

Angolan *Cymbopogon citratus* used for therapeutic benefits: Nutritional composition and influence of solvents in phytochemicals content and antioxidant activity of leaf extracts

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ABSTRACT

Folk medicine is a relevant and effective part of indigenous healthcare systems which are, in practice, totally dependent on traditional healers. An outstanding coincidence between indigenous medicinal plant uses and scientifically proved pharmacological properties of several phytochemicals has been observed along the years.

This work focused on the leaves of a medicinal plant traditionally used for therapeutic benefits (Angolan *Cymbopogon citratus*), in order to evaluate their nutritional value. The bioactive phytochemical composition and antioxidant activity of leaf extracts prepared with different solvents (water, methanol and ethanol) were also evaluated.

The plant leaves contained 60% of carbohydrates, protein (20%), fat (5%), ash (4%) and moisture (9%). The phytochemicals screening revealed the presence of tannins, flavonoids, and terpenoids in all extracts. Methanolic extracts also contained alkaloids and steroids. Several methods were used to evaluate total antioxidant capacity of the different extracts (DPPH[•], NO[•], and H₂O₂ scavenging assays, reducing power, and FRAP). Ethanolic extracts presented a significantly higher antioxidant activity ($p < 0.05$) except for FRAP, in which the best results were achieved by the aqueous extracts. Methanolic extracts showed the lowest radical scavenging activities for both DPPH[•] and NO[•] radicals.

Keywords: *Cymbopogon citratus*, Phytochemical screening, Nutritional value, Bioactive compounds, Antioxidant activity assays

1. Introduction

Since ancient times, the medicinal properties of plants have been investigated due to their powerful pharmacological properties. Over the centuries, people have been living in close association with the environment and relying on its flora and fauna as a source of food and medicine (Moshi et al., 2012; Cheikhoussef et al., 2011; Gurib-Fakim, 2006). Crude plant extracts (e.g. infusion, tincture, decoction or others) are traditionally used by populations all over the world for medicinal purposes. Although their effectiveness and mechanisms of action have not been scientifically tested

in the majority of the cases, they often mediate beneficial responses due to their bioactive chemical components (Barnes et al., 2007). The organic compounds usually related with physiological actions on the human body include alkaloids, phenolics, flavonoids, tannins, terpenoids, and steroids (Yadav and Agarwala, 2011).

Popular African culture include powerful indigenous therapies that can surpass medicines from industrialized countries. All over the world, Republic of Angola is recognized for its remarkable plants biodiversity, which can be explored in a rational way for humankind benefits. Several species from the Angolan flora, traditionally selected for medicinal uses, have been the target of current investigations searching for therapeutically effective drugs with antioxidant, antimicrobial, and antifungal properties (Nema et al., 2012; Kennedy and Wightman, 2011; Silva et al., 2011; Lulekal et al., 2008; Atawodi, 2005).

Cymbopogon citratus (Poaceae family) is an Angolan medicinal plant widely used by local people for treating high fever, stomach, gut problems and headache. Due to the pleasant aroma of its

infusion, this plant is widely known and used in tropical and sub-tropical countries. It can also act as an antidepressant and as a mood enhancer. Other researches also reported hypoglycemic, hypolipidemic, anxiolytic, sedative (Blanco et al., 2009) and antioxidant properties (Pereira et al., 2009; Figueirinha et al., 2008; Cheel et al., 2005; Cheah et al. 2001).

C. citratus has shown potential as a source of chemotherapeutic compounds. Figueirinha et al. (2008) reported that *C. citratus* leaves exhibit antiradical capacity against the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]) and identified tannins, phenolic acids (caffeic and *p*-coumaric acid derivatives) and flavone glycosides (apigenin and luteolin derivatives). In another study, isoorientin, isoscoparin, swertijaponin, isoorientin 2⁰⁰-O-rhamnoside, orientin, chlorogenic acid, and caffeic acid were isolated from *C. citratus* extracts (Cheel et al., 2005).

This aromatic plant has also been reported by its antimicrobial activity against *Aspergillus* species, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *S. pyogenes*, (Matasyoh et al., 2011; Aboaba et al., 2006).

Although there are few studies in literature regarding proximal composition of Nigerian *C. citratus* leaves and respective extracts (Asaolu et al. 2009; Oloyede, 2009; Akande et al., 2012), as far as we know, there are no studies so far concerning the nutritional value of the Angolan *C. citratus*. Knowing that the geographical origin and edaphoclimatic conditions may significantly affect plants composition, the aim of this study was to characterize the nutritional composition of the leaves of this plant in order to evaluate eventual differences from those previously described in literature. The contents of bioactive phytochemicals and the total antioxidant activity of different extracts of the Angolan *C. citratus* were also studied in order to predict potential industrial applications (food, supplementation or cosmetic fields) of this plant.

2. Material and methods

2.1. Reagents and standards

Hydrochloric acid, Sodium nitrate, ferric chloride, aluminum chloride, TPTZ solution, catechin, 2,6-dichlorophenol (Tillmans reagent), sodium carbonate, oxalic acid, ethanol, petroleum ether, ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Methanol, the Folin–Ciocalteu reagent, sodium hydroxide, and gallic acid were purchased from Panreac Química S.L.U. (Barcelona, Spain). Anhydrous sodium carbonate, sodium hydroxide and ethanol were obtained from Merck (Darmstadt, Germany). All chemicals and reagents used were of analytical grade and used without further purification. All aqueous solutions were prepared with Milli Q filtered water (resistivity > 18 MX cm) (Millipore, Bedford, MA).

2.2. Samples

Cymbopogon citratus leaves were collected in Benguela, a city located in Western Angola (coordinates: 120° 33' S, 130° 25' E). Plants were identified in the Polytechnic Superior Institute of Benguela, Angola. The leaves were picked and washed with water to remove all unwanted materials, dried in the dark (25 °C ± 2 °C) and stored in an airtight container for further use before being ground to fine powder.

2.3. Nutritional composition

The *C. citratus* dried leaves were analyzed for nutritional composition using AOAC procedures (1995). For moisture, powdered leaves (1 g) were placed within a porcelain capsule in a stove (WTC

binder Klasse 2.0, Tuttlingen, Germany) at 105 °C ± 1 °C, until constant weight was achieved. The ash content was determined by incineration at 600 ± 10 °C. The crude protein content was estimated by the Kje-

Percentage (%)

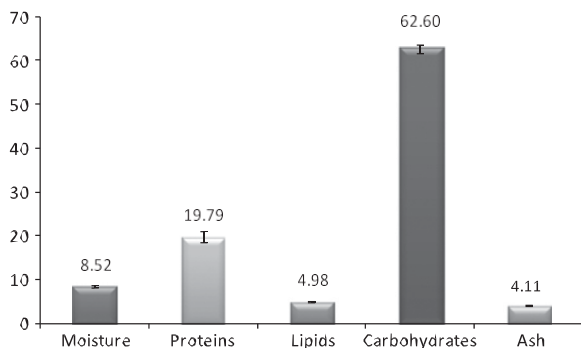


Fig. 1. Nutritional composition of Angolan *Cymbopogon citratus* leaves.

2.4. Phytochemical screening

A small portion of dried powdered leaves was used for the phytochemical screening tests in order to detect the presence of tannins, flavonoids, steroids, alkaloids, and terpenoids, according to different methods previously described with some modifications (Vinha et al., 2012; Harborne, 1998; Sofowara, 1993; Trease and Evans, 1989).

Briefly, 1.0 g of sample was mixed with 10 ml of three different solvents (distilled water, ethanol and methanol) to obtain three different extracts, which were subsequently filtered (using Whatman No. 1 filter paper).

A blue coloration resulting from the addition of ferric chloride reagent indicated the presence of tannins in the extract.

About 2 ml of the extracts were mixed with 2 ml of methanol and heated, followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange coloration was indicative of flavonoids presence.

For alkaloids screening, 2 ml of the plant extracts were added into 5 ml of 1% HCl on a steam bath and 1 ml of the final filtrate was treated with few drops of Dragendorff's reagent. Turbidity or precipitation was

taken as indicative of a positive reaction. Total protein was calculated using the general factor 6.25. Crude fat was determined by extracting a known aliquot of sample (50 g) with petroleum ether, using a Soxhlet apparatus. Total carbohydrates were calculated by difference. Results for each parameter were expressed in percentage (%). The energy value of the leaves was determined by using the Atwater factors. Results are reported in Fig. 1.

About 1 ml of each extract was mixed with 3 ml of acetic anhydride. Concentrated H₂SO₄ was carefully added to the filtrate to promote the phase's separation. Blue or green color formation indicated the presence of steroids. For the detection of terpenoids (Salkowski test), 3 ml of sample extracts were mixed in 2 ml of chloroform and 3 ml of concentrated H₂SO₄. A red-dish brown color at the interface was taken as positive for terpenoid.

Results are reported in Table 1.

2.5. Bioactive phytochemicals determination

2.5.1. Plant extracts preparation

A 5 g of powdered sample were added into 50 ml of three different solvents (distilled water, ethanol, and methanol) and allowed to stand at 40 °C, under constant agitation during 1 h. The resultant extracts were then filtered (using double filter paper) for further experiences. Dilutions were performed if necessary.

2.5.2. Total phenolics

Total phenolics were determined colorimetrically according to Vinha et al. (2012). Briefly, 200 μl of each extract was mixed with 1.5 ml of Folin-Ciocalteu reagent (1:10), and allowed to stand at 22 °C ± 1 °C, for 5 min. A 1.5 ml of sodium bicarbonate solution (8%) was added to the mixture. After 60 min at 22 °C ± 1 °C, absorbance was measured at 725 nm using a UV-Vis spectrophotometer (Shimadzu, model UV-1800). Total phenolic contents were quantified through a calibration curve obtained from measuring the absorbance of known concentrations of a gallic acid standard ($r = 0.9992$). The results were expressed as mg of gallic acid equivalents (GAE)/ml of extract (Table 2).

2.5.3. Total flavonoids

Total flavonoids were measured using the aluminum chloride assay, previously described by Zhishen et al. (1999). Distilled water (4 ml) and 5% Na₂NO₃ (300 μl) were added to an aliquot (1 ml) of extract and to the catechin standard solutions

Table 1

Phytochemical constituents of leaves extracts of *Cymbopogon citratus*.^a

Plant extracts	Tannins	Flavonoids	Alkaloids	Steroids	Terpenoids
Aqueous	+	+			+
Ethanolic	+	+			+
Methanolic	+	+	+	+	+

^a (+) Presence of phytochemical compounds. () Absence of phytochemical compounds.

Table 2

Bioactive compounds analysis of the aqueous, ethanolic and methanolic extracts of Angolan *C. citratus* leaves.

Bioactive compounds	Cymbopogon citratus extracts		
	Aqueous	Ethanolic	Methanolic
Phenolics (mg GAE/ml)	4.28 ±	6.11 ± 0.11 ^a	3.26 ± 0.26 ^c
Flavonoids (mg CE/ml)	0.24 ^b	6.62 ± 0.65 ^a	3.47 ± 0.49 ^c
Tannins	5.04 ±	1.88 ±	2.32 ± 0.16 ^a

*Values expressed as mean ± standard deviation obtained from 3 measurements. For each group of compounds (within each table line), different letters (a, b or c) represent significant differences at $p < 0.05$. The same letter indicates that there are no significant differences ($p > 0.05$) between results.

($r = 0.9987$). A volume of 300 μ l of 10% AlCl₃ was added after 5 min at quiescent, followed by 2 ml of NaOH (1 M) after another minute. The total volume was fulfilled till 10 ml. The resultant solution was homogenized and absorbance measured at 510 nm against a reagent blank. Total flavonoid content was expressed as mg catechin equivalents (CE)/ml of extract (Table 2).

2.5.4. Tannins

The content of tannins was determined according to a previously described procedure (Schneider, 1976). 2 ml of plant extract were mixed with 8 ml of water and 10 ml of acetate buffer (solution 1, S1). An aliquot of this solution (10 ml) was subsequently shaken with 50 mg of casein during 60 min and, after that, filtered (solution 2, S2). 1 ml of each previously prepared solution (S1, S2) was mixed separately with 500 μ l of Folin-Denis reagent and, then, both solutions were diluted to 10 ml with sodium carbonate decahydrate solution (33%). The absorbance of such prepared solutions was measured against a blank sample at 720 nm. The tannins content was evaluated upon three independent analyses. Absorbance values obtained for S1 corresponded to total polyphenol content. Differences between absorbance of solutions S1 and S2 correspond to content of casein-adsorbed tannins in *C. citratus* extracts ($r = 0.9945$). The contents of tannins were expressed as mg of catequin equivalents (CE)/ml of extract (Table 2).

2.5.5. Ascorbic acid content

Ascorbic acid content was determined according to the modified 2,6-dichloro-phenolindophenol (DIP) method (Franck et al., 2003). 20 ml of oxalic acid (0.4%) were added to each sample extract (5 ml) and mixed for 1 h. The final solution was filtered and 5 ml were added to 10 ml of oxalic acid and 1 ml of HCl (1 M). Quantification was obtained from a standard curve ($r = 0.9993$) of ascorbic acid (AA) per ml of extract (Table 2).

2.6. Antioxidant activity

2.6.1. Reducing power assay

The reducing power of each extract was evaluated according to the method of Chou et al. (2009). Extracts (250 μ l) were mixed with 250 μ l of sodium phosphate buffer (0.2 M, pH 6.6) and 250 μ l of potassium ferric cyanide (1%, w/v). The resulting mixture was incubated at 50 °C for 20 min. After, 250 μ l of trichloroacetic acid (10%) were added to the mixture to stop the reaction, followed by centrifugation at 4000 rpm during 10 min. The upper layer of the solution (500 μ l) was mixed with 400 μ l of deionized water and 100 μ l of FeCl₃ (0.1%). The

control solution was prepared in the same conditions but without plant extracts. The absorbance was measured at 700 nm in a microplate reader (Synergy HT BioTek.). The reducing power of the plant extract shows a linear correlation with the concentration of the sample. Final results were expressed as mg of ascorbic acid equivalents (AAE)/ml of extract (Fig. 2).

2.6.2. Scavenging of diphenyl-picrylhydrazyl (DPPH[•]) radical

The scavenging activity of the extracts was determined based on DPPH[•] scavenging assay described by Molan et al. (2009). Briefly, 25 μ l of each plant extract was allowed to react with 250 μ l of 0.2 mM DPPH[•] in 95% ethanol in a 96-well microplate. The absorbance was measured at 550 nm using a microplate reader plate (after incubation in dark at room temperature for 30 min). All determinations were performed in triplicate in two separate experiments. Ascorbic acid was used as standard and final results were expressed in terms of ascorbic acid equivalents (AAE)/ml of extract (Fig. 2).

2.6.3. Ferric reducing antioxidant power assay (FRAP)

The capacity to reduce ferric ions was determined using the Ferric Reducing Antioxidant Power (FRAP) assay as described by Benzie and Strain (1996), with slight modifications (Molan et al., 2009). Briefly, an aliquot of 8.5 μ l of each plant extract was added to 275 μ l of diluted FRAP reagent (1:1 with water) directly in

a microplate. The plates were incubated at 37 °C during 15 min and the absorbance was read at 595 nm. Final results were expressed as ascorbic acid equivalents (AAE)/ml of extract (Fig. 2).

2.6.4. Nitric oxide (NO) radical scavenging assay

Nitric oxide radical scavenging method was determined based in previous works (Badami et al., 2005; Govindarajan et al., 2003). 2.0 ml of sodium nitroprusside ($\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}] \cdot 2\text{H}_2\text{O}$) in phosphate buffer (10 mM) were added to 500 μl of each

plant extract. The final solution was then incubated at 25 °C (water bath) during 60 min, and after incubation, 2.5 ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylene and diamine dihydrochloride in 2% H_3PO_3) were added. Absorbance of the chromophore formed was registered at 540 nm. NO \cdot radical scavenging

activity was expressed in terms of ascorbic acid equivalents (mg AAE)/ml of extract (Fig. 2).

2.6.7. Hydrogen peroxide (H_2O_2) scavenging assay

The ability of *C. citratus* extracts to scavenge hydrogen peroxide was determined according to Ruch et al. (1989). A hydrogen peroxide solution (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4) and 2 ml of the final solution was added to 1 ml of plant extracts (1:20 dilution). The absorbance was measured after 10 min of incubation at 230 nm, against a blank solution. H_2O_2 radical scavenging activity was expressed in terms of ascorbic acid equivalents (mg AAE)/ml of extract (Fig. 2).

2.7. Statistical analysis

Data are reported as mean \pm standard deviation of three independent measurements. Statistical analyses were performed using the statistical package SPSS v15.0 (SPSS for Windows; SPSS Inc., Chicago, IL). One-way ANOVA was used to compare groups, and post hoc Dunnett's test was performed for simultaneous paired comparisons. Simple linear regression analysis was used to evaluate correlations between different parameters.

3. Results and discussion

Medicinal plants are of great importance for both individual and community health. It is well known that medicinal and aromatic plants production is challenging and involves a wide variety of issues, including agricultural, commercial, ecological, pharmacological, as well as social (Vinha et al., 2012). Angola is acknowledged for its botanical richness, by resorting to natural therapies, and also by its limited information about their endemic plants (Schoeman et al., 2013).

C. citratus species, currently known as lemon grass, besides the medicinal use, may be also used in the food industry (for preservative and aromatic purposes), as well as in cosmetics and pharmaceutical industries (Mirghani et al., 2012; Sessou et al., 2012). Recently, there has been a considerable interest in extracts from aromatic plants with antioxidant activities for potential industrial applications. In this work, we tried to demonstrate the high potential of the endemic Angolan *C. citratus* leaves.

3.1. Nutritional characterization

The proximate composition of the leaves of *C. citratus* is shown in Fig. 1. On average, the increasing order of the analyzed nutrients was: ash < fat < moisture < protein < carbohydrates. The moisture and ash contents of the leaves were 8.52% and 4.11%, respectively. Crude protein and fat levels were 19.79% and 4.98%, correspondingly, and total carbohydrate represented about 60% of *C. citratus* leaves. The calculated energetic value of *C. citratus* leaves corresponded to 374 kcal/100 g.

These results are slightly different than those described by Oloyede

(2009) for Nigerian *C. citratus* leaves which contained a lower lipid amount (rv1%). Comparatively, the Angolan leaves analyzed in this study are a better source of the so appreciated lemongrass essential oil. Oloyede (2009) also reported a higher ash amount (rv13%). The higher mineral content reported by Oloyede(2009) is mainly due to high P contents that can be related to the soil type or cropping practices. Referring to the total carbohydrate, our values are in accordance with those described by

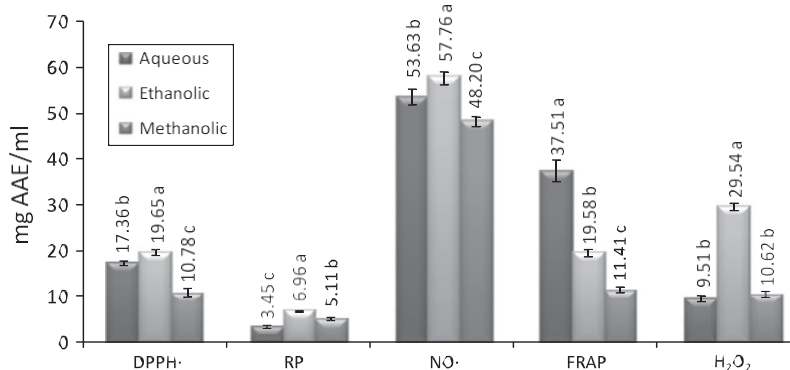


Fig. 2. Total antioxidant capacity of aqueous, ethanolic, and methanolic extracts obtained by *C. citratus* leaves. Legend: AAE, ascorbic acid equivalents; DPPH·, diphenyl– picrylhydrazyl radical scavenging assay; RP, reducing power assay; NO·, nitric oxide radical scavenging assay; FRAP, ferric reducing antioxidant power assay; H₂O₂, Hydrogen peroxide scavenging assay. For each assay, different letters indicate statistical significant differences ($p < 0.05$) caused by the solvent extraction.

Oloyede (2009). Although fiber content was not analyzed in this study, high fiber content in leaves (Oloyede, 2009) and leaf extracts (Asaolu et al., 2009) are described in literature (28% and 55%, respectively), showing that this plant could be an interesting source of fiber for a potential application in food and food supplements industries.

3.2. Phytochemical screening

Preliminary screening tests are useful in the detection of bioactive principles and subsequently may lead to drugs discovery and development. In the present study, several phytochemical constituents were evaluated qualitatively and quantitatively.

Table 1 shows the results obtained for the phytochemical screening analysis conducted on *C. citratus* extracts. The presence of tannins, flavonoids and terpenoids was observed in all the 3 extracts analyzed, while alkaloids and steroids were absent in aqueous and ethanolic extracts.

Extraction is a fundamental step in the medicinal plants analysis, since it is essential to obtain and purify the desired chemical constituents from the plant material for further characterization. Extraction with different solvents is frequently used for isolation and quantification of antioxidant compounds, and both extraction yield and antioxidant activity of the extracts are strongly correlated with the solvent employed. This is mainly due to the different polarity of those compounds, once each matrix-solvent system shows a particular behavior that cannot be predicted (Al-Farsi and Lee, 2008). For this reason, in this work, different solvents were assayed for the extraction of *C. citratus* leaves (water, ethanol and methanol).

Our results support that the edaphic and climatic conditions may promote differences in the synthesis of secondary metabolites which may contribute to the medicinal value as well as physiological activity of the plant part evaluated (Edoga et al., 2005). Indeed, an Indian study reported that both methanolic and aqueous *C. citratus* extracts contained no alkaloids or steroids (Dama et al., 2011). On the other hand, we obtained a similar profile to *C. citratus* leaf extracts studied in Nigeria (Asaolu et al., 2009; Danlami et al., 2011; Ewansiha et al., 2012).

Considering the variability of the extracted chemical compound classes described in Table 1, methanol seems to be the best extractor solvent; however, regarding its toxicity and a potential domestic therapeutic application, the extracts obtained with water and ethanol seem to be quite satisfactory alternatives. Moreover, depending on the type and amount of alkaloids present in the methanolic extract, this could present some kind of toxicity that should be characterized and monitored, if necessary.

3.3. Bioactive compounds

Most of the polyphenolic compounds in nature have antioxidant activities. Water and alcohol were primarily selected as the extraction solvents since both are more innocuous (in comparison with other solvents) and commonly used in the food industry. Nevertheless, methanol was also used as extractor solvent in this work, since previous studies reported better extraction rates for several bioactive compounds in this type of extracts (Bae et al., 2012; Cuanii et al., 2012; Akowuah et al., 2002).

As shown in Table 2, flavonoids were the major components of *C. citratus* extracts, while tannins represented, generally, the minor group. Phenols and polyphenols are secondary metabolites found in high amount in plants. The lowest contents of tannins may be due to the fact that these compounds belong to the flavonoid group which basic chemical structure is C₆–C₃–C₆. So, our results indicated the presence of polymeric tannins, which in small amounts do not have toxicity.

Significant differences ($p < 0.05$) were observed between the total phenolic and flavonoid contents among the different plant extracts. The ethanolic extract was found to have the highest total phenolic and flavonoid amounts, while methanolic one presented the lowest contents ($p < 0.05$) of both groups of compounds. On the other hand, this last extract contained significantly higher levels ($p < 0.05$) of tannins. The highest content of ascorbic acid was found in the aqueous extract, as expected, due to the high hydro-solubility of the molecule.

3.4. Antioxidant activities

Natural antioxidants are considered to be multifunctional and of high interest as alternatives to synthetic antioxidants to reduce oxidation in complex food systems. Their activity depends on several parameters such as the multiplicity and heterogeneity of the plant matrix, edaphic and climatic conditions of the production region and the experimental conditions used for the product achievement (Vinha et al., 2012). It is well known that the antioxidant properties of plant extracts cannot be evaluated by one single method due to the complex nature of phytochemicals. Instead, thorough antioxidant assays should involve several activity studies, with dissimilar and complementary mechanisms of action (Giotti et al., 2009).

In this work, five methods were used to evaluate total antioxidant capacity of the different extracts (DPPH·, NO·, and H₂O₂ scavenging assays, reducing power method, and FRAP). Results are depicted in Fig. 2.

In general, significant differences ($p < 0.05$) were observed among the antioxidant activities of the three extracts evaluated. The ethanolic extract presented a superior total antioxidant capacity, with significantly higher results ($p < 0.05$) for all assays, except for the FRAP method, in which the aqueous extract showed the best results. The FRAP mechanism is based in an electron transfer, being very useful to distinguish dominant mechanisms of antioxidant action, in combination with the other methods (Ronald et al., 2005). On the other hand, the aqueous extract showed the lowest reducing power. The methanolic extract, although often referred as the best solvent to extract antioxidant compounds from vegetable matrixes, presented in this study the lowest radical scavenging activities against both DPPH \cdot and NO \cdot radicals.

Our results are slightly different from those reported by Pereira et al. (2009) that found similar DPPH \cdot inhibition for methanolic and ethanolic extracts of *C. citratus* and lower scavenging activity for aqueous extracts. These differences should be mainly due to the different procedures used to prepare sample extracts, since Pereira et al. (2009) macerated the sample in the dark for 7 days with methanol or ethanol and prepared the aqueous extracts by infusion just before use. On the other hand, Figueirinha et al. (2008) found higher DPPH \cdot inhibition for infusion, followed by 80% aqueous ethanol, decoction, and methanol extracts, by this order. In another study, Tapia et al. (2007) identified the most active DPPH \cdot scavenger compounds from *C. citratus* cultivated in bioreactors by the temporary immersion principle as caffeic acid, chlorogenic acid, neochlorogenic acid, and the flavonoid luteolin 6-C-fucopyranoside.

Hydrogen peroxide when converted into hydroxyl radicals (\cdot OH) can present toxicity to living cells, by reacting with biomolecules, and cause tissue damage and cell death (Khan et al., 2012). No significant differences ($p > 0.05$) were observed between H₂O₂ scavenging activity of aqueous and methanolic extracts. In this case, ethanol extraction achieved better results.

3.5. Correlations

According to the previously discussed results, distinct solvents produced *C. citratus* extracts with significantly different antioxidant activities. Concordantly, the extracts composition regarding bioactive phytochemicals content also differed between samples. It was possible to observe some high correlations between the analyzed parameters, which are detailed in Table 3. The DPPH \cdot results showed a positive correlation with total phenolics ($r = 0.8944$), and flavonoids ($r = 0.9178$). In addition, NO-scavenging activity was also significantly correlated to flavonoids ($r = 0.9263$) and total phenolics ($r = 0.9051$). Obviously, a high correlation coefficient was observed between results of these both antioxidant activity assays ($r = 0.9618$). On the other hand, the levels of tannins were inversely correlated with values obtained in FRAP assay ($r = 0.8046$), while total phenolics and flavonoids showed very low correlation coefficients. This behavior was probably due to different mechanisms of antioxidant action of the analyzed phytochemicals.

A validation of the antioxidant capacity approach is crucial for investigating the role, in human health, of natural antioxidants present in foods. This work intended to approve that the content of different secondary metabolites of *C. citratus* and other plant matrices are dependent upon the solvent used to extract them, an effect that is most likely due to the different phytochemical profiles of the respective extracts. Our results are in agreement with Xu and Chang (2007) who reported that total phenols and flavonoids contents in soybeans and their effects on the FRAP, ORAC, and DPPH \cdot assays may vary with the extraction solvent choice.

Table 3

Correlation coefficients between the different parameters

analyzed Parameters	Parameters	
DPPH \cdot Phenolics		0.8944
DPPH \cdot Flavonoids		0.9178
DPPH \cdot Tannins		0.5914
DPPH \cdot Ascorbic acid		0.1349
RP Phenolics		0.6422
RP Flavonoids		0.5062
RP Tannins		0.2581
RP Ascorbic acid		0.7744
NO \cdot Phenolics		0.9051
NO \cdot Flavonoids		0.9263
NO \cdot Tannins		0.3817
DPPH \cdot RP		0.2670
DPPH \cdot NO \cdot		0.9618
DPPH \cdot FRAP		0.5348
DPPH \cdot H ₂ O ₂		0.6585
RP NO \cdot		0.4246
RP FRAP		0.6267
RP H ₂ O ₂		0.8903
NO \cdot FRAP		0.3407
NO \cdot H ₂ O ₂		0.7639
NO \cdot H ₂ O ₂		0.2623
NO \cdot Ascorbic acid		0.3345
FRAP Phenolics		0.1517
FRAP Flavonoids		0.2982
FRAP Tannins		0.6284
FRAP Ascorbic acid		0.7959
H ₂ O ₂ Phenolics		0.9061
H ₂ O ₂ Flavonoids		0.7959
H ₂ O ₂ Tannins		0.6422
H ₂ O ₂ Ascorbic acid		0.7065
Phenolics Flavonoids		0.9389
Phenolics Tannins		0.3863
Phenolics Ascorbic acid		0.4191
Flavonoids Tannins		0.4401
Flavonoids Ascorbic acid		0.3659
Tannins Ascorbic acid		0.4720

^aDPPH \cdot , scavenging of diphenyl-picrylhydrazyl radical; RP, reducing power assay; NO \cdot , nitric oxide radical scavenging assay; FRAP, ferric reducing antioxidant power assay; H₂O₂, hydrogen peroxide scavenging assay.

4. Conclusion

Plant phytochemicals are used due to their medicinal and physiological properties. Further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

The Angolan *C. citratus* leaves analyzed in this study were mainly constituted by carbohydrates, followed by protein, fat, ash and moisture. Tannins, flavonoids, and terpenoids were detected in all the extracts prepared, though methanolic ones also revealed the presence of alkaloids and steroids.

Ethanolic extracts contained higher amounts of total phenolics and flavonoids while tannins content was higher in methanolic extracts. Aqueous extracts presented higher levels of ascorbic acid. A significantly higher antioxidant activity ($p < 0.05$) was found in ethanolic extracts for all assays, except for the FRAP method, in which the aqueous extract showed the best results. The aqueous extract showed the lowest reducing power and the methanolic ones presented the lowest radical scavenging activities for both DPPH \cdot and NO \cdot radicals.

In general, significant differences ($p < 0.05$) were observed between the antioxidant activities of the three types of extracts studied. The differences found between assays result not only from the chemical composition of the extracts (which vary with the extracting solvent) but also from the different antioxidant mechanisms tested in each assay.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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