

The Effects of Solvents Polarity on the Phenolic Contents and Ferric Reducing Antioxidant Power of *Cassytha filiformis*, *Commiphora schlechteri*, *Ochna natalitia* and *Pavetta assimilis*

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ABSTRACT— *In the present study, the levels of Total Phenols compounds (TPC) and the antioxidant capacity of different extracts from leaves of Cassytha filiformis (C. filiformis), Commiphora schlechteri (C. schlechterii), Ochna natalitia (O. natalitia) and Pavetta assimilis (P. assimilis) were evaluated by the Folin-Ciocalteu and ferric ion reduction methods, respectively. The extracts evaluated were prepared with solvents of different polarities (n-hexane, dichloromethane, ethyl acetate and aqueous methanol). The total phenols found in all samples increase with solvent polarity. The reduction power found was linearly proportional to the concentration and is greater the greater the content of total phenolics. All extracts of the species O. natalitia (DCM, EtAcO and MetOH) showed higher antioxidant capacity than the other species. Thus, the promising results found in the present study suggest a high potential of these species as a source for the development of therapeutically useful natural antioxidants.*

Keywords— Phenolic contents, Ferric reducing power, *Cassytha filiformis*, *Pavetta assimilis*, *Ochna natalitia*, *Commiphora schlechteri*.

1. INTRODUCTION

Natural products were the basis of the ancient system of traditional medicine, with emphasis on the systems of Chinese, Ayurvedic and Egyptian medicine [1, 2]. The use of herbal medicines and supplements has increased tremendously over the past three decades, with no less than 80% of people worldwide relying on them for some part of primary health care [3]. The development of new products from natural sources is also encouraged, as it is estimated that of the 300,000 species of plants existing in the world, only 15% have been evaluated to determine their pharmacological potential [4].

In Mozambique, about 10% of the 5500 species of plants collected and registered are used in traditional medicine. During the colonial period (1891 - 1975) or even in the post-independence period (1975 - 1990), the use of medicinal plants was not encouraged, as they were related to superstitions and beliefs. But since the last decades of the 20th century, traditional medicine has gained more respect from governments and primary care providers, which resulted in the creation, in 1990, of the Association of Traditional Healers (AMETRAMO) [5]. Although the published bibliographies mention the ethnobotanical aspects of Mozambican plants used in traditional medicine [5, 6, 7], some studies on the phytochemical and biological potential were developed by some national authors [8, 9]. However, taking into account the rich plant diversity and the vast knowledge of Mozambican communities about the use of plants in the therapeutic scenario, this investigation is still far from being considered significant, so more phytochemical and ethnopharmacological studies of Mozambican medicinal plants need to be urgently carried out.

Thus, the present research is based on the idea that several compounds present in plants, including phenolic compounds, have biological actions in different systems of the body, thus being responsible for the prevention or cure of

various diseases. For example, the antioxidant action described for phenolic compounds is extremely important in the prevention of degenerative diseases [10]. Epidemiological studies indicate that populations that consume high levels of plant-derived foods have low incidence rates of various types of cancer [11]. This work aims to evaluate the preliminary phytochemistry, quantify the total phenol content, measure the ferric reduction capacity and compare the influence of the extractive solvent polarity on the variation of the antioxidant capacity of different extracts from leaves of *Cassipha filiformis*, *Commiphora schlechteri*, *Ochna natalitia* and *Pavetta assimilis*.

2. METHODS

2.1. Chemicals

Potassium ferrocyanide (98.5%), sodium chloride (99%), potassium chloride (99%), sodium bicarbonate (99.7%) and ferric chloride (99%) were purchased from Sigma – Aldrich Co (St. Louis, USA); Folin-Ciocalteu reagent was obtained from Pehedi Medical & Lab Suppliers (Kenilworth, Johannesburg, South Africa); trichloroacetic acid (99%) was purchased from Riedel-de-Haen (Wunstorfer, Germany); monobasic sodium phosphate (97%), dibasic sodium phosphate (99.5%) and hydrochloric acid (37%) were obtained from Panreac Química SA (Barcelona, Spain), all solvents, n-hexane (99.8%); dichloromethane (99.8%), ethyl acetate (99.8%) and methanol (99.8%) were purchased from Merck Co. (Darmstadt, Germany).

2.2. Plant collection and pre-treatment

The material was collected in November 2021, in the District of Chipenhe (Gaza, Mozambique) and positively identified with *Cassipha filiformis* (specimen identification number: 102/ 2852), *Commiphora Schlechteri* (specimen identification number: 139/4151), *Ochna natalitia* (specimen identification number: 182/5112) and *Pavetta assimilis* (specimen identification number: 170/8383) in the Herbarium of the Department of Botany, Instituto de Investigação Agrária de Moçambique - IIAM. The samples were dried at room temperature at the Institute of Traditional Medicine (IMT) of the Ministry of Health (MISAU) for fifteen days, ground in a hammer mill (Bison, MMRB-20, Mexico) in the laboratory of the Faculty of Engineering of the Eduardo Mondlane University (UEM) and stored in hermetically sealed bottles for further analysis.

2.3. Extract preparation

The extracts were obtained separately by successive maceration for 72 hours of 50.0 g of leaf powder from each plant with 300 ml of each solvent (n-hexane (**nHex**), dichloromethane (**DCM**), ethyl acetate (**EtAcO**) and a methanol/water (95/5) (**MetOH**). The resulting residues were decanted and filtered through Whatman No. 6 filter paper (Sigma-Aldrich), concentrated to dryness on a rotary evaporator (Buchi Rotavapor R-100, Germany) under vacuum at 45°C.

2.4. Qualitative phytochemical and Total phenolics content (TPC)

Qualitative phytochemical analysis was performed using colorimetric or precipitation methods described by Gul et al. (2017) and applied to the identification of secondary metabolites such as steroids, terpenes, glycosides, flavonoids and tannins. Total phenols (TPC) were determined by the Folin-Ciocalteu spectrophotometric method described by Cumbane et al. (2021). Briefly, 500 μ L of 1 mg/mL of each extract (dissolved in methanol) was mixed with 500 μ L of Folin-Ciocalteu reagent (diluted 10 times with distilled water), shaken vigorously and allowed to stand for 5 minutes, followed by the addition of 1 mL of 7.5% (w/v) sodium carbonate solution and 2 mL of distilled water and subsequent incubation for 2 hours. The absorbances of the resulting blue colored solutions were read at 760 nm using the UV-Vis spectrophotometer Thermo Scientific™ Orion™ AquaMate 7000 Vis Spectrophotometer. The total phenol content (TPC) was calculated as a function of the gallic acid calibration curve ($C = 40A - 4.224$; $R^2 = 0.9916$) constructed according to the procedure described for the extract in a concentration range ranging from (5 – 50 μ g/mL). All tests were performed in three replicates and the TPC was expressed as milligrams of gallic acid equivalents (mgGAE) per gram of dry extract (gDE). Where C - gallic acid concentration and A - gallic acid absorbance.

2.5. The Ferric Reducing Power (FRP)

The determination of the antioxidant activity by the method of reduction of ferric ions of the extracts was carried out according to the methodology described by Bhalodia et al. (2013) and Vijayalakshmi & Ruckmani (2016), with some modifications. According to this method, 1 mL of different extracts (100 – 500 μ g/mL) or standard (quercetin) were mixed with 2.5 mL of phosphate buffer pH = 6.6 (8 g of sodium chloride, 0.2 g of potassium chloride, 1.44 g of disodium hydrogen phosphate, 0.24 g of potassium dihydrogen phosphate was taken in a 1,000 mL standard flask and add 800 mL

of distilled water and adjust the pH 6.6 using hydrochloric acid and adjust the volume with deionized water) and 2.5 mL of potassium ferricyanide. 1%. The reaction mixture was incubated at 50°C for 20 minutes, after cooling, the reaction was stopped with the addition of 2.5 mL of 10% trichloroacetic acid and centrifuged at 3000 rotations per minute (rpm) for 10 minutes. 2.5 mL was taken from the supernatant and mixed with 2.5 mL of distilled water and 500 µL of 1 mg/mL of ferric chloride. The absorbances of extracts (A_s) or quercetin (A_r) were read at 700 nm using a UV-Vis spectrophotometer (Massachusetts, USA). The absorbance of quercetin was determined at a fixed concentration of 500 µg/mL. All tests were performed in three repetitions, and the results were used to calculate the reducing power of iron using the equation described below (equation 1). The values of the reducing power obtained were plotted with the respective concentrations to obtain the calibration curve used to calculate the efficient concentration (EC_{50}).

$$FRP (\%) = \left(1 - \frac{A_r - A_s}{A_r}\right) \times 100 \quad (1)$$

2.6. Statistical analysis

The results presented in this study correspond to the mean of three repetitions ($n=3$) ± standard deviation of the mean. For the assays of total phenols and antioxidant activity, the one-way ANOVA test was used followed by the Tukey test ($P < 0.05$) to analyze the differences between TPC and EC_{50} , respectively. The probability of $P < 0.05$ was considered significant. All calibration curves were determined using GraphPad 9.0 software (San Diego, USA) and calculations were performed in Microsoft Excel© 2016.

3. RESULTS AND DISCUSSION

3.1. Qualitative phytochemicals

The preliminary results of the phytochemical tests are presented in Table 1. Thus, in the *Cassytha filiformis* species, phytosterols, anthraquinones, tannins and flavonoids were identified in all analyzed extracts. In the *C. schlechteri* specie, phytosterols, anthraquinones, tannins, flavonoids and terpenoids were identified in the MetOH extract, while in the DCM and EtAcO extracts all metabolites present in the MetOH extract, except for terpenoids, are equally present. In the nHex extract, only phytosterols were detected. In the species *O. natalitia*, phytosterols, anthraquinones, tannins, flavonoids and terpenoids were found for the MetOH extract, in the DCM and EtAcO extracts, all metabolites present in the MetOH extract were also detected, except for anthraquinones. In the nHex extract, all tests were negative. In *P. assimilis*, all analyzed metabolites were positive for MetOH and EtAcO extracts. The DCM extract was positive for all metabolites except terpenoids, while the nHex extract only phytosterols were detected.

Table 1. Phytochemical tests of extracts from leaves of the species *C. filiformis*, *C. schlechteri*, *O. natalitia* and *P. assimilis* in solvents of different polarities.

	<i>Cassytha filiformis</i>				<i>Commiphora schlechteri</i>			
	n-Hex	DCM	EtAcO	MetOH	n-Hex	DCM	EtAcO	MetOH
Phytosterols	+	+	+	+	+	+	+	+
Anthraquinones	+	+	+	+	-	+	+	+
Tannins	+	+	+	+	-	+	+	+
Flavonoids	+	+	+	+	-	+	+	+
Terpenoids	-	-	-	-	-	-	-	+
	<i>Ochna natalitia</i>				<i>Pavetta assimilis</i>			
	n-Hex	DCM	EtAcO	MetOH	n-Hex	DCM	EtAcO	MetOH
Phytosterols	-	+	+	+	+	+	+	+
Anthraquinones	-	-	-	+	-	+	+	+
Tannins	-	+	+	+	-	+	+	+
Flavonoids	-	+	+	+	-	+	+	+
Terpenoids	-	-	+	+	-	-	+	+

(+): present, (-): absent, **nHex**: n-hexane, **DCM**: dichlorometahne, **EtAcO**; ethyl acetate, **MetOH(aq)**: aqueous methanol.

3.2. Total Phenolic Content (TPC)

Phenolic compounds (flavonoids, phenolic acids and tannins) are known to be the main active agents responsible for the antioxidant effect of medicinal plants or foods of plant origin [12, 13]. Thus, the quantification of these compounds is an indispensable parameter in predicting the antioxidant capacity of plant extracts [14]. These compounds can be determined in their entirety in a complex matrix using the Folin-Ciocalteu method [15, 16]. In this method, the phenolic compounds of the sample are oxidized with the Folin-Ciocalteu reagent, which consists of a mixture of heteropoly acids, phosphomolybdic and phosphotungstic acids in which molybdenum and tungsten are in a 6+ oxidation state. In the reaction with a reductant, molybdenum blue and tungsten blue are formed and the average oxidation state of the metals is between 5 and 6 [15]. The blue color produced by the oxides has maximum absorption at 760 nm and is proportional to the total phenolic concentration [8]. In the present study, the quantification of total phenols (TPC) was performed for MetOH, DCM and EtAcO extracts from leaves of *C. filiformis*, *C. schlechteri*, *O. natalitia* and *P. assimilis* species. However, the species *C. filiformis* had its phenolic compounds present in the nHex extract measured and the results are presented in table 2.

Table 2. Total phenolic contents of extracts from leaves of *C. filiformis*, *C. schlechteri*, *O. natalitia* and *P. assimilis* with different polarities.

Total Phenols (TPC) in mg GAE/gDE				
	<i>C. filiformis</i>	<i>C. schlechteri</i>	<i>O. natalitia</i>	<i>P. assimilis</i>
nHex	185.897 ± 0.458 ^{aA}	-	-	-
DCM	271.647 ± 0.458 ^{aB}	297.323 ± 0.459 ^{bB}	408.217 ± 0.917 ^{cB}	316.117 ± 0.916 ^{dB}
EtAcO	443.947 ± 0.917 ^{aC}	464.853 ± 0.917 ^{bC}	469.307 ± 0.458 ^{cC}	465.643 ± 0.046 ^{dC}
MetOH(aq)	473.854 ± 0.794 ^{aD}	561.723 ± 0.458 ^{bD}	687.282 ± 0.630 ^{cD}	685.909 ± 0.200 ^{dD}

Different lowercase letters on the same line indicate significant differences between TPC values in different samples using the same extractive solvent. Different capital letters in the same column indicate significant differences between TPC values of the same sample in different extractive solvents. For all analyzes a significance level of $p < 0.05$ was considered.

Gallic acid was used to construct the calibration curve used to obtain the equation $C = 40A + 4.224$ ($R^2 = 0.9916$) in $\mu\text{g/mL}$. The TPC content was obtained by replacing the value of A in the equation by the absorbance value (A) of each sample analyzed. All tests were performed in three replications and the results expressed in mg of gallic acid equivalent per gram of dry extract (mgGAE/gDE). In all the botanical species studied, the TPC content showed significant variations, with a tendency to increase as the solvent polarity increases. Thus, for the species *C. filiformis*, the total phenol content measured was 185.897 ± 0.458 mg GAE/gDE. In the other species, the phenolic contents were not measured for the nHex extract, as they were not detected in the preliminary phytochemistry. For DCM extracts, the phenolic contents measured for the species *C. filiformis*, *C. schlechteri*, *P. assimilis* and *O. natalitia* were, respectively, 271.647 ± 0.458 , 297.323 ± 0.459 , 316.117 ± 0.916 and 408.217 ± 0.9178 mg GAE/gDE. While for the extracts of the solvents EtAcO and MetOH, the phenolic contents measured for the same species were 443.947 ± 0.917 , 464.853 ± 0.917 , 465.643 ± 0.046 and 469.307 ± 0.458 mg GAE/gDE for the MetOH extract and 473.854 ± 0.794 , 561.723 ± 0.458 , 685.909 ± 0.200 mg GAE/gDE and 687.282 ± 0.630 , respectively. The variation of TPC in the four species in all solvents was observed in the order, *O. natalitia* > *P. assimilis* > *C. schlechteri* > *C. filiformis*. The polarity of extractive solvents can greatly influence the concentration of phenolic compounds in plant extracts [17]. The variations in the phenolic contents of the different extracts from the leaves of the species studied for solvents with different polarities are not surprising. Because such differences have already been reported by several authors in many studies [17, 18, 19]. Barchan et al. (2014) found a significant increase in TPC levels of six species (*Mentha crispata*, *Mentha pulegium*, *Mentha piperita*, *Mentha crispata*, *Mentha pulegium* and *Mentha piperita*) for the solvents nHex, DCM, MeOH(aq) and water respectively. While Babbar et al. (2014) studied the influence of solvent polarity on the extraction of phenolic compounds present in tomato skins, potato skins, pea pods and cauliflower residue using four solvents with increasing polarity (nHex, chloroform, EtAcO and MetOH). The results of these authors revealed that MetOH and EtAcO were better than the other two solvents in the extraction of phenolic compounds. In the present study, the data show that the extractive capacity of phenolic compounds was higher in solvents with higher polarity (MetOH and EtAcO). However, MetOH showed greater efficiency in the extraction of phenolic compounds in relation to the other solvents. Factors such as solvent selectivity for a given group of metabolites can profoundly affect the concentration of these metabolites in plant extracts. High polarity compounds such as flavonoids (especially glycosides) and tannins are easily extracted by polar solvents, including hydroalcoholic mixtures (methanol or ethanol) [20]. The influence of water on the extractive efficiency of the hydroalcoholic mixture also seems to be associated with its ability to swell (hydrate) the plant material, facilitating the interaction of alcohol with the compounds of interest [21]. Furthermore, it should be noted that the TPC content can be influenced by some reducing constituents, including reducing sugars, ascorbic acid,

amino acids tyrosine, tryptophan and cysteine, compounds with high affinity for polar solvents and which can also be oxidized by Folin- Ciocalteu [22, 23]. Therefore, the results obtained in the present study should be evaluated with caution, until the absence of these interferents is considered.

3.3. The Ferric Reducing Power (FRP)

Phenolic compounds such as flavonoids, tannins and others are considered the main contributors to the antioxidant capacity of several medicinal plants, hence the growing interest in the search for such substances from natural sources [24, 25]. Growing epidemiological evidence points to the role of antioxidant foods in preventing certain diseases [26, 27]. Several studies have described the reducing power of phenolic compounds present in various plant species, correlating them with the reducing capacity of free radicals produced in various pathological processes in the human or animal biosystem [28]. The reducing power of plant extracts is associated with antioxidant activity and can serve as a significant reflection of the antioxidant activity of the samples tested. Compounds with reducing potentials indicate their availability to donate electrons and can reduce biomolecule intermediates such as lipids in an oxidized state [29]. In the present study, the reducing capacity of the thirteen extracts was measured by the ferric ion reduction method and calculated in relation to the quercetin standard. Calibration curves were obtained by plotting concentrations versus ferric reduction capacity for each concentration. The equations of each curve were used to calculate the values of the efficient concentrations of each sample (EC_{50}), which is the concentration of the extract corresponding to a reducing power of 50%. The thirteen extracts studied clearly showed concentration-dependent reducing power (figures 1 - 2).

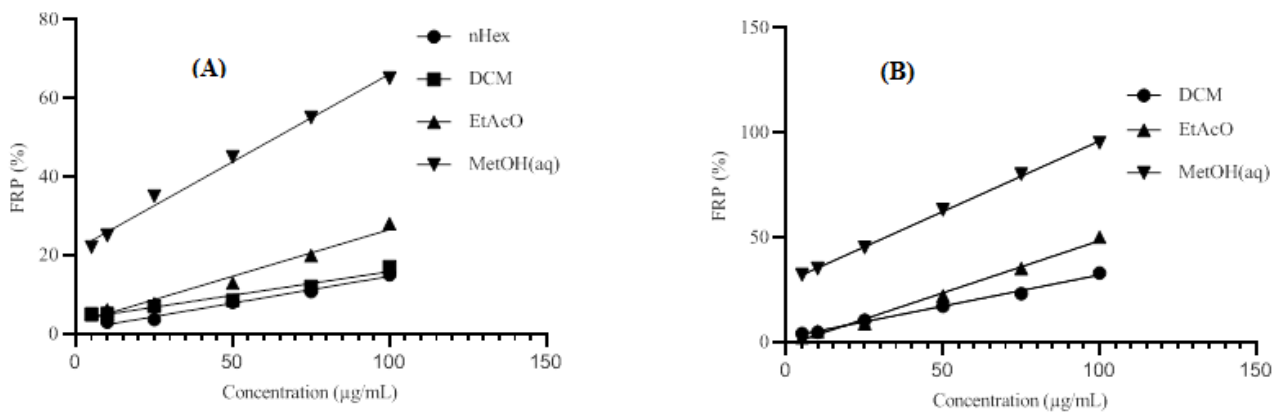


Figure 1. FRP (%) in different concentrations of *C. coliformis* (A) and *C. schlechter* (B) extracts using four different solvents

The reducing power of nHex extracts was measured only for *C. filiformis* specie, while for DCM, EtAcO and MetOH extracts it was determined for all four species (*C. filiformis*, *C. schlechteri*, *O. natalitia* and *P. assimilis*). The antioxidant activity exhibited by all samples showed a significant increase as the polarity of the extractive solvent increases (nHex < DCM < EtAcO < MetOH) and seems to be closely linked to the phenolic content found for these samples. Extracts with higher phenolic contents showed lower EC_{50} values (Tables 2 and 3), consequently, higher antioxidant activity. The measured EC_{50} values for the four species ranged from $358.73 \pm 0.34 - 63.95 \pm 0.96 \mu\text{g/mL}$ for the specie *C. filiformis*, $160.59 \pm 0.65 - 31.78 \pm 0, 58 \mu\text{g/mL}$ for the specie *C. schlechteri*, $58.68 \pm 0.77 \mu\text{g/mL}$ for the specie *O. natalitia* and $108.31 \pm 0.19 - 49.51 \pm 0.96$ for the specie *P. assimilalis*. Comparing the ferric reducing power between species, for each solvent we have the following order, *C. filiformis* < *C. schlechteri* < *P. assimilalis* < *O. natalitia*, *C. filiformis* < *C. schlechteri* < *P. assimilalis* < *O. natalitia*, *C. foliformis* < *P. assimilalis* < *C. schlechteri* < *O. natalitia*, for DCM, EtAcO and MetOH extracts, respectively.

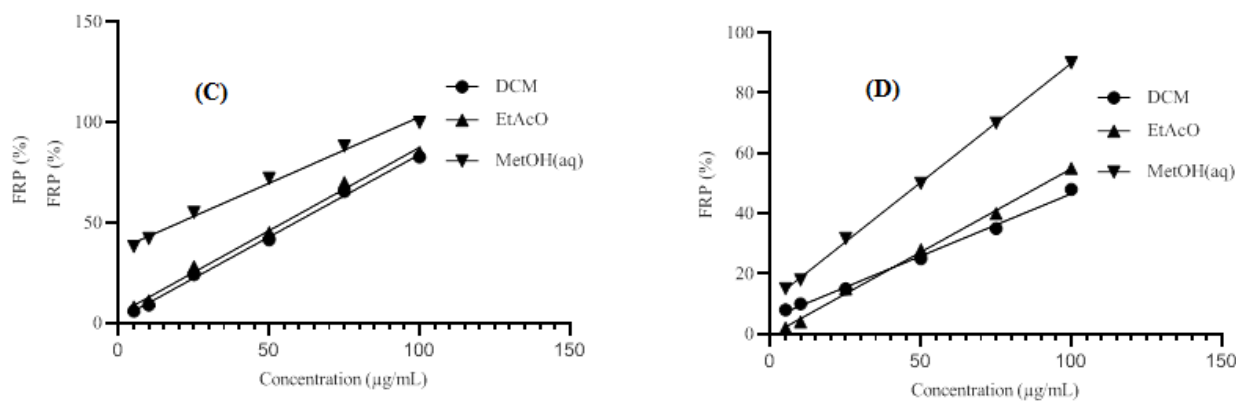


Figure 2. FRP (%) in different concentrations of *O. natalitia* (C) and *P. assimilis* (D) extracts using four different solvents

Thus, the highest antioxidant activity was achieved by the specie *O. natalitia*, in all solvents measured (DCM, EtAcO and MetOH). Polyphenolic molecules such as flavonoids, tannins and phenolic acids are important antioxidant constituents present in plants responsible for inactivating reactive species, including free radicals produced in biological systems [30, 31].

Table 3. Ferric reducing power (FRP) of extracts from leaves of *C. filiformis*, *C. schlechteri*, *O. natalitia* and *P. assimilis* with different polarity.

Half maximal effective concentration (EC ₅₀) in µg/mL				
	<i>C. filiformis</i>	<i>C. schlechteri</i>	<i>O. natalitia</i>	<i>P. assimilis</i>
n-Hex	383.34 ± 0.36 ^{aA}	-	-	-
DCM	358.73 ± 0.34 ^{aB}	160.59 ± 0.65 ^{bB}	58.68 ± 0.77 ^{cB}	108.31 ± 0.19 ^{dB}
EtAcO	198.67 ± 0.57 ^{aC}	103.37 ± 0.52 ^{bC}	54.82 ± 0.88 ^{cC}	91.19 ± 0.46 ^{dC}
MetOH	63.95 ± 0.96 ^{aD}	31.78 ± 0.58 ^{bD}	20.20 ± 0.48 ^{cD}	49.51 ± 0.96 ^{dD}

Different lowercase letters on the same line indicate significant differences between EC₅₀ values in different samples using the same extractive solvent. Different capital letters in the same column indicate significant differences between EC₅₀ values of the same sample in different extractive solvents. For all analyzes a significance level of $p < 0.05$ was considered.

Research in recent years strongly points to the role of polyphenols in preventing degenerative diseases, particularly cancer, cardiovascular disease, and neurodegenerative diseases [31, 32]. Different reports in the literature indicate a direct correlation of total phenolic content with antioxidant capacity using different in vitro antioxidant models [30, 33]. In the present work, extracts with higher phenolic content showed greater ferric reduction capacity, which also allows us to conclude that the antioxidant activity of these samples is correlated with the phenolic content. All samples analyzed showed a linear correlation of antioxidant capacity as a function of concentration, as shown in the graphs in Figures (1 – 4). This correlation is not surprising, as Khaled-Khodja et al. (2014) determined the reducing power of methanolic extracts from *Ajuga iva*, *Marrubium vulgare*; *Mentha pulegium* and *Teucrium polium* and found a linear correlation between concentration and reducing capacity. The same correlation was found by Jayanthi and Lalitha (2011) for extracts of different polarities of the specie *Eichhornia crassipes* (Mart.) Solmes. The extracts of the four species studied by us showed strong reducing capacity and showed significant differences between them ($p < 0.05$). The species *O. natalitia* showed the highest antioxidant activity in all solvents, while the specie *C. filiformis* showed the lowest reducing power. The data found in the present study, although not conclusive because a single method was used to measure the antioxidant capacity, give a clear indication of the antioxidant potential of these species (*C. schlechteri*, *P. assimilis*, *O. natalitia* and *C. filiformis*) and, therefore, its use in low doses may constitute an alternative resource in the primary prevention of diseases related to the oxidation of biomolecules.

4. CONCLUSION

Based on the results obtained in the present study, it is concluded that the hydromethanolic, ethyl acetate, dichloromethane and n-hexane extracts from the leaves of *C. filiformis*, *C. schlechteri*, *O. natalitia* and *P. assimilis* contain high levels of phenols and exhibit high antioxidant power in vitro. As solvent polarity increases, solvent extraction power for phenolic compounds and antioxidant activity significantly increase. The antioxidant activity is higher the higher the total phenolic content. The high levels of TPC together with the strong antioxidant activity demonstrated for all four species is an indication that these plants may be a useful source of therapeutic substances useful in the primary prevention or slowing of the progress of various pathological conditions associated with oxidative stress.

Due to the promising results found in the present study, further studies are being carried out in other in vitro and in vivo models to consolidate the knowledge generated in the present study and isolate the bioactive compounds responsible for such activities.

5. COMPETING INTERESTS

All authors declare no conflict of interest.

6. ACKNOWLEDGEMENT

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