence of condensed tannins in the Venezuelan integral sorghum, as well as, condensed and hydrolyzable tannins in the acetone / water extracts of sorghum brans and grains, corroborating with previous report [19].

Furthermore, specific colorimetric tests revealed the presence of polyphenols and flavonoids in the sorghum extracts, which were further identified by HPLC analysis. We believe that the highest content of quercetin, gallic acid and ellagic acid found in the acetone/water extracts of sorghum grains and brans could explain their higher effect on the inhibition of DPPH$^{\bullet}$ radical and reduction of Mo (VI) and $Fe^{+3,}$ since these compounds are able to transfer electrons [20]. It is known that ellagic and gallic acids possess the pyrogallol group in the presence of π-conjugated structures, which confer greater capacity to reduce metallic ions and favor the reduction potential of quercetin [20, 21]. This structure probably activates the mechanism of electron donation and proton release resulting the formation of a semiquinone, which can donate another electron and release another proton to form a quinon molecule [20, 21]. Hence, these bioactive compounds present in the acetone / water extracts may be responsible to inhibit DPPH$^{\bullet}$ by proton transfer, forming quinones that can be stabilized by resonance, and reduce Mo (VI) and Fe^{+3} by electrons release.

Interesting, *in vitro* studies have postulated quercetin as the flavonoid with the highest sequestering power of reactive oxygen species [22]. Higher percentages of Fe^{+2} chelation were observed in the aqueous extracts of grains and brans. This effect may have been attributed to the high concentrations of rutin, luteolin, caffeic and chlorogenic acids in the aqueous extracts of sorghum brans; as well as, the content of rutin, quercitrin, and caffeic and chlorogenic acids in aqueous extracts of sorghum grains. These compounds possess the catechol groups in their chemical structure, which may favor the complete complexation of Fe^{+2} ions.

In the case of rutin, its antioxidant activity is improved when it is complexed to metal ions [23]. The formation of the complex between catechol groups and a metal ion could cause a decrease in the hydrogen pKa from the second phenolic hydroxyl and thus, favor a complete Fe-catechol complexation [24]. Similarly, the Fe^{+2} chelating activities of acetone / water extracts can be explained in part by the synergistic effect of catechin, rutin, quercitrin, luteolin, caffeic and chlorogenic acids.

It is worthy of note that the body lacks effective strategies to excrete the iron excesses. Thus, the ability of the aqueous and acetone / water extracts from sorghum to chelate Fe^{+2} ions could lead to novel strategies in minimizing Fe^{+2} overload and the consequent generation of free radicals, reinforcing the importance of its use as a functional food [25, 26].

The inhibition of oxidative degradation of 2-DR by all the sorghum extracts showed a dose dependent response, indicating an antioxidant action by the chelation of Fe^{+2} ions and the prevention of Fe^{+2} to participate in the generation of free radicals [27]. The synergistic electron donation of quercetin, gallic and ellagic acids in addition to the formation of semiquinones and quinones in acetone / water extract of sorghum brans can explain, at least in part, the greater inhibition of 2-DR oxidative degradation induced by H_2O_2 [27, 28].

In a similar manner, aqueous extract of sorghum grains exhibited a greater inhibition of 2-DR oxidative degradation when compared to acetone/water extract. This could be due to the higher levels of chlorogenic and caffeic acids which, when dissociated, are able to neutralize $^{\bullet}$OH radicals by forming covalent bonds between the negatively charged oxygen atoms and the oxygen atoms from the $^{\bullet}$OH, hence forming peroxyacids R(C=O)OOH. Constant protection levels of 2-DR by varying its concentration against fixed concentrations of Fe^{+2} / H_2O_2 reconfirm in general terms that the set of compounds present in the acetone / water extracts acted as Fe^{+2} chelating agents, thereby preventing the formation of H_2O_2 and $^{\bullet}$OH.

However, such a chelating mechanism does not exclude a free radical scavenger component, evidenced by other authors [29, 30]. In addition, chelates formed by these extracts might be able to sequester of $O_2^{\bullet-}$ particular importance, there is an indication that the antioxidant activity of rutin may be improved when it is complexed to metal ions [23].

CONCLUSION: The results obtained here showed that the grains and brans extracts from the sorghum [*Sorghum bicolor* (L.) Moench] hybrid Guanipa 71, exhibited considerable *in vitro* antioxidant activities, which were confirmed through the

DPPH$^{\bullet}$ radical scavenging capacity, Fe^{+2} chelating activity, Fe^{+3} reducing power, ability to inhibit oxidative degradation of (2-DR), and relative antioxidant capacity. Such properties could make sorghum a promising plant to be evaluated in *in vivo* antioxidant studies, and outline the use of grains and brans from the whole cereal in human food with important antioxidant properties. Sorghum brans, like the brans of other traditional grains, are commonly discarded in the process of producing human and animal food. However, this study revealed bioactive compounds in its aqueous and acetone / water extracts, including flavonoids and phenolic acids that conferred relevant *in vitro* antioxidant activity. These results make the bran as an important plant material to be considered in the human food production.

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CONFLICT OF INTEREST: The authors have declared that there is no conflict of interest associated with this publication.

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