Phytochemical Screening and comparison of DPPH radical scavenging from different samples of coffee and Yerba Mate beverages

Rayzah Teodoro de Oliveira[*], Jair Marques Junior[**], Diego Vinicius do Nascimento[*], Ricardo Stefani[*]

[*] Laboratorio de Estudos de Materiais (LEMAT), Instituto de Ciencias Exatas e da Terra, Campus Universitario do Araguaia, Universidade Federal de Mato Grosso. Barra do Garcas – MT. Brazil
[**] Departamento de Policia Cientifica Civil (POLITEC), Rua Manoel Camerino, 791. Barra do Garcas – MT. Brazil

***Abstract*- A** phytochemical screening of commercial samples of roasted coffee, soluble coffee and yerba maté prepared as tereré and chimarrão consumed in South America was evaluated. All samples were subjected to a qualitative assay for phytochemical screening in order to detect classes of phenolic compounds, as well as FT-IR analysis of the dried crude extracts, Antioxidant potential was conducted with DPPH assay. The results were similar to each sort of samples according to the presence of phenolic compounds classes, meanwhile the antioxidant potential percentage were varied.

***Index Terms*-** Ilex paraguariensis, Coffee, Phytochemical screening, Antioxidant potential

I. Introduction

Plants are widely used as medicinal agents and many extracts from plants around the world have an antioxidant activity higher than synthetical antioxidants, due to their high contents of phenolic compounds [1]. Many natural antioxidant agents, such as hydroxycinnamic acids, flavonols, anthocyanins and their oligomers act primarily by free radical scavenging [2]. Free radicals have been linked to chronic inflammation, origin and pathology of cancer, as well as neurodegenerative diseases. Thus, researches about dietary antioxidants are developed to targeting oxidative damage [3]. Coffee is an important dietary antioxidant source and is reported to be among the most widely consumed beverages around the world [4]. Its flavor, aroma, and caffeine content play a role in its popularity. Beverages based on coffee are complex chemical mixture and is reported to contain more than a thousand different chemical compounds, including carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids and phenolic compounds. Recently, coffee consumption has been associated with reductions of several chronic diseases as type 2 diabetes mellitus, Parkinson disease and colorectal cancer prevention [5].

Ilex paraguariensis is a native plant from South America and its beverages have been widely consumed as infusions, which are popularly known as chimarrão, tereré (both from green dried leaves, the first with hot water and the second with cold water) and mate tea (with roasted mate leaves). Research has proven its pharmacological effects as chemopreventive activity, cholerectic effect and intestinal propulsion, vasodilatation effects, inhibition of glycation and as a free radical scavenger, associated with its bioactive compounds present in the leaves and aqueous infusions like phenolic compounds, caffeine and saponins [6]. Despite approximately 550 to 660 Ilex species are known, only three is reported to be edible: *Ilex angustifolia* (yerba mate periquita), *Ilex amara* (yerba mate crioula), and *Ilex paraguariensis* [7].

In order to evaluate the antioxidant potential of folk beverages that come from plant sources, this study compares test results of commercial coffee and yerba mate samples

II. Methodology

1. Preparation of extracts

Extracts from samples acquired in local Brazilian market of soluble coffee, roasted coffee, yerba maté (tereré) and yerba maté (chimarrão) were prepared. All samples were prepared in triplicate. Soluble coffee samples were prepared by dissolving 50 g in 500 mL of water at 80 °C. Roasted coffee samples were prepared by leaching 300 mL of water in a paper filter containing 50 g of the roasted coffee powder. Tereré samples were prepared by maceration, leaving 50 g of yerba maté in 300 mL of water at 17 °C for two hours and later filtering; while chimarrão samples were prepared by mixing 50 g of yerba maté in 50 mL of water at 25 °C, then adding 250 mL of water at 80 °C, leaving for one hour and later filtering. Then, all the aqueous extracts were fractionated three times with 50 mL of ethyl acetate. All ethyl acetate fractions were dried at 35 °C in oven.

2. Qualitative phytochemical screening

Phytochemical screening was carried with the colorimetric methodology purposed by Matos [8], which were detailed in the following sections. For each assay, 5-10 mg of each dried extract was dissolved in approximately 3.0 mL of distilled water in a test tube, obtaining test solution for each extract.

2.1 Phenols and tannins

To test the presence of phenols and tannins, three drops of an alcoholic 5% ferric chloride ($FeCl_3$) solution was added to the test solution. Color change of the solution to blue or red indicates the presence of phenolic compounds. A darker blue precipitate indicates the presence of pyrogallic tannins, while a green precipitate indicates the presence of condensed tannins.

2.2 Anthocyanins, anthocyanidins and flavonoids

For each extract, the test proceeded in three test tubes containing some milligrams extract dissolved in 1 mL ethanol, one was acidulated to a pH 3.0 with an HCl solution, while the others were alkalinized to a pH 8.5 and 11.0 with a NaOH solution. Color changes were observed as shown in Table 1.

Table 1. Qualitative assay for anthocyanins, anthocyanidins and flavonoids.

Constituents	Color		
	Acid (3.0)	Alkaline (8.5)	Alkaline (11.0)
Anthocyanins and anthocyanidins	Red	Lilac	Purplish blue
Flavones, flavonols and xanthones	-	-	Yellow
Chalcones and aurones	Red	-	Purplish red
Flavononols	-	-	Orange red

2.3 Leukoanthocyanidins, catechins and flavanones

A test tube was acidulated by concentrated HCl until it reaches pH 1.0-3.0; another test tube was alkalinized with sodium hydroxide solution (NaOH, 1 mol/L) until it reaches pH 11.0. Both were heated in a water bath for two minutes. Change or intensification of color indicates the groups of compounds, as shown in Table 2.

Table 2. Assay for Leukoanthocyanidins, catechins and flavanones.

Constituents	Color	
	Acid (1.0 a 3.0)	Alkaline (11.0)
Leukoanthocyanidins	Red	-
Catechins	Brown-yellow	-
Flavanones	-	Orange red

2.4 Confirmation of Catechins

A little piece of wood was wetted with the extract, evaporating the solvent. After that, the wood was wetted with concentrated HCl, it was heated for two minutes in a Bunsen burner. The presence of red or reddish brown color in acidulated side of the wood confirms catechins.

3. Quantitative analysis of antioxidant potential by using DPPH

2,2-diphenyl-1-picrylhydrazyl (DPPH) is a free radical used to assess the free radical scavenging property of natural products. It is characterized as a stable free radical by the delocalization of the unpaired electron over the molecule as a whole, so the molecule do not dimerize, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color. When a DPPH solution is mixed with a compound that can donate a hydrogen atom, then this gives rise to the reduced form, diphenyl-picriyl-hydrazine, with loss of the violet color. Furthermore, this assay has the advantage of independence of the substrate polarity [9, 10, 11, 12]. To quantify the activity by using DPPH radical (2,2-diphenyl-1-picrylhydrazyl) methodology was based on the description of [9] with the following adaptations.

3.1. Sample dilution and the positive control

0.05 g of dried extract of each sample was diluted in 25 ml of ethanol, obtaining a concentration of 2000 $\mu g.mL^{-1}$ as stock solution. From this stock solution were obtained the concentrations of 500.0; 250.0; 200.0; 150.0; 100.0; 50.0; 25.0; e 10.0 $\mu g.mL^{-1.}$ Same procedure was used to Rutin (positive control).

3.2. Preparation of DPPH solution

A stock solution of DPPH was prepared diluting 0.0125 g of DPPH in 50 ml of ethanol. A final solution of 250 $\mu g.mL^{-1}$ was obtained subsequently prepared for testing and construction of the DPPH calibration curve of DPPH. The solution that was used in the reaction with samples and control had the concentration of 40 $\mu g.mL^{-1}$.

3.3. Antioxidant potential assay

0.3 mL of each sample concentration and positive control was added to 2.7 mL of DPPH solution at 40 $\mu g.mL^{-1}$. Reaction occurred inside tubes covered with aluminum paper, in a dark environment at 25 °C. The decrease of absorbance was read after 30 min of incubation in a PerkinElmer UV-Vis spectrophotomer at 517 nm. The assay for each sample was proceeded in triplicate. As blank, solutions containing 2.7 mL of ethanol and 0.3 mL of extracts dilutions or positive control at same concentrations were used, corresponding to each reaction mixture.

3.4. Calculation the antioxidant percentage

The antioxidant potential percentage of each concentration is determined by equation 1, where Abs control is the initial absorbance of DPPH, and Abs Sample is the absorbance of the reaction mixture (DPPH + sample) as shown in Equation 1.

$$\%DPPH = (Abs\ Control - Abs\ Sample)/Abs\ Control \times 100 \quad \text{(Equation 1)}$$

4. FT-IR analysis

FT-IR analysis was conducted using PerkinElmer Model Spectrum 100 equipment equipped with an Attenuated Reflectance accessory.

III. RESULTS AND DISCUSSION

1. Phytochemical screening

Table 3 shows the results of the phytochemical screening. The main constituents of all samples were catechins, flavonoids, tannins and xanthones. Thus, the phytochemical screening profile was similar for all samples. Moreover, except the third sample of Tereré (3), all samples have the expected phenolic compounds according to literature. The third sample from Yerba Mate Tereré (3) has a different result on its constituents, having as main

constituents leukoanthocyanidins and catechins. A different profile for this sample was expected, since this sample is a bitterest herb named Ilex amara [7]. According to the literature, *Coffea arabica* L. or *Coffea canephora* Pierre ex A. Froehner, have in the green seeds carbohydrates, lipids, proteins, unsaponifiable lipids, sterols, hydrocarbons, tocopherols, diterpenic alcohols, and phenolic acids. Caffeine levels are variable, depending on roasting processes. After roasting, the chemical composition has some alterations: the polysaccharides are degraded, and has a complex mixture of alcohols, aldehydes, phenols, furanic and pyrrolic derivate compounds, thiophenes, and others [13, 14]. Condensed tannins are the main compounds in coffee pulp, while in coffee seed are present as main constituents the chlorogenic acids. In coffee seed, other phenolic compounds such as tannins, lignans and anthocyanins are also present in minor amounts [15].

Ilex paraguariensis is documented as having in its chemical composition vitamins A, B complex, C and E, amino acids, tannins triterpenic saponins, other phenolic compounds as catechins, chlorogenic acid and its oxidation products and methylxanthines [16, 17] Previous work [18] showed that *Ilex paraguariensis* has a higher content of caffeoyl derivatives and flavonoids comparing with other Ilex spp. In the other hand, *Ilex amara* is a Brazilian plant species that is also popularly known as mate, because its leaves has a similar morphology and aroma to that in *Ilex paraguariensis*. That specie has triterpene saponins and flavonoids as major constituents [19].

Table 3. Phytochemical Screening results

Sample	Constituents	Samples	Constituents
Roasted Coffee (1)	Flavononols, catechins, flavanones.	Tereré (1)	Condensed tannins, flavones, flavonols, xanthones.
Roasted Coffee (2)	Flavononols, catechins, flavanones.	Tereré (2)	Condensed tannins, flavones, flavonols, xanthones, catechins.
Roasted Coffee (3)	Condensed tannins, flavononols, catechins, flavanones.	Tereré (3)	Leukoanthocyanidins, catechins.
Soluble Coffee (1)	Flavononols, catechins, flavanones.	Chimarrão (1)	Condensed tannins, catechins.
Soluble Coffee (2)	Condensed tannins, flavononols, flavanones, catechins.	Chimarrão (2)	Flavones, flavonols, xanthones.
Soluble Coffee (3)	Condensed tannins, flavononols, flavanones, catechins.	Chimarrão (3)	Condensed tannins, flavones, flavonols, xanthones, catechins.

2. FT-IR analysis

All of the extracts showed similar spectra (Fig. 1 and 2), showing large bands near 3400-3300 cm-1 from –OH; absorptions approximately in 1750-1800 cm^{-1} from C=O, corresponding to esters and chlrogenic acids. All spectra showed bands at 1500-1600 cm^{-1}, indicating the presence of phenolic compounds [20, 21, 22, 23]. FT-IR results corroborate the results from phytochemical screening.

Fig. 1. Roasted and Soluble Coffee Spectra

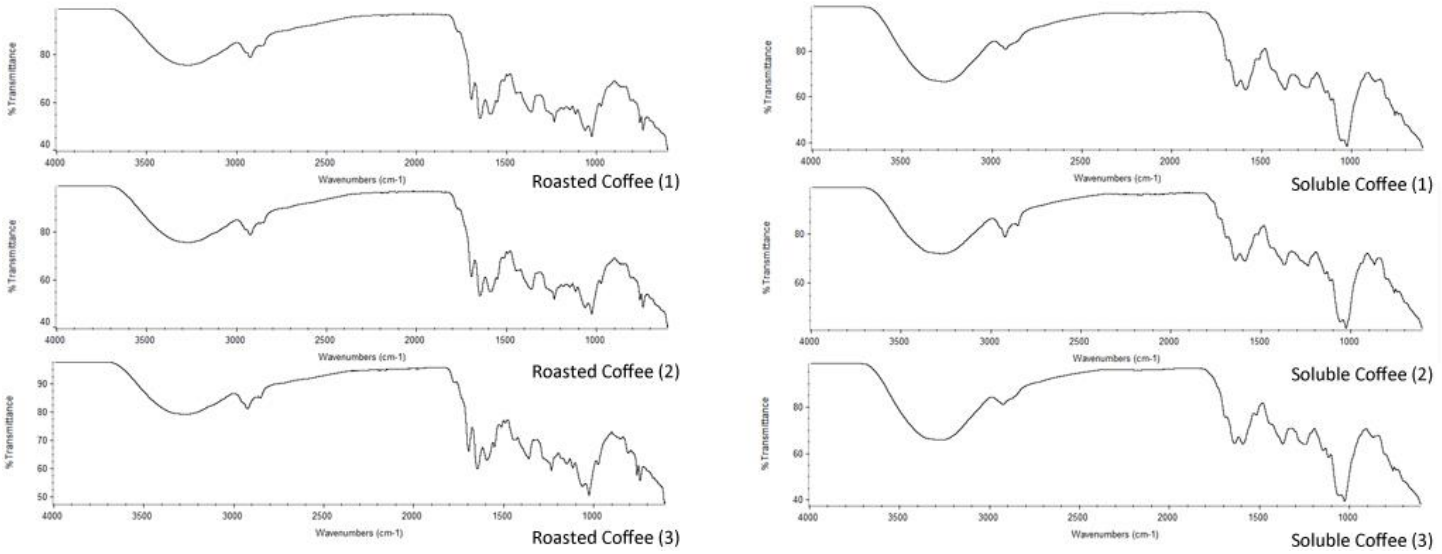
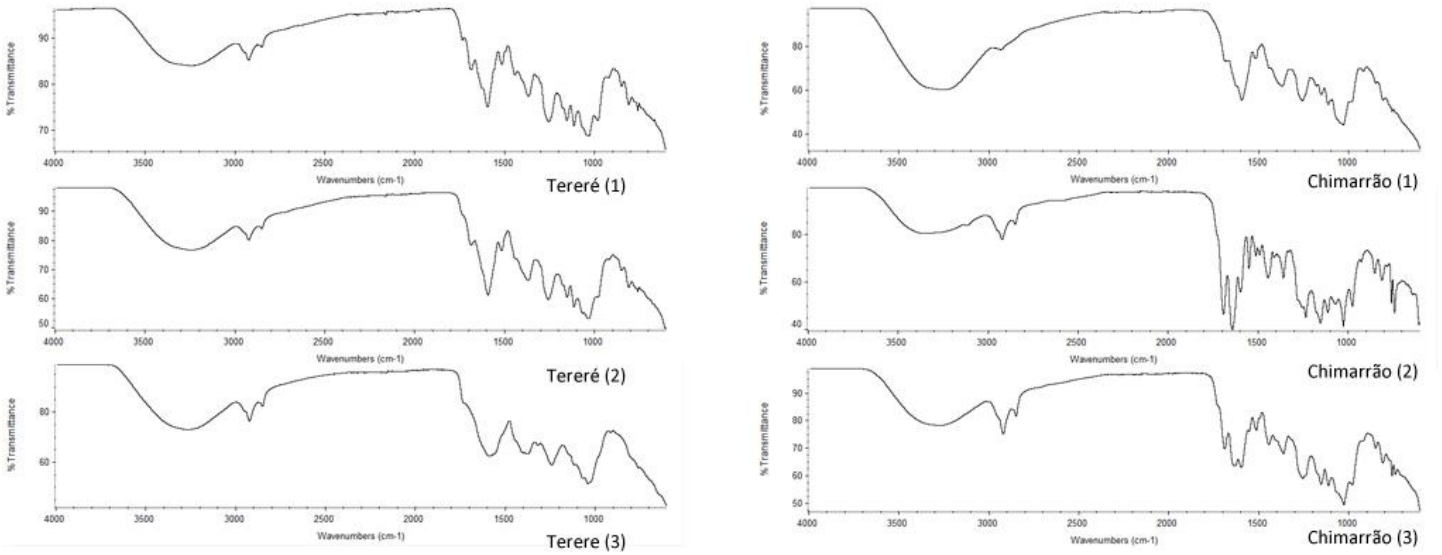


Fig. 2. Yerba-maté Spectra



3. Antioxidant Potential Assay by using DPPH

According to Table 4, in comparison to Rutin, the extracts with greater antioxidant potential in vitro were (2) and (3) of roasted coffee, (2) of soluble coffee, (2) of tereré, and (2) and (3) of chimarrão. In this study, The two samples (Soluble Coffee (2) and Terere (2)) that showed highest antioxidant potential showed in the phytochemical screening the presence of catechins and condensed tannins, which are among the class of phenolic compounds and since the antioxidant potential of such extracts depends on the class and concentration of phenolic compounds, which are present in such extracts.

Table 4. Antioxidant Potential Percentage

Antioxidant Potential (%)								
Sample	Concentration (μg.mL^{-1})							
	500	250	200	150	100	50	25	10
Roasted Coffee (1)	30.3 ± 0.004	21.2 ± 0.006	18.2 ± 0.006	18.2 ± 0.006	15.15 ± 0.006	12.1 ± 0.006	9.1 ± 0.004	2.1 ± 0.004
Roasted Coffee (2)	48.5 ± 0	33.33 ± 0	30.3 ± 0.001	24.25 ± 0	24.25 ± 0.001	18.2 ± 0.003	18.2 ± 0.004	18.2 ± 0
Roasted Coffee (3)	51.5 ± 0	33.3 ± 0	30.3 ± 0.001	27.3 ± 0	24.24 ± 0	21.2 ± 0	18.2 ± 0	15.15 ± 0
Soluble Coffee (1)	36.35 ± 0.008	27.3 ± 0.007	24.25 ± 0.006	21.2 ± 0.004	15.15 ± 0.005	9.1 ± 0.004	3 ± 0.003	3 ± 0.002
Soluble Coffee (2)	63.6 ± 0	60.6 ± 0	57.6 ± 0.001	51.5 ± 0	42.4 ± 0.001	30.3 ± 0.003	27.3 ± 0.004	24.25 ± 0
Soluble Coffee (3)	30.3 ± 0	12.1 ± 0.001	12.1 ± 0.002	12.1 ± 0.002	9.1 ± 0.001	0 ± 0.001	0 ± 0.002	0 ± 0.001
Tereré (1)	33.33 ± 0	18.2 ± 0	15.15 ± 0	12.1 ± 0	9.1 ± 0	6.05 ± 0.002	6.05 ± 0.001	3 ± 0
Tereré (2)	63.6 ± 0	57.6 ± 0.002	51.5 ± 0.001	48.5 ± 0.002	39.4 ± 0.003	30.3 ± 0.002	27.3 ± 0.002	24.25 ± 0.002
Tereré (3)	18.2 ± 0.006	12.1 ± 0.002	12.1 ± 0.001	12.1 ± 0.001	9.1 ± 0	6.05 ± 0	6.05 ± 0	6.05 ± 0
Chimarrão (1)	15.15 ± 0.002	9.1 ± 0	6.06 ± 0.001	6.06 ± 0.001	3.03 ± 0.001	3.03 ± 0	3.033 ± 0	0 ± 0
Chimarrão (2)	60.6 ± 0	51.5 ± 0.002	45.45 ± 0.002	42.4 ± 0.002	30.3 ± 0.003	24.25 ± 0.001	21.2 ± 0.001	15.15 ± 0
Chimarrão (3)	51.5 ± 0.002	42.4 ± 0.002	36.35 ± 0	33.3 ± 0	33.3 ± 0	33.3 ± 0	30.3 ± 0	30.3 ± 0
Positive Control (Rutin)	63.6 ± 0	63.6 ± 0	63.6 ± 0	63.6 ± 0	60.6 ± 0	42.4 ± 0.002	27.3 ± 0.001	27.3 ± 0

In addition to data shown in Tables 3 and 4, the literature about phenolic compounds may explain the results. According to [24], flavonols have the highest antioxidant in some *Ilex spp* [25]. Previous work [26], evaluated the participation of caffeine, chlorogenic acid, caffeic acid and rutin in the antioxidant activity of mate and noticed that caffeine did not present any scavenging activity on DPPH, meanwhile chlorogenic acid showed antioxidant activity, followed by caffeic acid and rutin. The antioxidant activity of those compounds is also reported by other authors [27, 28, 29]. Other soluble coffee products has been studied and all of them possessed antioxidant potential conferred by balanced concentrations of phenolic compounds, caffeine and melanoidins, and the antioxidant activity was unaffected by roasting conditions, since the degradation of 5-caffeoylquinic acid was balanced by formation of melanoidins [30]. Furthermore, the content of 5-caffeoylquinic acid in the chimarrão beverage was higher than the content in tereré and maté tea [31].

In this study, the two samples that showed highest antioxidant potential, which showed in the phytochemical screening the presence of catechins and condensed tannins. The dietary flavanols catechin and epicatechin commonly occur in combination with gallic acid as epigallocatechin gallate or epicatechin gallate, found in condensed tannin polimers [32]. It was suggested that tannins or polymeric polyphenolics may be much more potent antioxidants than are simple monomeric phenolics [33]. Phenolic compounds can be obtained from vegetal sources by an ethanolic extract from the dried or fresh vegetal [34].

The extraction method can also have influence on the antioxidant potential exhibited by the obtained extracts. Since polar solvents are frequently employed for recovering phenolic compounds from a plant matrix, the choice of the most suitable of these solvents, which include aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate in different proportions can influence on the availability of antioxidant compounds. Thus, differences in extract yields are found [35]. This can influence the content antioxidant compounds and antioxidant activity, considering that the extraction in these beverages only uses water as solvent [36, 4, 37].

IV. CONCLUSION

Some of the analyzed samples had a comparable antioxidant activity to the positive control (rutin), which can support the consumption of these beverages as antioxidant sources. As the studied beverages are very popular, people can have a good source of antioxidant compounds through the consumption of such beverages. Morevover, the results of the present study can support further phytochemical investigation in order to determine the compounds responsible for the antioxidant properties of such beverages.

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AUTHORS

First Author – Rayzah Teodoro, Bachelor degree in Pharmaceutical Science, Instituto de Ciencias Exatas e da Terra, Campus Universitario do Araguaia, Universidade Federal de Mato Grosso. Barra do Garcas – MT, rayzahteodoro@gmail.com.
Second Author – Jair Marques Junior, Bachelor degree in Pharmaceutical Science, Departamento de Policia Cientifica Civil (POLITEC), jairmatquesjunior@yahoo.com.br.
Third Author – Diego Vinicius do Nascimento, MSc. In Materials Science, B.Sc. in Chemistry, Instituto de Ciencias Exatas e da Terra, Campus Universitario do Araguaia, Universidade Federal de Mato Grosso. Barra do Garcas – MT, diegovinicius@ufmt.br.
Fourty Author – Ricardo Stefani, Associate Professor, of organic chemistry has a PhD in Organic chemistry, Instituto de

Ciencias Exatas e da Terra, Campus Universitario do Araguaia, Universidade Federal de Mato Grosso. Barra do Garcas – MT, rstefani@ufmt.br.

Correspondence Author – Ricardo Stefani, rstefani@ufmt.br, rstefani.ufmt@gmail.com, +55668129-0449.