Research, Society and Development, v. 11, n. 8, e15911830495, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i8.30495

Phytochemical screening, total phenolics and antimicrobian action from the brazilian cherry, black mulberry and blueberry fruits' pulps

Prospecção fitoquímica, fenólicos totais e ação antimicrobiana de polpas dos frutos pitanga, amora preta e mirtilo

Prospección fitoquímica, fenoles totales y acción antimicrobiana de pulpas de pitanga, mora y arándano

Received: 05/17/2022 | Reviewed: 06/10/2022 | Accept: 06/13/2022 | Published: 06/14/2022

Marcos Bertani Gazola
ORCID: https://orcid.org/0000-0001-8167-3714
Universidade Tecnológica Federal do Paraná, Brazil
E-mail: marcosbg@gmail.com
Cledes Terezinha de Oliveira
ORCID: https://orcid.org/0000-0002-2759-1002
Instituto Federal do Paraná, Brazil
E-mail: cledes.oliveira@ifpr.edu.br
Mariane Carolina Pesenti
ORCID: https://orcid.org/0000-0003-2162-6080
Universidade Tecnológica Federal do Paraná, Brazil
E-mail: mariane_pesenti@hotmail.com
Rodrigo Hinojosa Valdez
ORCID: https://orcid.org/0000-0002-4420-8327
Instituto Federal do Paraná, Brazil
E-mail: rodrigo.valdez@ifpr.edu.br
Edimir Andrade Pereira
ORCID: https://orcid.org/0000-0002-9793-5552
Universidade Tecnológica Federal do Paraná, Brazil
E-mail: edimir@utfpr.edu.br
Sirlei Dias Teixeira
ORCID: https://orcid.org/0000-0003-1135-6785
Universidade Tecnológica Federal do Paraná, Brazil
E-mail: sirlei@utfpr.edu.br

Abstract
The demand for functional foods with exotic flavors is emerging among consumers. The fruits brazilian cherry, black mulberry and blueberry have demonstrated considerable potential as inhibitors of the growth of microorganisms. The phytochemicals present in different extracts were obtained from fruits (pulps), employing specific tests to detect classes of compounds besides Infrared. Tests were realized for antimicrobial activity and quantification of total phenolics. In the phytochemical tests, the presence of mainly the flavonoid group was observed, in agreement with the high values of total phenolics found. The 70% ethanol is the most indicated solvent because it presents greater extraction of polar clusters, besides providing signals of absorption of O-H bonds in all pulps studied. The blueberry showed fungistatic action for *Candida tropicalis.* The diffusion of the extract through the culture medium is difficult, consequently, the formation of a halo, visible as that of the control, too is difficult. Therefore, results obtained in this study are considered screening and not as definitive results.
Keywords: *Eugenia uniflora*; *Morus nigra*; *Vaccinium spp*.; Microbiological characterization; Teaching.

Resumo
A demanda por alimentos funcionais com sabores exóticos está surgindo entre os consumidores. Os frutos pitanga, amora preta e mirtilo têm demonstrado considerável potencial como inibidores do crescimento de microrganismos. Os fitoquímicos presentes nos diferentes extratos foram obtidos de frutos (polpas), empregando-se testes específicos para detectar classes de compostos além do infravermelho. Foram realizados testes de atividade antimicrobiana e quantificação de fenólicos totais. Nos testes fitoquímicos, observou-se a presença principalmente do grupo flavonóide, concordando com os altos valores de fenólicos totais encontrados. O etanol 70% é o solvente mais indicado por apresentar maior extração de aglomerados polares, além de fornecer sinais de absorção de ligações O-H em todas as polpas estudadas. O mirtilo apresentou ação fungistática para *Candida tropicalis*. A difusão do extrato pelo meio de

cultura é difícil, consequentemente, a formação de um halo, visível como o do controle, também é difícil. Portanto, os resultados obtidos neste estudo são considerados de triagem e não como resultados definitivos.
Palavras-chave: *Eugenia uniflora*; *Morus nigra*; *Vaccinium spp*.; Caracterização Microbiológica; Ensino.

Resumen
La demanda de alimentos funcionales con sabores exóticos está surgiendo entre los consumidores. Los frutos de pitanga, zarzamora y arándano han mostrado un considerable potencial como inhibidores del crecimiento de microorganismos. Los fitoquímicos presentes en los diferentes extractos se obtuvieron de frutas (pulpas), mediante pruebas específicas para detectar clases de compuestos más allá del infrarrojo. Se realizaron pruebas de actividad antimicrobiana y cuantificación de fenoles totales. En las pruebas fitoquímicas se observó la presencia del grupo flavonoide, en concordancia con los altos valores de fenoles totales encontrados. El etanol al 70% es el solvente más adecuado por presentar mayor extracción de aglomerados polares, además de brindar signos de absorción de enlaces O-H en todas las pulpas estudiadas. El arándano mostró acción fungistática contra *Candida tropicalis*. La difusión del extracto a través del medio de cultivo es difícil, por lo que también es difícil la formación de un halo, visible como en el control. Por tanto, los resultados obtenidos en este estudio se consideran cribado y no resultados definitivos.
Palabras clave: *Eugenia uniflora*; *Morus nigra*; *Vaccinium spp*.; Caracterización Microbiológica; Enseñanza.

1. Introduction

Berry fruits are small fleshy and succulent fruits that are commercially grown and commonly consumed in Brazil, *in natura* or processed form. We can include among these fruits, the brazilian cherry (*Eugenia uniflora* L.), native plant, and the exotic ones like black mulberry (*Morus nigra*) and blueberry (*Vaccinium* spp.).

The brazilian cherry tree is a widely distributed tree species in Brazil, Argentina, Uruguay and Paraguay. Several isolated metabolites of this plant have been studied, Antimicrobial, antiviral, anti-fungal and antioxidant effects on metabolism have been reported for this plant, (Fidelis *et al*., 2022).

For the production of fruits, three main species of mulberry are used: black mulberry (*M. nigra* L.), red (*M. rubra* L.) and white (*M. alba* L.), (Hojjatpanah *et al*., 2011). *M. nigra* L. is one of the most important species because its fruit has substantial levels of the derived from benzoic and cinnamic acid (phenolic acids), quercetin glycosides (flavonoids), cyanidin glycosides and pelargonidin (anthocyanins), which are responsible for potential biological activities on human health (Sang *et al*., 2017), and for animals too, due to their analgesic and anti-inflammatory effects (Lim and Choi, 2019). Secondary metabolites responsible for plant defense against microbial pathogens may be helpful as antimicrobial drugs in humans, (Saboia *et al*., 2022).

The blueberry belongs to the family *Ericaceae*, genus *Vaccinium* (Sinelli *et al*., 2008), is native to several regions of Europe and North America, and has recently been introduced in Brazil, in the beginning of the 1980s (Sarkis *et al*., 2013).

It is known that fruit composition varies with a number of factors including species, variety, crop, region, weather, maturation, harvest time and storage conditions, (Faniadis *et al*., 2010). This study aimed to identify the similarities and qualitative differences between the pulps of brazilian cherry, black mulberry and blueberry collected in the southwestern region of the state of Paraná, Brazil, due to the presence or absence of certain specialized metabolites in extracts obtained with solvents in a polarity gradient. For that, the analysis of the infrared spectra of these pulps was used, associated to the phytochemical prospection. Although it is well established that fruits in the form of berries are sources of bioactive compounds, Pengkumsri *et al*., (2019), for example, report that polyphenols, present in vegetables, fruits, legumes and cereals, are secondary metabolites are extensively studied for their having possible applications in food and the pharmaceutical industries, the study proposed here also verified total phenolic content and antimicrobial action in strains of bacteria and yeasts (pulps lyophilized).

2. Methodology

2.1 Acquisition and processing of fruits

The brazilian cherry fruits, native to the Araucaria Forest (S: 26°15'28.07"; W: 52°48'22.20") and exotic black mulberry (S: 26°14'41.8"; W: 52°47'5.92") were purchased in extractive way in the municipality of Vitorino and those of blueberry in commercial cultivation in the municipality of Palmas (S: 26°24'19.02"; W: 52°3'59.12") (GPS), southwest region of the state of Paraná. The fruits were classified according to their quality attributes (color, uniformity, degree of maturation, disease exemption), then sanitized (solution with 250 ppm of chlorine for 15 minutes), rinsed, drained, pulped and packaged (low density polyethylene) being stored frozen (-18 °C).

2.2 Obtaining extracts

The *in natura* pulps were lyophilized (*Liotop* L101 lyophilizer), weighed and subjected to cold exhaustive extraction with solvents in increasing order of polarity (petroleum ether, ethyl ether and 70% ethanol) under stirring for 72 h each solvent. The extract was concentrated using a rotary evaporator (*Fisatom*® 802) at low temperature (30 ºC).

2.3 Infrared Analysis

Each extract was lyophilized and subjected to infrared analysis on a PerkinElmer FT-IR Frontier Spectrophotometer using liquid film of each sample resolubilized in dichloromethane in potassium bromide (KBr) cells. The spectra were obtained in the range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and accumulations of 64 scans using *Perkin Elmer*® Spectrum software version 10.03.07.0112.

2.4 Phytochemical tests

A phytochemical study of each extract was carried out, according to a methodology proposed by Matos (1997), for the detection of phenols and tannins; anthocyanins and anthocyanidins; flavones, flavonols and xanthones (free or their heterosides); chalcones and aurones; flavanonols; leucoantocyanidins; catechins (catheter tannins); flavanones.

2.5 Total phenolics

The determination of total phenolics of lyophilized pulps occurred through the Folin-Ciocalteu method based on procedures described by Ubeda *et al*. (2011).

The total phenolic content was calculated using the regression equation obtained with the read absorbance values, proceeding with the calculation of the average value of the duplicates and respective standard deviations.

2.6 Tests of antimicrobial activity

The lyophilized pulps were submitted to antimicrobial activity tests for some species of yeasts and bacteria. The initial test using the diffusion disc method was based on Mahesh *et al*. (2008), with modifications. For the experiment with the bacteria, Petri dishes with Mueller Hinton Agar and for Candidas, plates of Sabouraud Agar were used.

In this experiment, ATCC (American Type Culture Collection) strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 15442, *Bacillus subtilis* ATCC 6623, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, *Candida tropicalis* ATCC 28707, as well as *Salmonella typhimurium* NEWP 0028.

Initially, a solution containing 10,000 μg of each lyophilized extract (brazilian cherry, black mulberry and blueberry), 100 μL of dimethylsulfoxide (DMSO) and, after homogenization, 900 μL of sterile saline solution was prepared in *eppendorf*.

In another *eppendorf* were placed 1,000 µg of antifungal or antibiotic, 10 µL of (DMSO) and 990 µL of saline solution. Then, 100 µL of each prepared solution was transferred to a second *eppendorf* which also received 900 µL of sterile saline, this dilution being used in analyzes of antimicrobial activity on diffusion disks.

For the analyzes of lyophilized extracts, three dilutions were prepared with concentrations of 1,000 µg mL^{-1}, 500 µg mL^{-1} and 100 µg mL^{-1}, referred to as SP1 (standard solution 1), SP2 (standard solution 2) and SP3 (standard solution 3), respectively. In parallel, a small amount of the microorganism studied was transferred to a sterile saline solution. After homogenization the turbidity of the solution was verified (scale 0.5 of Mac Farland). This suspension was prepared for all tests. In the next step, swab was used to transfer the microorganism from the test tube to a petri dish containing culture medium suitable for the organism under study. A few paper filter discs (one for each tested solution) were placed in a petri dish with flanged forceps. Then, 10 µl was added on each paper disk of the solutions to be tested. Each plate was incubated at 35 °C, and readings were taken at 24 and 48 hours, measuring the disk halos in mm. Fluconazole and ketoconazole were used as antifungals, and as antibiotics, tetracycline, norfloxacin and erythromycin. The experiment was carried out in triplicate. In each replicate, there were duplicates of each dilution of the pulps, totalizing six responses for each dilution used (1,000 µg mL^{-1}, 500 µg mL^{-1} and 100 µg mL^{-1}).

2.7 Minimum inhibitory concentration (MIC)

The determination of the minimum inhibitory concentration (MIC) was performed based on the methodology applied by Ushimaru *et al*. (2007), with some modifications. A 96-well microplate was used. The other materials used were autoclaved (Prismatec autoclave).

In an *eppendorf*, 10,000 µg (0.010 g) of lyophilized pulp was transferred, it was added 100 µL of DMSO and after homogenization 900 µL of sterile saline solution were added forming Stock 1 (SE1) solution. In another *eppendorf*, 1,000 µg (0.001 g) of antifungal or antibacterial material was added, plus 10 µL of DMSO and after homogenization 990 µL of sterile saline solution were added forming the standard solution (SE2).

For each pulp, 7 dilutions were performed (with 500 µL of Sabouraud broth), obtaining the following concentrations: 2,500 µg mL^{-1}; 1,250 µg mL^{-1}; 625 µg mL^{-1}; 312.50 µg mL^{-1}; 156.25 µg mL^{-1}; 78.12 µg mL^{-1} and 34.05 µg mL^{-1}. In relation to the control, only the dilution corresponding to 250 µg mL^{-1} was prepared. Each well of the plates received 100 µL of Sabouraud broth (when tests were performed with yeast), 100 µL of the dilution of the pulp, and 5 µL of the suspension with the microorganism to be tested. In the control wells (total of 3 wells for each antimicrobial) were placed 100 µL of Sabouraud broth and 100 µL of fungal suspension (or 100 µL Mueller Hinton broth and bacterial suspension) and 100 µL of diluted antimicrobial suspension.

The plate was then packed in a closed container containing a piece of damp cotton and incubated at 35 °C. Results were read in 24 and 48 h. Solutions (10 µL) of the wells that showed a positive result (inhibition) were seeded in a plate containing Sabouraud agar in relation to the dilutions of SE1. The plates were incubated for 24 h. After this period, it was verified when in a certain dilution, if the pulp acted as biostatic or biocide. It should be noted that six-replicates were used for the pulps and triplicate for the controls, divided into three experiments for each microorganism studied. The halos diameter readings occurred 24 and 48 hours after incubation. The trials were carried out between the months of October/2012 to December/2012.

2.8 Statistical analysis

The mean and standard deviations of the diameters of the inhibition halos observed during antimicrobial activity experiments, in the determination of antioxidant capacity and total phenolics were obtained by the algorithm from the

statistical software, (*Statistica*® 8.0 software). The results of the analysis were also submitted to analysis of variance and the comparison of means by the Tukey test at the 5% level of significance.

3. Results and Discussion

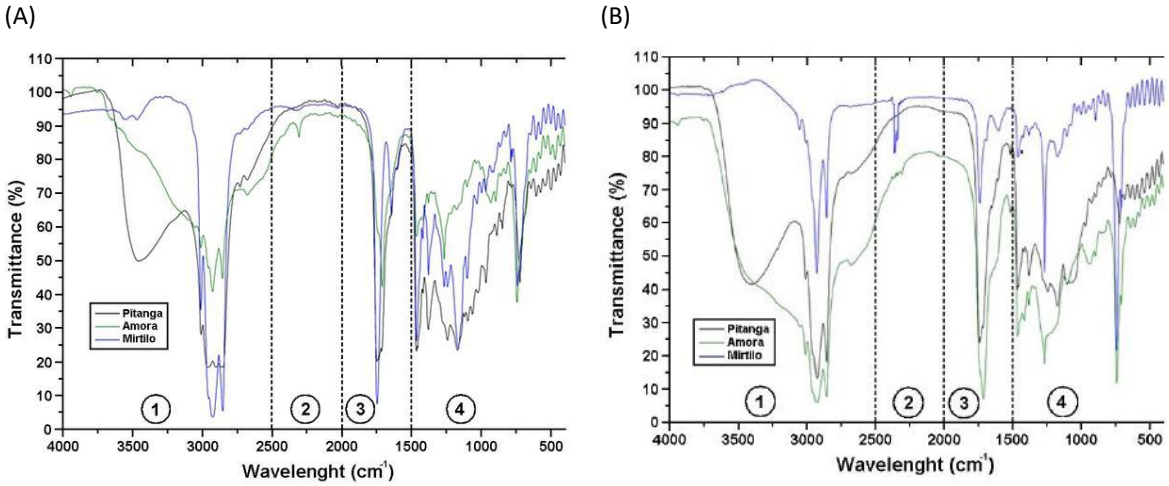
3.1 Infrared analysis

The analysis of the infrared spectra of the extracts was done according to guidelines of Silverstein *et al*. (2000), and IR-FT spectra were divided into four regions, following the model proposed by Mc Murry (2010). Region 1 comprises the range of 4000 to 2500 cm^{-1} and corresponds to absorptions caused by stretches of N−H, C−H, and O−H single bonds, region 2, from 2500 to 2000 cm^{-1} is assigned to triple stretch of nitriles and alkynes, region 3 comprises the range of 2000 to 1500 cm^{-1} and corresponds to the absorptions of the double bonds of C=O, C=C and C=N, while region 4 is below 1500 cm^{-1} and is known as the fingerprint region, it corresponds to a large number of absorptions due to a variety of vibrations of single bonds C−C, C−O, C−N and C−X.

Petroleum ether extract (Figure 1 (A)), shows that in the three pulps analyzed there were signs referring to the stretching of the C−H bond of alkane (3000-2840 cm^{-1}) and C=O (1800-1700 cm^{-1}), as well as symmetrical angular strain in the CH_2 plane (~1465 cm^{-1}), C−O (1300-1000 cm^{-1}) and C−X (alkyl halide) vibrations in 1000-500 cm^{-1}. The brazilian cherry pulp is distinguished from the others by the presence of an O−H axial deformation band of alcohol or phenol (3650-3100 cm^{-1}). In the black mulberry pulp, low intensity absorption of aromatic compounds in the 3100-3000 cm^{-1} region and in blueberry stretch the N−H (3471 cm^{-1}) of primary amine were observed. C=C binding stretch (1639 cm^{-1}) can be observed in brazilian cherry and blueberry pulps.

In the extracts of ethyl ether (Figure 1 (B)), the presence of O−H of alcohol or phenol (3650-3100 cm^{-1}) is evident only in the brazilian cherry pulp, whereas salts of amines, due to the absorption of N−H (2260-2220 cm^{-1}), is checked on the blueberry pulp. In the three pulps, C−H (alkanes), C=O, CH_2, C−O and C−X (alkyl halides) are present.

Figure 1 – (A) Spectra of lipophilic extracts (petroleum ether) from brazilian cherry, black mulberry e blueberry; (B) Spectra of hydrophilic extracts (ethyl ether) from brazilian cherry. black mulberry and blueberry pulps.

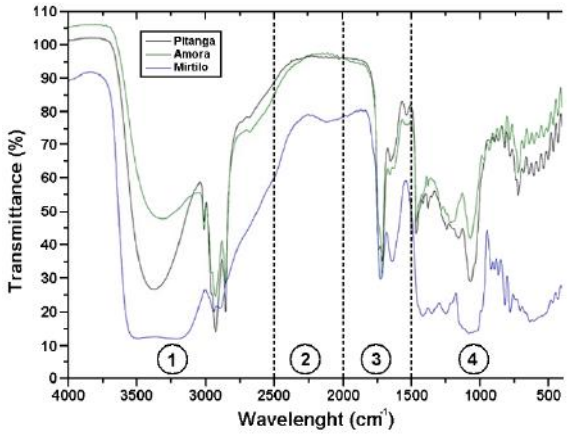


Source: Authors.

However, in the spectra of 70% ethanol (Figure 2), the O−H stretch is present in the three pulps, with greater absorption for the blueberry. In the three spectra, C−H (alkanes), C=O, CH_2, C−O and C−X (alkyl halides) are observed, these signals being more evident in brazilian cherry and black mulberry pulps.

In view of the obtained results, it is verified that the solvent used will involve the extraction of compounds with characteristic functional groups, and should be correctly chosen according to the interest in probable secondary metabolites present in the extracts. For example, in the investigation of total phenolics, it is known that the basic structure of these compounds has polar groups, so hydrophilic solvents are more suitable for the extraction and isolation processes of these substances, in these specific cases the 70% ethanol extract.

Figure 2 − Spectra of the hydrophilic extracts (ethanol 70%) from brazilian cherry, black mulberry and blueberry pulps.



Source: Authors.

3.2 Phytochemical prospecting

By performing phytochemical tests of petroleum ether extracts, of ethyl ether and of 70% ethanol of the three fruits studied (brazilian cherry, black mulberry e blueberry), it was possible to identify the presence of secondary metabolites of the class of flavonoids.

Table 1 shows that hydrophilic solvent (70% ethanol) presented higher extractive power of secondary metabolites with phenols, tannins, flavones, flavonols and xanthones, as well as flavanones and catechins in **brazilian cherry pulp**. In the extract − ethyl ether, the flavanones were also identified with strong intensity, besides the observation with medium intensity of phenols and flavanonols.

The presence of flavonoids and tannins in the ethyl acetate, flavonoids and terpenes fractions in the chloroform fraction and terpenes in the hexane fraction were observed in the powder of the leaves of *E. uniflora* by Fiuza *et al*. (2008). Anthocyanins and anthocyanidins were not identified in any of the extracts obtained, but it cannot be affirmed that these metabolites did not exist, since for some reason, even for low levels, they could not be detected by the methods used. In this case, it is possible to explain the absence of the chemical constituents in the samples analyzed, as well as the environmental factors, (Viana *et al*., 2012).

As observed in the brazilian cherry, the extract − ethanol 70% presented higher extraction power of the compounds in the **black mulberry pulp**, as shown in Table I. The phenols and the tannins were moderately identified in the ethyl ether extract, same as that of petroleum ether.

The anthocyanins and anthocyanidins were strongly identified in the 70% ethanol extract, being absent in the extracts of petroleum ether and ethyl ether. Mulberries show high concentrations of anthocyanins and phenolic compounds, (Khalid *et al*., 2011). Flavones, flavonols and xanthones, in the 70% ethanol extract, these metabolites were observed in higher concentrations. The chalcones, aurones, flavanonols, leucocyanidins and flavanones were strongly evidenced in the 70% ethanol too. In catechins, flavonoids of the flavonols class, at carbon 3 it present a mandatory hydroxyl, which characterizes

them as flavan-3-ol. In the synthesis of condensed tannins, the catechins are considered intermediate, together with the leucoanthocyanidins, (Rodrigues *et al*., 2010). Therefore, the absence or simultaneous presence of catechins and tannins in the extracts studied is justified.

Table 1 − Results of the phytochemical prospection of lipophilic (petroleum ether), lipophilic medium polarity (ethyl ether) and hydrophilic (70% ethanol) extracts of brazilian cherry, black mulberry and blueberry.

Constituents	Petroleum ether extract			Ethyl ether extract			70% Ethanol extract		
	By01	By02	By03	By01	By02	By03	By01	By02	By03
Phenols	NI	NI	NI	++	++	NI	+++	+++	+++
Tannins	NI	NI	NI	NI	++	+	+++	NI	NI
Anthocyanins and Anthocyanidins	NI	NI	NI	NI	NI	NI	NI	+++	+++
Flavones, Flavonols and Xanthones	+	+	+	+	+	NI	++	+++	+++
Chalcones and Aurones	+	+	+	NI	NI	NI	NI	+++	+++
Flavanonols	+	+	+	++	+	+	+	+++	+
Leucoanthocyanidin	+	+	NI	NI	NI	NI	NI	+++	+
Catechins	+	+	NI	NI	+	NI	++	NI	NI
Flavanones	+	+	NI	+++	NI	NI	++	+++	NI

Caption: +: weak; ++: medium; +++: strong; NI: not identified; By01: brazilian cherry; By02: black mulberry; By03: blueberry.

Source: Authors.

In Table I, the identified secondary metabolites (presence or absence) are shown too in the lyophilized **blueberry pulp**. The hydrophilic solvent showed good extraction of secondary metabolites, such as phenols, anthocyanins, anthocyanidins, flavones, flavonols, xanthones, chalcones and aurones. Tannins, anthocyanins, flavonols, catechins, phenolic acids were quantified in several blueberry cultivars, by Yi *et al*. (2005).

The non-identification of catechins in extracts may be related to the type of cultivar, maturity, geographical origin, growth stage, culture techniques, post-harvest storage and processing, (Lata *et al*., 2005). According to Pertuzatti *et al*. (2007), the blueberry has significant levels of phenolic compounds, wich are in greater quantity in the peel of the fruit. Knowing that the pulp used contained blueberry peel, the anthocyanins may have been diluted with the other present compounds, altering their quantification and, consequently, interfering in the phytochemical prospecting tests. According to Dembinska-Kiec *et al*. (2008), studies infer that anthocyanins may decrease glucose uptake in the gut, postponing the release of glucose during digestion.

3.3 Total Phenolics

The total phenolic content of the pulps was statistically different, except the brazilian cherry and blueberry pulps in the solvent acetone. Ethanol had lower phenolic extraction power, ranging from 9.02 mg EAG g^{-1} (brazilian cherry pulp) to 14.88 mg EAG g^{-1} (blueberry pulp). The highest total phenolic content was observed for the blueberry pulp, and the lowest for the brazilian cherry pulp. In the pulp of red brazilian cherry, Bagetti *et al*. (2011) found 0.21 mg EAG g^{-1}. Moyer *et al*. (2012)

obtained 0.72 mg EAG g^{-1} in blueberry pulp, while Cho *et al*. (2005) observed a variation of 2.27 to 3.70 mg EAG g^{-1}, using solvent methanol: formic acid: water.

Using jambolan pulp (*Syzygium cumini*), fruit also found in Brazil, Vital et al. (2022), quantified the total phenolic compounds and found values from 134.91 to 167.94 mg EAG g^{-1}, comparatively higher than those presented in this work.

In summary, it can be stated that, through the methodology used, the total phenolic content differ from those presented in other scientific studies. This discrepancy may be related to the climatic/environmental conditions, harvesting season and genotype of the plants whose fruits were sampled, justifying the acceptability of the presented results.

3.4 Tests of antimicrobial activity and Minimum inhibitory concentration (MIC)

According to the data obtained, none of the lyophilized pulps tested showed inhibitory growth potential of the bacterial group studied. Khalid *et al*. (2011), observed zones of inhibition of black mulberry juice (*M. nigra*) 100 µL mL^{-1} in eight different strains of microorganisms. There was no zone of inhibition for *C. albicans* and *E. coli*. None of the applied pulps and antifungals showed a halo of inhibition against yeast *Candida albicans*.

The black mulberry and brazilian cherry pulps also did not inhibit the growth of the other two yeasts used in the assays. However, the blueberry pulp showed inhibition halo for *C. tropicalis* and *C. parapsilosis*, reaching 32.50 mm at the concentration of 1,000 µg mL^{-1}, before *C. tropicalis*.

For the blueberry pulp, therefore, the Minimum Inhibitory Concentration (MIC) was determined in three experiments. For the blueberry pulp, MIC of 625 µg mL^{-1} was observed and 1250 µg mL^{-1}. Therefore, 10 µL of MIC of 625 µg mL^{-1} and 10 µL of MIC of 1,250 µg mL^{-1} were seeded separately in Petri dishes containing Sabouraud agar and incubated for 24 h. After this period, growth of colonies of *C. tropicalis* on the plates was observed, indicating that in these concentrations the blueberry pulp is considered fungistatic, that is, it has the capacity to inhibit the growth of this yeast.

Shen *et al*. (2014) further explains that at low pH the phenolic compounds can form complexes with outer membrane proteins of microorganisms, altering the tolerance of bacteria in low osmosis environments, leading to the death of the same.

4. Conclusion

Regarding the results obtained: through the infrared analysis, it was verified that 70% ethanol is the most indicated solvent, because it enables the extraction of polar metabolites such as flavonoids; the presence of flavonoids makes it possible to use the studied plant species as anti-inflammatory; the highest total phenolic content was observed for the blueberry pulp; regarding the antimicrobial analysis, it should be noted that the results found apply to the methodology used and do not necessarily mean that brazilian cherry and black mulberry do not present antimicrobial activities and that blueberry only has action against *C. tropicalis* (the blueberry pulp was considered fungistatic). Therefore, studies with extracts of these fruits, indicate that there is the possibility of elaborating a food product oriented towards the utilization of the detected nutraceutical characteristics / properties.

In this way, as future work, it is suggested to carry out the same analyzes reported in this work, of cherry, blueberry, and blackberry pulps incorporated into a food product.

Acknowledgments

CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; UTFPR – Universidade Tecnológica Federal do Paraná – Câmpus Pato Branco; PPGTP – Programa de Pós-Graduação em Tecnologia de Processos Químicos e Bioquímicos.

References

Bagetti, M. *et al*. (2017). Physicochemical characterization and antioxidant capacity of pitanga fruits (*Eugenia uniflora* L.). *Ciência e Tecnologia de Alimentos*, 31(1), 147-154. 10.1590/S0101-20612011000100021

Cho, M. J. *et al*. (2005). Flavonol glycosides and antioxidant capacity of various blackberry and blueberry genotypes determined by high-performance liquid chromatography/mass spectrometry. *Journal of the Science of Food and Agriculture*, (85), 2149-2158. 10.1002/jsfa.2209

Dembinska-Kiec, A. *et al*. (2008). Antioxidant phytochemicals against type 2 diabetes. *British Journal of Nutrition*, 99 (1), 109-117. doi:10.1017/S000711450896579X

Faniadis, D. *et al*. (2010). Effects of cultivar, orchard elevation, and storage on fruit quality characters of sweet cherry (*Prunus avium* L.). *Scientia Horticulturae*, (125), 301–304. 10.1016/j.scienta.2010.04.013

Fidelis, E. M. *et al*. (2022). Pitanga (*Eugenia uniflora* L.) as a source of bioactive compounds for health benefits: A review. *Arabian Journal of Chemistry*, (15), 103691. 10.1016/j.arabjc.2022.103691

Fiuza, T. S. *et al*. (2008). Caracterização farmacognóstica das folhas de *Eugenia uniflora* L. (Myrtaceae). *Revista Eletrônica de Farmácia*, (2), 01-11.

Hojjatpanah, G. *et al*. (2011). Evaluation of the fouling phenomenon in the membrane clarification of black mulberry juice. *International Journal of Food Science & Technology*, (46), 1538–1544. 10.1111/j.1365-2621.2011.02651.x

Khalid, N. *et al*. (2011). Antimicrobial activity, phytochemical profile and trace minerals of black mulberry (*Morus nigra* L.) fresh juice. *Pakistan Journal of Botany*, (43), 91-96.

Lata, B. *et al*. (2005). Effect of cultivar and harvest date on thiols, ascorbate and phenolic compounds content in blueberries. *ACTA Scientiarum Polonorum, Hortorum Cultus*, 4 (1), 163-171.

Lim, S. H. and Choi, C. (2019). Pharmacological Properties of *Morus nigra* L. (Black Mulberry) as a Promising Nutraceutical Resource. *Nutrients*, 11 (2), 437. 10.3390/nu11020437

Mahesh, B. *et al*. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World Journal of Agricultural Science*s, (4), 839-843.

Matos, F. J. de A. *Introdução à fitoquímica experimental*. (2a ed.), Edições UFC, 1997.

Mc Murry, J. *Química Orgânica*. Pioneira Thomson Learning, Cengage Learning, 2010.

Moyer, R. A. *et al*. (2002). Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium, Rubus*, and *Ribes*. *Journal of Agricultural and Food Chemistry*, (50), 519-525. 10.1021/jf011062r

Pengkumsri, N. *et al*. (2019). Influence of Extraction Methods on Total Phenolic Content and Antioxidant Properties of some of the commonly used Plants in Thailand. *Pak. J. Biol. Sci*., 22, (3), 117-126. 10.3923/pjbs.2019.117.126

Pertuzatti, P. B. *et al*. (2007). Relação de fitoquímicos na casca e polpa de mirtilo (*Vaccinium ashei* Reade). In: *XVI CONGRESSO DE INICIAÇÃO CIENTÍFICA, IX ENPOS*, Pelotas: Universidade Federal de Pelotas.

Rodrigues, S. *et al. (2010).* Prospecção fitoquímica e atividade moluscicida de folhas de Momordica charantia L. *Cadernos de Pesquisa*, 17 (2).

Saboia, C. da S. *et al*. (2022). Caracterização química e atividade antimicrobiana do óleo essencial e do extrato bruto de capim limão (*Cymbopogon citratus*). *Research, Society and Development*, 11(7), e37611730064. 10.33448/rsd-v11i7.30064

Sang, J. *et al*. (2017). Development and validation of green chromatography for the determination of anthocyanins in haskapberry, mulberry and blackberry. *Anal. Methods*, (9), 2535–2545. 10.1039/c7ay00229g

Sarkis, J. R. *et al*. (2013). Effects of ohmic and conventional heating on anthocyanin degradation during the processing of blueberry pulp. *LWT – Food Science and Technology*, (51), 79-85. 10.1016/j.lwt.2012.10.024

Shen, X. *et al*. (2014). Antimicrobial effect of blueberry (*Vaccinium corymbosum* L.) extracts against the growth of *Listeria monocytogenes* and *Salmonella Enteritidis*. *Food Control*, (35), 159-165. 10.1016/j.foodcont.2013.06.040

Silva, S. *et al*. (2013). Evaluation of the antimicrobial activity of aqueous extracts from dry *Vaccinium corymbosum* extracts upon food microorganism. *Food Control*, (34), 645-650. 10.1016/j.foodcont.2013.06.012

Silverstein, R. M. *et al. Identificação Espectrométrica de Compostos Orgânicos*. (6a ed.), LTC, 2000.

Sinelli, N. *et al*. (2008). Evaluation of quality and nutraceutical content of blueberries (*Vaccinium corymbosum* L.) by near and mid-infrared spectroscopy. *Postharvest Biology and Technology*, (50), 31-36. 10.1016/j.postharvbio.2008.03.013

Ubeda, C. *et al*. (2011). Evaluation of antioxidant activity and total phenols index in persimmon vinegars produced by different processes. *LWT – Food Science and Technology*, (44) 1591-1596. 10.1016/j.lwt.2011.03.001

Ushimaru, P. I. *et al*. (2007). Antibacterial activity of medical plant extracts. *Brazilian Journal of Microbiology*, (38), 717-719. 10.1590/S1517-83822007000400024.

Viana, F. C. *et al*. (2012). Identificação fitoquímica de flavonoides e taninos em folhas de pitanga (*Eugenia uniflora* l.) utilizadas tradicionalmente na região sul da Bahia. *InterPHacis*, (1), 28-37.

Vital, A. R. *et al*. (2022). Caracterização química e compostos bioativos do sorvete de polpa de jambolão. *Research, Society and Development*, 11(6), e23011628900. 10.33448/rsd-v11i6.28900

Yi, W. *et al*. (2005). Phenolic compounds from blueberries can inhibit colon cancer cell proliferation and induce apoptosis. *Journal of Agricultural and Food Chemistry*, (53), 7320-7329. 10.1021/jf051333o