In Vitro Antioxidant Capacity, Total Phenolic and Ascorbic Acid Contents of Crude Extracts from wild Fruits of *Mimusops Caffra, Strychnos Madagascariensis* and *Vangueria infausta*

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ABSTRACT— ***Ethnobotanical, phytochemical, ethnopharmacological and toxicological studies are being used by different authors to support indigenous knowledge and to provide scientific evidence of the beneficeffects of native fruits and other natural products on health. In the present research, antioxidant capacity of hydroethanolic extracts of fruit pulp from Mimusops caffra (family Sapotaceae), Strychnos madagascariensis Poir. (family Loganiaceae) and Vangueria infausta Burch. subsp. infausta (Rubiaceae) was studied. The total phenolic compounds and total ascorbic acid were quantified by the spectrophotometric methods of Folin-Ciocalteu and 2,4-Dinitrophenyl Hydrazine respectively. The antioxidant capacity of the extracts was evaluated using reducing methods (phosphomolybdenum antioxidant assay, ferric reducing ability of plasma, metal chelating activity and ferric reducing power assay) and free radical scavenging method (DPPH and ABTS assay). The results showed significant differences (p <0.05) between the three samples analyzed. In the quantification of total phenols, the highest value (355,814 ± 4,167 mgEAG/gES) was found for the hydroethanolic extract of M. caffra while the hydroethanolic extract of V. infausta was the one that exhibited the highest content of ascorbic acid (120,146 ± 0.224). The highest total antioxidant activity was also exhibited by the fruit extract of M. caffra. The results found in the present study show that the fruits of the species of M. caffra and S. madagascariensis have secondary metabolites with a strong antioxidant activity, which suggests a beneficial effect on health, resulting from consumption of these fruits, especially in communities with limited resources. On the other hand, they can be used as an alternative to synthetic additives in the food processing industries or in pharmaceutical laboratories for the conservation of their formulations.***

Keywords— Antioxidants; DPPH method; *M. caffra*; *S. madagascariensis*; *V. infausta*

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1. INTRODUCTION

Historically the development of most drugs has its origin associated with a natural product (microorganism, plant, animal or marine resources) or its derivative. This led to the recognition of natural products as the main source of therapeutic products. According to the World Health Organization a significant number of the world population still rely on traditional medicine for their health care needs [18][19].

Until few decades ago, more than 80% of drugs and substances with therapeutic properties were derived from natural products or their derivatives, number that decreased with the increased use of combinatorial chemistry in this area. Natural products present a huge structural and chemical diversity and represent the largest source of bioactive substances and are, therefore, substances with a huge potential for the discovery of new drugs and production of supplements. They have relatively few problems of toxicity or other side effects, or their effects are known due to their use and test for a longer period [8][15][18][23].

Therapeutic effect of medicinal plants includes antioxidant capacity, a property resulting from the presence of components which neutralize or reduce the effect of free radicals and other reactive species. These species are responsible for the occurrence of mutagenic transformations and the consequent development of degenerative diseases like cancer, atherosclerosis, cardiovascular and inflammatory diseases, Parkinson, Alzheimer and other neurodegenerative diseases [5][7][29]. Antioxidants include compounds like minerals, vitamins and phytochemicals. Its effect results from the capacity to break the chain reactions were free radicals are involved, by converting them to a stable and unreactive form and inhibit further reactions [9].

Use of synthetic antioxidants is sometimes associated, on a long term, with health problems like skin allergies, gastrointestinal tract problems and, in some cases, increased risk of cancer [3][12][20]. The need for alternative

antioxidants, promoted research aiming to explore the potential of antioxidants available in fruits, vegetables and other products from a natural origin. In this research we studied the antioxidant potential of fruit pulp from *Mimusops caffra*, *Strychnos madagascariensis* e *Vangueria infausta*. Research carried out included a qualitative and quantitative phytochemical analysis (total phenols, total flavonoids, total tannins and ascorbic acid).

These native species grow spontaneously, particularly in Southern Mozambique, and have a nutritive and therapeutic potential within rural communities. The flesh of *Strychnos madagascariensis* is used as food and as an anemic, while its roots are used to heal inflammation and wounds [24]. Pulp of *Mimusops caffra* is widely consumed due to its sweet taste and other organoleptic properties. In South Africa the bark is used to treat wounds, the roots to treat sexually transmitted diseases, while the leaves show a potential antimalarial activity [4]. *Vangueria infausta* is used, traditionally, to treat malaria, wounds, menstrual and uterine diseases [22][28], gastrointestinal disorders, cough, snakebites, infertility, candidiasis and abdominal pains [20][22].

2. MATERIALS AND METHODS

The fruits of Mimusops caffra, Strychnos madagascariensis and *V. infausta* were collected in Manhiça and Marracuene, respectively, two districts in the province of Maputo, south of Mozambique, during the month of October 2016. The fruit species were authenticated by botanists from the National Herbarium of the Mozambican Agricultural Research Institute (IIAM).

For the preparation of hydroethanolic extracts, 50 grams of dried pulp powder were macerated with hydroethanolic solution (80 %) at room temperature for 24 h. The process was repeated up until the negative reaction with Folin-Ciocalteu (10 %) reagent in sodium carbonate solution (7.5 %). The extracts were filtered and concentrated under reduced pressure at 50°C.

After preparation of the extracts, a preliminary phytochemical screening was carried out following the procedure described by [13], which results were classified using a qualitative assessment based on presence (+) or absence (-) of a certain component. Qualitative phytochemical assessment was followed by the quantitative determination of total flavonoids, using the aluminum chloride method [25]; total phenolic compounds based on the oxidation of samples using the Folin-Ciocalteau reagent [1] and total tannins by the Folin-Ciocalteau method [1].

Content of ascorbic acid was determined using a spectrophotometric method based on measurement of the absorbance, at 521 nm, of a red complex, resulting from transformation of ascorbic acid [14][15]. Antioxidant activity was determined using six common methods namely: 1) DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay [16], 2) ABTS Radical Scavenging Assay [31], 3) Ferric Reducing Antioxidant Power (FRAP) assay [2] 4) Ferrozine-Antioxidant Assay [34], 5) Phosphomolybdenum Antioxidant Assay [27] and 6) Reducing Power Assay method [33].

All experiments were carried out in triplicates and data reported are mean ± standard error of the mean. Data were analyzed using ANOVA test and means of significant differences were separated using Student t test at the 0.05 level of probability. Linear regression and correlation analyses were carried out using GraphPad Prism (Version 7.0).

3. RESULTS AND DISCUSSION

Table 1 presents the results of the qualitative phytochemical analysis, while table 2 presents results of the content of total phenols, total flavonoids, total tannins and ascorbic acid. The highest contents of total phenolic, total flavonoids and total tannins were found in the Mimusops caffra extract ($p < 0.05$) with values estimated at 355.814 ± 4.167 mg of gallic acid per gram of dry extract, 74.670 ± 2.280 mg of quercetin per gram of dry extract and 40.027 ± 0.306 mg tannic acid equivalent per gram of dry extract respectively. Curiously, no phenolic compounds were found in the fruit extract of V. infausta. The content of ascorbic acid in the extract of *Vangueria infausta* (120.146 ± 0.224 mg AA per g DE) is higher ($p < 0.005$) than that of the extracts of M. caffra (111.63 ± 0.991 mg AA / g DE) and *S. madagascariensis* (87.648 ± 0.507 mg AA / 100 g DE).

Table 1. Result of the qualitative tests for Phyto-constituents of the hydroethanolic extract of the fruits of *Mimusops caffra*, *Strychnos madagascariensis* and *Vangueria infausta*.

Phyto-constituents	***Mimusops caffra***	***Strychnos madagascariensis***	***Vangueria infausta***
Alkaloids	+	-	-
Tannins	+	-	-
Flavonoids	+	+	-
Triterpenes/steroids	-	-	+
Ascorbic acid	+	+	+
Reducing sugars	+	+	+

Table 2. Total phenolic, total flavonoids, total tannins and total ascorbic acid content from hydroethanolic extracts from *M. caffra*, *S. madagascariensis* and *V. infausta* fruits.

Phenolics or ascorbic acid	*Mimusops caffra*	*S. madagascariensis*	*V. infausta*
Total Phenols (mg GAE per grams of dry extract)	355.814 ± 4.167[a]	118.366 ± 0.247[b]	ND
Total Flavonoids (mg QE per grams of dry extract)	74.670 ± 2.280[a]	24.968 ± 0.016[b]	ND
Total Tannins (mg GAE per grams of dry extract)	40.027 ± 0.306[a]	18.629 ± 0.512[b]	ND
Ascorbic acid (mg per grams of dry extract)	111.63 ± 0,991[a]	87.648 ± 0,507[b]	120.146 ± 0.224[c]

Each value in the table is represented as mean ± SE (n = 3).
Means not sharing the same letter are significantly different at $p < 0.05$ probability level in each line. ND - not determined.

Reducing power observed with plant and fruit extracts is associated by different authors with the ability to sequester free radicals produced in pathological processes occurring in human and animal organisms [26] and can, therefore, be seen as a contribution to the antioxidant capacity.

Total phenolic content is seen as a measure of the antioxidant capacity of an extract. [30] and [11] found a good correlation between total phenolic content and antioxidant activity. This result is seen as a proof of the significant contribution of total phenolic compounds on antioxidant activity.

Results of the antioxidant activity by different methods are presented in table 3 (*M. caffra*), table 4 (*S. madagascariensis*) and table 5 (*V. infausta*). There results were obtained from extracts with concentrations ranging from 10 to 50 µg/mL. Although the tree extracts show different antioxidant values for the different concentrations, values obtained by different methods for the same extract show a strong correlation of the antioxidant capacity values obtained by different methods (Figure 1).

Table 3. Antioxidant activity of the hydroethanolic extract of *M. caffra*.

	Antioxidant activity (%)					
(µg/mL)	DPPH	FRAP	FRP	PPC	ABTS	FERR
10	53.622 ± 0.094	34.110 ± 1.031	11.540 ± 0.040	52.474 ± 0.139	25.332± 0.294	19.804 ± 0.189
20	59.227 ± 0.000	45.103 ± 0.863	21.233 ± 0.586	57.440 ± 0.000	29.697 ± 0.061	30.088 ± 0.452
30	65.769 ± 0.069	56.293 ± 0.958	29.433 ± 0.379	59.898 ± 0.029	34.888 ± 0.113	36.386 ± 1.332
40	73.844 ± 0.053	66.673 ± 0.029	37.127 ± 0.046	65.471 ± 0.032	39.014 ± 0.049	42.832 ± 1.561
50	78.935 ± 0.043	78.203 ± 0.023	47.167 ± 2.376	69.699 ± 0.638	42.079± 0.021	49.505 ± 0.080

DPPH and *ABTS* - percentage inhibition of the DPPH and ABTS radicals respectively; *FRAP*, *RRP* and *FERR* - percentage of iron reduction in complexes $Fe^{(III)}$-TPTZ (TPTZ = 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride), $[Fe(CN)_6]^{3-}$ and $Fe^{(III)}$ - FZ (FZ = ferrozine)] respectively; *PPC* – percentage reduction of phosphomolybdenum (VI) acid.

Table 4. Antioxidant activity of the hydroethanolic extract of *S. madagascariensis*.

	Antioxidant activity (%)					
(µg/mL)	DPPH	FRAP	FRP	PPC	ABTS	FERR
10	10.09 ± 0.00	14.36 ± 0.53	18.02 ± 0.57	18.85 ± 0.59	13.08± 0.49	23.75 ± 0.75
20	12.36 ± 0.33	22.64 ± 0.08	21.67 ± 0.91	24.16 ± 0.56	18.22 ± 0.16	27.09 ± 0.18
30	14.97 ± 0.03	32.02 ± 0.50	26.17 ± 0.65	31.27 ± 0.11	22.46± 0.02	30.72 ± 0.03
40	17.77 ± 0.12	38.22 ± 0.08	30.57 ± 0.83	41.01 ± 0.90	27.24 ± 1.10	34.62 ± 0.79
50	19.36 ± 0.37	46.38 ± 1.71	34.63 ± 0.91	46.55 ± 0.97	31.78 ± 1.54	39.22 ± 0.11

Table 5. Antioxidant activity of the hydroethanolic extract of Vangueria infausta.

	Antioxidant activity (%)					
(µg/mL)	DPPH	FRAP	FRP	PPC	ABTS	FERR
10	18.99 ± 0.66	12.21 ± 0.50	22.24 ± 0.26	8.62 ± 0.66	12.04 ± 0,17	9.90 ± 0.48
20	20.00 ± 0.59	15.08 ± 0.54	24.42 ± 0.27	10.36 ± 0.59	15.19 ± 0.13	15.04 ± 0.23
30	20.90 ± 0.41	19.27 ± 0.23	26.17 ± 0.73	12.78 ± 0.41	19.28 ± 0.05	18.19 ± 0.39
40	22.23 ± 0.43	22.62 ± 0.18	28.61 ± 0.53	15.34 ± 0.43	22.22 ± 0.03	21.42 ± 0.39
50	23.05 ± 0.34	26.09 ± 0.14	30.88 ± 0.39	17.27 ± 0.34	26.08 ± 0.29	24.74 ± 0.58

Figure 1 presents a correlation of antioxidant capacity results obtained by the DPPH method, on one side, and each of the other methods tested in this study (FRAP, FRP, PPC, ABTS and Ferrozine methods) on the other side. Within used methods, DPPH and ABTS are considered the most reliable methods [18], but existing correlation of results obtained by different methods (Figure 1) shows a potential good performance of other methods on determination of antioxidant capacity. This is particularly acceptable since antioxidant capacity is evaluated by comparison with a standard. Further studies comparing different methods, under standard conditions, can help establish accepted conditions for the reliable determination of antioxidant activity using the different methods.

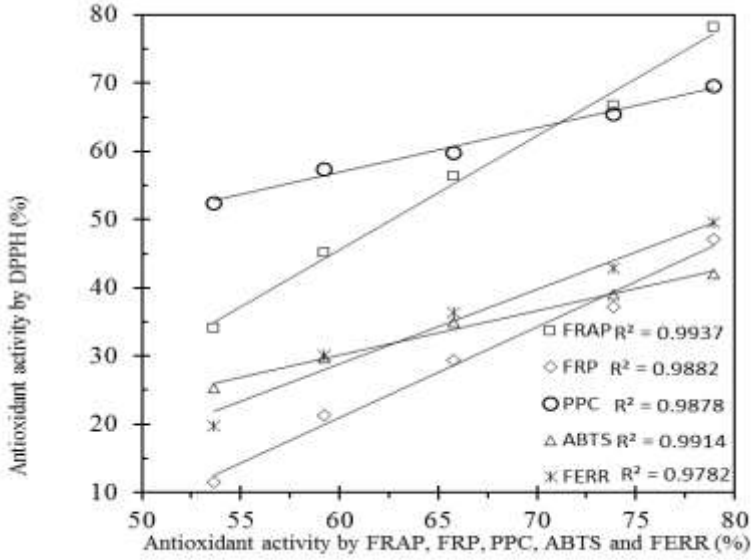

Figure 1: Correlation of antioxidant capacity of results obtained by the DPPH method and the other methods tested in this research.

Table 6 resumes the values of IC_{50} obtained with the different methods. The highest antioxidant activity using the DPPH method was observed with the *Mimusops caffr*a extract (EC_{50} = 5.0480 ± 0.098 µg/mL), with inhibition values ranging from 53.622 ± 0.094 – 78.935 ± 0.043, while the lowest antioxidant activity was observed in the extract of *Vangueria infausta* (EC_{50} = 309.874 ± 0.368 µg / mL), with the inhibition values varying between 18.990 ± 0.659 - 23.048 ± 0.340. Despite a greater inhibition interval shown by the fruit extract of *Vangueria infausta*, when compared to *Strychnos madagascariensis* (10.091 ± 0.003 – 19.358 ± 0.365%), (EC_{50} = 176.516 ± 0.368 µg / mL), the differences between two consecutive inhibitions [by example AA (20 µg / mL) - AA (10 µg / mL)] are higher for the extract of *S. madagascariensis*, which justifies the lower EC_{50} value and the consequent higher antioxidant activity of this extract against the *V. infausta* extract. Antioxidant capacity of *V. infausta* extract, where no phenolic compounds were identified, points for a significant contribution of ascorbic acid on the antioxidant capacity.

Table 6. Half maximal effective concentration (EC50) in µg/mL

Sample	Half maximal effective concentration (EC_{50}) in µg/mL					
	DPPH	FRAP	FRP	PPC	ABTS	FERR
M. caffra	5.048 ± 0.01[a]	24.45 ± 0.68[a]	54.87 ± 0.03[a]	4.08 ± 0.10[a]	66.90 ± 0.32[a]	49.81 ± 1.16
S. madagascariensis	176.52 ± 0.37[b]	54.23 ± 0.94[b]	86.48 ± 0.42[b]	54.41 ± 0.53[b]	95.18± 1.45[b]	79.211 ± 1.437[b]
V. infausta	309.87± 0.37[c]	235.34 ± 0.55[c]	134.08 ± 0.86[c]	196.72 ± 4.99[c]	236.89 ± 3.80[c]	119.08 ± 2.35[c]

Each value in the table is represented as mean ± SE (n = 3). Means not sharing the same letter are significantly different at $p < 0.05$ probability level in each column.

Antioxidant capacity (measured by EC_{50} values) of fruit extracts from *S. madagascariensis* was significantly higher ($p < 0.05$) in the ABTS method. However, the *M. caffra* extract showed an efficiency of about 13 times greater in the DPPH method. These findings seem to converge with those of a study by [6] in which they compare the use of the DPPH method and the ABTS method in the determination of the antioxidant capacity of different foods rich in antioxidant compounds popularly consumed in the United States. These authors found a greater antioxidant capacity for the ABTS method in fruits, vegetables and beverages compared to the DPPH method and concluded that the ABTS assay may be more useful than the DPPH assay in the determination of the antioxidant capacity in a variety of foods. However, this conclusion does not seem to be consensual. [32] found greater effectiveness of the DPPH method in the study of the

antioxidant capacity of the ethanolic extracts of bracts, leaves and floral stem from artichokes (*Cynara cardunculus* L.). This conclusion was supported by [10], who found also a predominant efficacy of the DPPH method for different fruit extracts of *M. buxifolia*. More studies are needed to produce a conclusive evidence about.

4. CONCLUSIONS

All extracts analyzed demonstrated, for all methods, a significant reducing capacity. The highest antioxidant activity was exhibited by the fruit extract of *M. caffra*, for all methods, followed by the fruit extract of *S. madagascariensis* and finally the fruit extract of *V. infausta*. This increased activity is certainly associated with high values of total phenols, total flavonoids and total flavonoids. Results obtained with *V. infausta*, where no phenols and flavonoids have been detected, show a significant contribution of ascorbic acid for the antioxidant activity.

Better performance of *M. caffra* on antioxidant capacity has been observed with all testing methods, thus, again, showing no contradictory results when different methods are used. Observed correlation and the good performance of different methods in comparison to selected standards can help establish reliability of the further methods used hereby on the determination of antioxidant capacity. Promising results produced in this and similar study show a potential for promotion of antioxidants from a natural origin, as alternative to the synthetic ones.

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