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Phenolic content, antibacterial and antioxidant activities of herbal infusions

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Abstract

Natural products derived from plants are considered the oldest medicine in the world. Teas are an accessible source of antioxidant compounds, especially polyphenols that impart flavor, aroma and pharmacological properties. Considering the context of using natural products for health maintenance, the present study aimed to analyze the content of total phenolic compounds, antibacterial and antioxidant activities of infusions using *Morus nigra*, *Casearia sylvestris*, *Hibiscus sabdariff* and *Moringa oleifera* commercialized in Muzambinho/MG. The infusions were prepared by adding 100 mL of distilled water at approximately 100ºC over 1 g of herb at room temperature for 10 minutes. The analysis of total phenolic compounds was performed using Folin-Ciocalteau. For the analysis of antioxidant activity, the free radical DPPH was used and the results were expressed as a percentage of scavenging activity. For the analysis of antibacterial activity, the microdilution in broth assay was performed in order to find the minimum inhibitory concentrations. It was possible to conclude that the infusion with the highest levels of phenolic compounds were *C. sylvestris* and *M. oleifera*, while those that showed a greater antioxidant activity were *M. oleifera* and *M. nigra*. As for antibacterial activity, all infusions were able to inhibit *E. coli*, especially the infusion of *C. sylvestris*, which was also able to inhibit *S. aureus*. Although all of the herbs showed antioxidant and antibacterial activities, and also phenolic compounds, *C. sylvestis* was the herb that stood out in this work because of its phenolic composition and antibacterial activity. In sum, it is concluded that future work is needed to elucidate the exact chemical composition of the infusions and other biological activities.

Keywords: Teas. *C. sylvestris. M. nigra. M. oleifera. H. sabdariffa*.

Introduction

Plant-derived natural products are considered the oldest medications in the world. They were traditionally used to treat different disorders, including cancer and cardiovascular disease (TALIB *et al*., 2020). It was first introduced in China as a traditional medicine and spread to the world because of its therapeutic properties. Today, tea is one of the most widely consumed beverages in the world. (MALONGANE; MCGAW; MUDAU, 2017; POSWAL *et al*., 2019).

Clinical evidence indicates that individuals need to prioritize plant materials like fruits, vegetables, grains, nuts and oils and lower the intake of red meat and processed sugary drinks to achieve a healthy diet (SCHULZE *et al*., 2018). In order to protect the health of the population, the World Health Organization encourages countries to include the use of traditional medicine, including increased the consumption of herbs, in its 2014-2023 strategy. Because herbs are effective and affordable alternatives to medicine, and is a healthcare choice coherent with people's cultural practice, it is largely used in countries from the Africa and Asia continents (WHO, 2013).

Tea is a source of antioxidant compounds with traces of proteins, carbohydrates, amino acids, lipids, vitamins and minerals. It also contains polyphenols which account for the aroma and beneficial health effects of tea (KHAN; MUKHTAR, 2019). Polyphenols are known to interfere with several physiological processes, assisting in the absorption and action of vitamins, acting in the healing process, as antioxidants, and having antimicrobial activity (RAJARAM; JONES; LEE, 2019).

Aerobic organisms produce constantly reactive species and free radicals during cellular process. Naturally, cells have mechanisms to balance the production of oxidative compounds and antioxidant compounds. However, when the balance between the production of free radicals and reactive species and the antioxidant defenses of the cell is unstable, an oxidative process occurs on the cell. The oxidative process is responsible for causing damage in cells and vital biomolecules, thus associated with induction of chronic inflammation and the development of many chronic diseases, including cancer, diabetes, cardiovascular, neurological and pulmonary diseases (HALLIEWLL, 2012; REUTER *et al*., 2010).

Tea consumption has been increasing steadily in Brazil, becoming an almost obligatory and year-round item in the diet of Brazilian consumers (GODOY *et al*., 2013). However, due to the concerns about the risks posed by COVID-19, in 2020 the growth in retails volume sales was significantly faster than in any other year. The most popular tea varieties include fruit/herbal teas (EUROMONITOR, 2021). In this context, *Moringa oleifera*, *Casearia sylvestris*, *Hibiscus sabdariffa* and *Morus nigra* are some of the common herbs used for herbal infusion in Brazil.

Moringa oleifera Lam. is a tree that belongs to the monogenic family Moringaceae and is known as horseradish tree, which grows widely in many tropical and subtropical countries. In some areas, immature seed pods are eaten, while the leaves are used as basic food because of their high nutrition content. *M. oleifera*. leaves are characterized to contain a desirable nutritional balance, containing vitamins, minerals, amino acids and fatty acids. In addition, it contains a range type of antioxidant compounds such as ascorbic acid, flavonoids, polyphenols, and carotenoids. Because of its chemical composition, *M. oleifera* is used for their anti-inflammatory, antihypertensive, diuretic, antimicrobial, antioxidant, antidiabetic, antihyperlipidemic, antineoplastic, antipyretic, antiulcer, cardioprotectant, and hepatoprotectans activities (DHAKAD *et al*., 2019; STOHS; HARTMAN, 2015).

Casearia sylvestris Swartz., which is popularly known as “guaçatonga”, belongs to Salicaceae family and is found mainly in South America region, including Brazil. This plant has been widely used to treat different diseases in traditional medicine. Some communities use *C. syvestris* for the treatment of snake bites, in wound healing and as a topical antiseptic. Pharmacological studies with ethanolic or hydroalcoholic extracts of its leaves have demonstrates antiulcerogenic, anti-inflammatory, antivenom and cytotoxic activities. Furthermore, no significant toxicological effects have been observed *in vitro* or *in vivo* (in animals). The chemical composition of *C. sylvestris*’ essential oils comprehend monoterpenes and sesquiterpenes, triterpenes, lapachol, cafeic, chlorogenic and vanillic acids, flavonoids, neolignans, ellagic and gallic acids derivatives (CLAUDINO *et al*., 2013; PEREIRA *et al*., 2017; WANG *et al*., 2010).

Hibiscus sabdariffa L. widely grows in tropical and subtropical regions around the world. This plant belongs to the family Malvaceae and is an annual or perennial herb (RIAZ; CHOPRA, 2018). Various parts of hibiscus plants have been used in traditional medicine to treat colds, urinary infections, toothaches, fever, liver disease, hypercholesterolemia, hypertension and microbial infections. In some countries such as India, it is used for curing diseases and as an ethnic food. Mexicans traditionally use the infusion of the calyces and leaves for curing hypertension and other diseases (ROCHA *et al*., 2014). The extract of the calyces and flower is also beneficial to cure diarrhea, dysentery, waist pain and gynecological disorders (especially in post-delivery cases) (SINGH; SUREJA; SINGH, 2006). The main chemical composition in the context of its pharmacological use is related to organic acids, anthocyanin, polysaccharides and flavonoids. It also contains dietary fiber, other proteins, vitamins and minerals (AURELIO; EDGARDO; NAVARRO-GALINDO, 2008; ROCHA *et al*., 2014).

Morus nigra L., also called blackberry, is a flowering plant belonging to the Moraceae family. It is native to Sothwestern Asia and has been grown throughout Europe and the Mediterranean regions (HUSSAIN *et al*., 2017). Its phytochemical composition includes flavonoids, tannins, coumarins, polyphenols, triterpenes and steroid substances, which contributes to its antioxidant, antimicrobial, hepatoprotective, hypotensive, antipyretic, analgesic, diuretic, anti-diabetic and progesteronic/estrogenic effects (ERCISLI; ORHAN, 2007; RODRIGUES *et al*., 2019; SOUZA *et al*., 2018).

Considering the appeal for natural compounds as alternatives for maintaining good health, the objective of this work is to elucidate the total phenolic composition, antioxidant and antibacterial activities of herbal infusions (*M. nigra*, *C. sylvestris*, *H. sabdariffa* and *M. oleifera*) commercialized in Muzambinho – MG.

Material and methods

Sample Collection and Preparation

All herbs used in this work were acquired in a natural products store in the city of Muzambinho – MG. Four different species of herbs were used: blackberry (*M. nigra*), guaçatonga (*C. sylvestris*), hibiscus (*H. sabdariffa*), and moringa (*M. oleifera*). The herbs were subjected to extraction in order to simulate a homemade extraction.

Infusions were prepared by the addition of 100 mL of distilled water (approximately ½ tea-cup) at 100ºC in 1 g of the herb for 10 minutes, following the procedure describe by Souza *et al*. (2011) as the best way to extract the phenolic compounds of the herbs.

The extracts were then filtered and the infusions stored in glass flasks at 5 ºC.

Total Phenolic Compounds

The total phenolic components were determined by using the Folin-Ciocalteau 1:10 reagent solution. The Folin-Ciocalteau was diluted in a 2.0 mL of 4% (w/v) Na_2Co_3 solution and mixed with 2.5 mL of the herbal infusion. Following two hours protected from light, at room temperature, absorbance was measured spectrophotometrically at 740 nm. The results were obtained by gallic acid standard and expressed as mg GAE g^{-1}. (SINGLETON; OTHOFER; LAMUELA-RAVENTOS, 1998).

Antioxidant activity by scavenging free radicals DPPH$^{\bullet}$

The blend was formed by adding 2.0 mL of herbal infusion and 0.5 mL DPPH at 0.5 mMol. Absorbance was measured at 517 nm following 45 minutes incubation protected from light, at room temperature.

This test was performed as percentage of antioxidant activity (AA), calculated through the rate of decline in the absorbance of the DPPH solution after 45 minutes of reaction (stable phase) regarding to the reference solution (DPPH in ethanol) calculated by the formula:

% Antioxidant activity = 100 - [(Sample - White) * 100/Control] at which: Sample = absorbance of the DPPH solution (samples); White = absorbance of the sample solution without adding DPPH; Control = absorbance of the DPPH reference solution (ethanol).

The results were expressed percentage of DPPH scavenging activity (BRAND-WILLIAMS; CUVELIER; BERSET, 1995).

Antibacterial activity

According to the protocol M07-A10 developed by the Clinical Laboratories Standardization Institute (CLSI, 2015), with modifications, the antibacterial activity was performed by determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). For the MIC and MBC analyzes, the microorganisms *Staphylococcus aureus* ATCC 0538 and *Escherichia coli* ATCC 8739 were grown in liquid Brain Heart Infusion (BHI). After incubation, they were adjusted to 1-2 × 10^8 CFU mL^{-1} in a 0.9 % NaCl solution by using the 0.5 Macfarland scale. Then, 52 uL of the bacterial suspensions was inoculated in 52 mL of the liquid BHI medium, in order to obtain a bacterial concentration around 1-2 × 10^5 CFU mL^{-1}. The technique was developed in 96-well microplates, which 180 μL of BHI broth previously achieved were added. Then, 10 μL of the herbal infusion was added in concentrations ranging from 1000 μg mL^{-1} to 7.8 μg mL^{-1} (serial concentration of ratio 2). For the color control, 180 μL of sterile BHI broth and 10 μL of the herbal infusions were added in the same concentrations already mentioned. The microplates were incubated at 37 ºC for 24 h. After incubation, 20 μL of the Resazurin dye (0.01% w/v) was added to verify bacterial growth. In wells where there was no change in the color of the dye compared to the control, the absence of viable bacteria was considered. Any evidence of color change was considered bacterial growth. For the determination of MBC, 10 μL aliquots of the culture medium from the wells considered inhibitory were placed in BHI agar and the plates were incubated at 37 ºC for 24h.

Statistical analysis

The experimental design was a completely randomized design with three replications. Each analysis was composed of three replications of each infusion. The statistical evaluation of the results was analyzed using the SISVAR 5.6 software by analysis of variance (ANAVA) and the Scott and Knott (1974) test was applied to observe the significant differences between the mean values (p value < 0.05) (FERREIRA, 2014).

Results and discussion

As shown in Table 1, the infusion with the higher phenolic compound content was *C. sylvestris* (18.27 mg GAE g^{-1}), followed by *M. oleifera* (15.70 mg GAE g^{-1}), *M. nigra* (11.34 mg GAE g^{-1}) and lastly *H. sabdariffa* (6.53 mg GAE g^{-1}).

Table 1. Content of phenolic compounds (mg GAE g^{-1} of sample) and antioxidant potential (% of DPPH scavenging) of the infusions. IFSULDEMINAS - Campus Muzambinho, Muzambinho / MG, 2018.

Samples	Phenolic content	Antioxidant Activity
Morus nigra	11.34 ± 1.05^c	50.04 ± 2.96^b
Casearia sylvestris	18.27 ± 0.25^a	47.71 ± 2.86^c
Hibiscus sabdariffa	6.35 ± 0.15^d	43.14 ± 2.55^d
Moringa oleifera	15.70 ± 0.18^b	57.34 ± 1.32^a

*Average followed by the same letters on the same column do not differ in the Scott Knott test (p<0.05).

Source: Elaborated by the authors (2018).

Bueno *et al*. (2015) analyzed the chemical composition of hydro-alcoholic extracts from *C. sylvestris* using UHPLC-DAD apparatus and found diterpenes and phenolic compounds as its main composition. Fernandes (2013) found phenolic compound content in *C. sylveris* (354.33 mg GAE g^{-1}) higher than those found in this work

(18.24 mg GAE g^{-1}), which is related to the fact that the author used ethanol as the extracting solvent.

When compared to the present work, *M. oleifera* leaves extracts showed a higher phenolic content in the works realized by Teles (2016) and by Nascimento *et al*. (2013), being 33.7 mg GAE g^{-1} and 53.69 mg GAE g^{-1}, respectively. These differences may be related to the extraction method since Teles (2016) and Nascimento *et al*. (2013) used hydro-alcoholic extracts and the present work used water infusion. The ethanol is used in place of water because it can extract polar substances and some organic apolar substance better than water.

Zeni *et al*. (2017) found an amount of total phenolic of 75.86 mg GAE g^{-1} on the infusion with *M. nigra* leaves, which was higher than the amount found in this work. The difference may be related to the extraction method as the authors used dried and crushed leaves in this work, which may interfere in the final phenolic content of the infusions.

Ramos *et al*. (2011) working with *H. sabdariffa* found an amount of total phenolic compounds of 6.72 mg GAE g^{-1}, which corroborates to the results of the present work (6.35 mg GAE g^{-1}).

Regarding the antioxidant potential (TABLE 1), it was observed that the antioxidant activity of *M. oleifera* was the highest one (57.34%), followed by *M. nigra* (50.04%), *C. sylvestris* (47.71%) and *H. sabdariffa* (43.14%).

M. oleifera showed the highest antioxidant activity but not the highest total phenolic compounds. This result suggests that the antioxidant activity of this herb may be related to other phytochemical compounds in this herb.

Zanco, Souza and Bonacorsi (2017) analyzed different concentrations of teas obtained by infusion and decoction of *M. nigra*, reporting values higher than that found in the present work, ranging between 74.5% to 96.4%. The authors used dried and crushed leaves, increasing the contact surface of the herbs with the water, which may have contributed to the greater amount of antioxidant compounds extracted by them. In the present work, it was used only dried leaves but not crushed. Also, the author used 10 g of herb in 100 mL of water instead of the 1 g of herb in 100 mL of water used in the present work.

A work from Ramos *et al*. (2011) found that the ethanolic extract of *H. sabdariffa* had a higher response than that obtained with the aqueous extract related to the percentage of free radical scavenging. The authors visualized that the ethanolic extracts showed about 66% of antioxidant activity while water extract showed about 40% of antioxidant activity. Those results found by the authors with aqueous extracts corroborates the results of the present work and suggests that ethanolic extracts may have higher antioxidants activities.

According to Melo *et al*. (2008), extracts with percentage of free radical scavenging above 70%, between 50% and 70%, and below 50% are classified as strong, moderate and weak, respectively. Thus, *M. oleifera* had a strong antioxidant activity, followed by *M. nigra* which showed moderate activity, and *C. sylvestris* and *H. sabdariffa* with weak activity.

As seen in Table 2, all of the tested herbs showed inhibitory activity against *E. coli* and most of them showed no activity against *S. aureus*. Also, none of the herbs presented bactericidal effect against both of the bacterial strains tested. Those results observed may be related to the differences between the chemical composition of each herb and the cellular structure of the bacteria analyzed.

Regarding the infusion of *M. nigra*, the present work found no inhibitory activity against *S. aureus* in the tested concentrations of the infusion, but we found MIC value between 500-1000 μg mL^{-1} against *E. coli*. According to Souza *et al*. (2018), the ethanolic extract of this herb needed a minimum concentration of 195 μg mL^{-1} to inhibit *E. coli*, which is lower than the values of the present work. This difference may have happened because many of the bioactive compounds of *M. nigra* are insoluble in water and are soluble in ethanol.

Table 2. Antibacterial activity (MIC) of the samples against *E. coli* and *S. aureus*. The results are expressed as μg mL^{-1} of the infusions. IFSULDEMINAS - Campus Muzambinho, Muzambinho / MG, 2018.

Samples	*S. aureus*	*E. coli*
Morus nigra	N.D.*	500-1000
Casearia sylvestris	500-1000	15,62-31,25
Hibiscus sabdariffa	N.D.*	500-1000
Moringa oleifera	N.D.*	15,62-31,25

*N.D.: not detected.

Source: Elaborated by the authors (2018).

The *C. sylvestris* infusion showed a good inhibitory activity against *E. coli* (15.62-31.25 μg mL^{-1}) and this was the only herb used in this work to inhibit *S. aureus* (1000-500 μg mL^{-1}). Spósito *et al*. (2019), working with ethanolic extracts of *C. sylvestris* observed MIC value of 1000 μg mL^{-1} against *Helicobacter pylori*, gram-negative bacteria that colonizes the human stomach. Also, Ribeiro *et al*. (2019), found antibacterial activity of *C. sylvestris* from the Atlantic Forest against *Streptococcus mutans*, a gram-positive bacteria, reducing 50% of the viable cells of this bacterial strain. Those results suggest that this herb may be efficient in the inhibition of gram-positive and gram-negative strains of bacteria, as seen in the present work.

In the present work, the infusion with *H. sabdariffa* showed no inhibitory effect against *S. aureus*, but showed a MIC value of 500-1000 μg mL^{-1} against *E. coli*. Ibrahim and Albadani (2014) tested a water and a methanolic extract of the same herb and observed no inhibitory activity of both extracts against *S. aureus* and *E. coli*, which corroborates partially our results.

Fouad, Elnaga and Kandil (2019) used different extracts of *M. oleifera* against bacteria isolated from a camel wound and the extract obtained with hot water was not efficient against *S. aureus* and *E. coli*, which corroborates the present results against *S. aureus*. Amanze *et al*. (2020) found MIC values of aqueous extracts from root bark of *M. oleifera* against *S. aureus* (25000 μg mL^{-1}) and *E. coli* (12500 μg mL^{-1}). Those values were higher than the values tested in the present work, and this might explain why we were unable to find any MIC value against *S. aureus*. Also, the authors cited above tested a different part of the plant. Finally, *S. aureus* and *E. coli* were resistant to the *M. oleifera* water extract in the work of Bagheri *et al*. (2020). In sum, this result against *E. coli* can be considered really promising when compared to other works.

Finally, the biological activities of those teas are related to their chemical composition, especially the polyphenols. Those compounds account for the aroma and beneficial health effects of teas (KHAN; MUKHTAR, 2019). Also, the difference observed with the herbs used in this work and other works found in the literature might be explained by the different methodology adopted to the extraction of the biological compounds, place where the plants were cultivated, and the climate also influences those chemical composition and biological activities. According to Zanco, Souza and Bonacorsi (2017), environmental and chronological conditions of the plant also influence the production of secondary metabolic compounds that even vary throughout the year. Additional works are needed to elucidate and isolate the compounds of those herbs in order to understand all the benefits of the consumption of those infusions.

Conclusion

Based on the present work, the herbs *C. sylvestris* and *M. oleifera* showed the highest phenolic contents. However, *C. sylvestris* did not show one of the highest antioxidant activities, being *M. nigra* and *M. oleifera* the herbs with higher antioxidant activity. *H. sabdariffa* showed the lowest phenolic content and antioxidant activity.

Regarding to the antibacterial activity, all the herbs showed inhibitory activity against *E. coli*, but only *C. sylvestris* showed inhibitory activity against *S. aureus*.

Although all of the herbs showed antioxidant and antibacterial activities, and also phenolic compounds, *C. sylvestis* was the herb that stood out in this work because of its phenolic composition and antibacterial activity. However, further studies are needed to investigate its phytochemical composition.

In sum, the infusions can be an affordable source of phenolic compounds, antioxidant and antibacterial activity, but it is necessary to realize future works to understand the exact chemical composition of those infusion and other biological activities they may have.

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