Indian Journal of Natural Products and Resources
Vol. 15(2), June 2024, pp. 294-301
DOI: 10.56042/ijnpr.v15i2.11492

Phytochemical profiles and cytotoxicity evaluation of the ethanolic extract of *Malpighia emarginata* leaves: Potential for its therapeutic use

Renato Constantino dos Santos Souza[1], Rodrigo Augusto Cristóvão Ferreira[1], Paula Reis Viola[1], Melina Iyomasa-Pilon[1], Mariana Balie[1,2], Ariane Harumi Yoshikawa[1] and Ana Paula Girol[1,2,3]*

[1]Experimental and Clinical Research Center (CEPEC), University Center Padre Albino (UNIFIPA), Catanduva 15809-144, Brazil

[2]São Paulo State University, (UNESP), Institute of Biosciences, Humanities and Exact Sciences (IBILCE), São José do Rio Preto Campus 15054-000, Brazil

[3]São Paulo Federal University (UNIFESP), São Paulo 04023-062, Brazil

Received 01 April 2024; revised received 19 May 2024; accepted 22 May 2024

The search for new natural compounds with relevant therapeutic properties has increased in recent years. The objective of this study was to investigate the biological properties of the ethanolic extract of *Malpighia emarginata* leaves. For this, phytochemical assays, high-performance thin layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), antioxidant activity analysis using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and cytotoxicity by hemolysis, and chorioallantoic membrane (CAM) studies were performed. Through qualitative analyses, phenolic compounds and alkaloids were identified, and by quantitative analyses, 92.52, 2.02, and 0.59 µg/mL were found for total flavonoids, total alkaloids, and total phenols, respectively. HPTLC showed the presence of the flavonoids catechin and gallic acid, while HPLC identified myricetin and quercetin. The extract showed a high antioxidant capacity of 80.77% by DPPH. Moreover, low cytotoxicity of the extract at 10% was observed by hemolysis and CAM assay. Our results indicate that the ethanolic extract of *M. emarginata* leaves is a potential candidate for therapeutic use due to the presence of important bioactive compounds, high antioxidant activity and low cytotoxicity.

Keywords: Acerola, Alcoholic extract, DPPH, HPLC, HPTLC, Phytochemical compounds

IPC code; Int. cl. (2021.01)– A61K 36/00, A61P, A61P 39/00

Introduction

Malpighia emarginata DC / *Malpighia glabra* L, popularly known as "acerola", belongs to the Malpighiaceae family and is a fruit-bearing plant native to Western India and the tropical region of South America[1]. This species is grown in several tropical countries and has a high vitamin C, ascorbic acid, vitamins A, B1, B2, B3, calcium, phosphorus and iron content. Acerola fruit also contains several phytonutrients such as carotenoids, phenolic compounds, flavonoids, and anthocyanins, and it has numerous biofunctional properties[2–5].

Research has demonstrated the great functional importance of this fruit due to its nutritional value and the presence of phytochemical compounds that act in various biological activities. It presents potential therapeutic effects, such as anti-inflammatory, antioxidant, antifungal, antibacterial activities, cancer prevention and degenerative diseases, including Alzheimer's and Parkinson's[4–10]. Therefore, the identification of phytochemicals and their therapeutic mechanism of action has been the focus of several studies around the world.

The saline extract of *M. emarginata* leaves also presents bioactive compounds that are important in biological activities[10]. However, knowledge about the properties of the organic leaf extract. For this reason, the present study aimed to investigate the phytochemical, antioxidant and cytotoxicity of the crude ethanolic extract of *M. emarginata* leaves.

Materials and Methods

Sample collection and identification

The collection of *M. emarginata* leaves was carried out on the main campus of Padre Albino University Center (UNIFIPA), Catanduva, SP, Brazil, in April 2021. Specimens were identified and deposited in the Irina Delanova Gemtchujnicov (BOTU) herbarium (number 36361). The leaves were washed, dried, and crushed until a fine powder was obtained.

*Correspondent author
Email: anapaula.girol@unifipa.com.br

Obtaining and standardising the extract

The extract was obtained by percolating the dry weight of 20 g of crushed leaves in 100 mL of 70% ethanol for 24 hours. The ethanol was evaporated by a rotary vacuum evaporator at a reduced pressure (175 ambar) with a maximum temperature of 45ºC, avoiding the loss of material properties. The crude extract obtained was weighed and diluted in distilled water at a concentration of 10%[11].

Phytochemical characterisation

Qualitative and quantitative analyses

Phytochemical analyses were carried out to identify the presence of the plant's secondary metabolites products of pharmacological interest. Initially, a qualitative analysis was conducted to identify terpenes, phenolic compounds and alkaloids[11].

To identify terpenes, the test for sesquiterpenes was performed by adding 1 mL of sulfuric acid to 2 mL of extract. The reddish colour indicates the presence of terpenoids with 15 C[12]. The presence of phenolic compounds of the Coumarin type was investigated using tubes containing 1 mL of the crude extract by moistening 3 areas of the filter paper with the extract, and after drying, 1 drop of aqueous NaOH solution (10%) was added to the moistened areas and taken to a water bath. After 10 min, the filter paper was observed under ultraviolet light. Blue-green fluorescence indicates the presence of coumarins[13].

The presence of alkaloids was determined using Bourchardat (yellow residue), Dragendorff (orange residue), Mayer (white precipitate) and Sheibler (light yellow residue) reagents after alkalinisation of the extract and separation of alcoholic and organic phases with the addition of chloroform[12-14].

Quantitative phytochemical analyses of the extract were carried out. The Folin-Ciocalteu method was used to evaluate total polyphenols in the samples[15]. Gallic acid (Dinâmica -1274) was used to construct the standard curve (0.25, 0.5, 1.0, 1.5, and 2.0 μg/mL), with 10% sodium carbonate ($NaCO_3$) (1.5 mL) and Folin-Ciocalteu (0.5 mL) as colourimetric developer reagents for the curve and samples, followed by the reading on a spectrophotometer at 760 nm.

The quantification of flavonoid-type phenolic compounds was performed through the stable reaction that forms Aluminum Chloride (AlCl3). Quercetin (Sigma - Q4951) was used to construct the standard curve (serial dilution from 0.25 to 0.0156 μg/mL). The reading of the mixture of 1 mL of the different concentrations of the curve or 1 mL of the samples added to 1 mL of 2% AlCl3 alcohol solution was taken at 415 nm in the spectrophotometer[15,16].

The total alkaloids quantification was carried out by the method in which the alkaloids that passed into a portion of chloroform after alkalinisation with 2N NaOH were revealed by the bromocresol green reagent diluted in a buffered solution at pH 4.7 with citric acid. A comparison curve (40, 60, 80, 100, 120, 140 μg/mL) was made with atropine (A0123 – Sigma) and analysed at 470 nm in the spectrophotometer[17].

Chromatographic studies

HPTLC chromatographic readings were carried out with the CAMAG Linomat 5 automatic applicator, with N gas (60 psi) and in thin layers (0.2 mm) of silica 10 x 10 cm (Ref. 818333 -Macherey-Nagel/Germany - ALUGRAM® Xtra SILG/UV254). The application method was the same for all analyses, using a 100 μL Hamilton syringe, a dosage speed of 50 nL/s and pre-dosage disposal of 0.2 μL. The bands were made using 10 μL, 8 mm in size and 5 mm in distance. The standards, eluents and developers used in chromatographic research are detailed in Table 1.

HPLC analysis of the crude extract was also performed, reproducing the chromatographic conditions of Jia *et al.*[18]. The column stationary phase (Phenomenex Luna - Torrance, CA - USA) used was C18 (250 mm × 4.6 mm - 5.0 μm), mobile phase with water and formic acid (100:0.1, v/v) (A) and Acetonitrile (B), gradient from 5 to 20% B in 30 min, 20 to 60% in 100 min and 60 to 80% in 120 min. Rate of 1 mL/min, injected volume of 10 μL, Shimadzu Prominence-i LC2030C-3D equipment (Kyoto, Japan). The peaks resulting from the chromatogram were read at 254 nm and compared with the aforementioned work.

Antioxidant activity

The antioxidant activity of the extract was determined by the free radical scavenging capacity using 2,2-diphenyl-1-picrylhydrazyl (DPPH)[4,19,20]. After adding the DPPH solution, the samples were

Table 1 — Standards and their dilutions, mobile phases and derivatization used in HPTLC

Standards	Eluents (Mobile Phase)	Developers
Catechin	Toluene / Ethyl Acetate / Methanol (30:70:10)	Vanillin 1% in ethanolic solution with H_3PO_4 (15%)
Gallic Acid	Toluene / Ethyl Acetate / Formic Acid (5:4:0,2)	$FeCl_3$ 0.5%

kept away from light for 30 min for subsequent readings on a spectrophotometer at 520 nm. Determinations were carried out in triplicate. The scavenging activity was calculated using the formula: % scavenging capacity = [(average absorbance of controls – average absorbance of samples) / average absorbance of controls] x 100.

Cytotoxicity

Hemolysis

Glycosylated solution (5%) of human blood (4%) was mixed in different concentrations (2, 4, 6, 8, and 10%) of the sample. For the positive control, a 0.1% Triton solution was prepared, and for the negative control, only saline solution was added to the red blood cell solution. Samples and controls were placed in a water bath at 37ºC for 15 min and centrifuged at 2,000 RPM for 10 min. Then, the reading was carried out using a spectrophotometer at 540 nm, zeroing the device with the glycosylated solution[11].

Chorioallantoic membrane assay – CAM

The CAM test of cytotoxicity was approved by the Research Ethics Committee on the use of animals – Padre Albino University Center/UNIFIPA (09/21). Fertilised chicken (*Gallus gallus*) eggs were incubated at 37 degrees Celsius and 50% relative humidity for 72 hours in the incubator. Embryonated eggs were divided into three experimental groups: Control, in which the eggs were injected with 50 µL of saline in the air chamber; and treated groups, in which 50 µL of CE at different concentrations (2, 4, 6, 8, 10, and 100%). After 72 hours, the egg shells around the air chamber were removed to evaluate the vascularisation in the chorioallantoic membranes and the morphological development of the embryos[11,21,22].

Results

Phytochemical characterisation and antioxidant activity

Qualitative phytochemical analyses showed the absence of sesquiterpenes but identified plant secondary metabolites of pharmacological interest, such as phenolic compounds and alkaloids. The results of quantitative phytochemical characterisation indicated the presence of 0.64 µg/mL of total polyphenols, 92.52 µg/mL of total flavonoids, and 2.02 µg/mL of total alkaloids in the CE. Furthermore, DPPH analysis showed that CE has high antioxidant activity (80.77%).

Chromatographic studies

HPTLC showed the presence of the phenolic compounds catechin (Fig. 1a and b) and gallic acid (Fig. 1c and d) in CE.

HPLC allowed identifying the presence of the flavonoids Myricetin (tR= 31.75 min, peak 68 and 39.76 min, peak 87) and Quercetin (tR= 36.41 min, peak 80) (Fig. 2).

Cytotoxicity assays

The hemolysis study showed that the CE presented low cytotoxicity at all concentrations studied when compared to the positive control (Fig. 3a and b).

CAM assay showed the absence of cytotoxicity up to a concentration of 10%. All embryos were alive at the time of shell opening, and no differences in membrane vascularisation or morphological

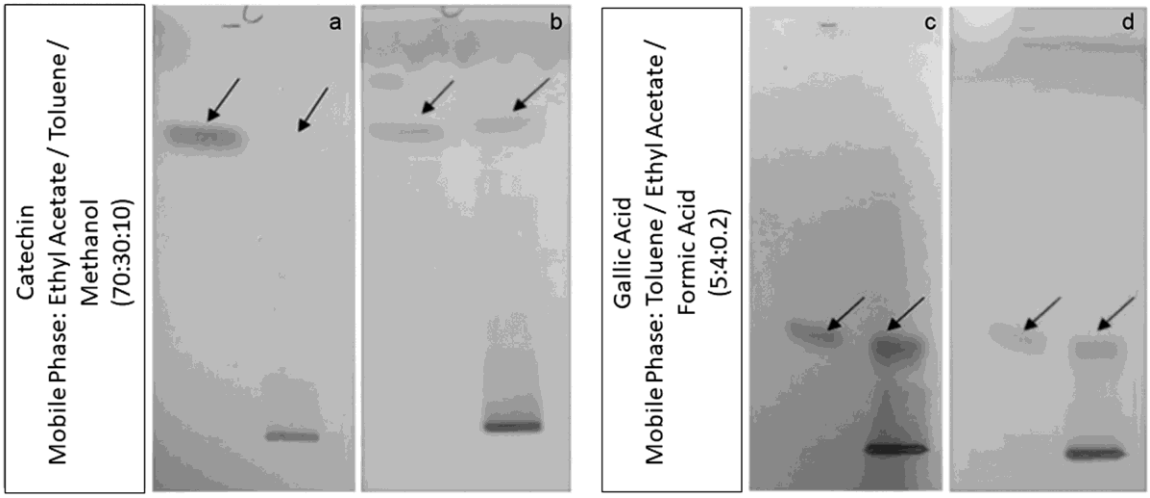

Fig. 1 — Results of phytochemical research by HPTLC. a) Arrows indicate bands corresponding to the catechin pattern in CE after derivatisation solution; b) Catechin revealed in ultraviolet light; c) Arrows show bands corresponding to the gallic acid pattern in CE after derivatisation solution; and d) Gallic acid revealed in ultraviolet light.

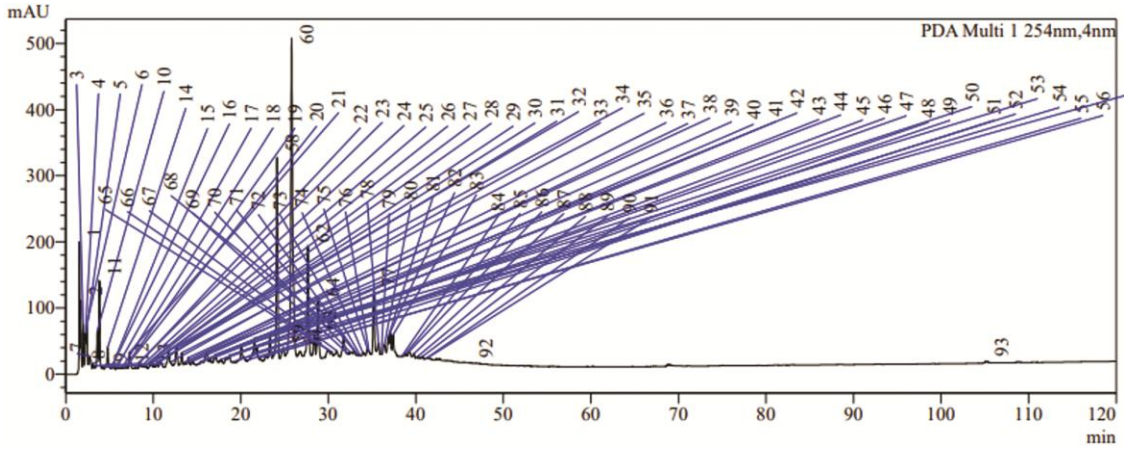

Fig. 2 — Results of phytochemical research by HPLC: in peaks 68 and 87, the compound Myricetin, and in peak 80, the compound Quercetin.

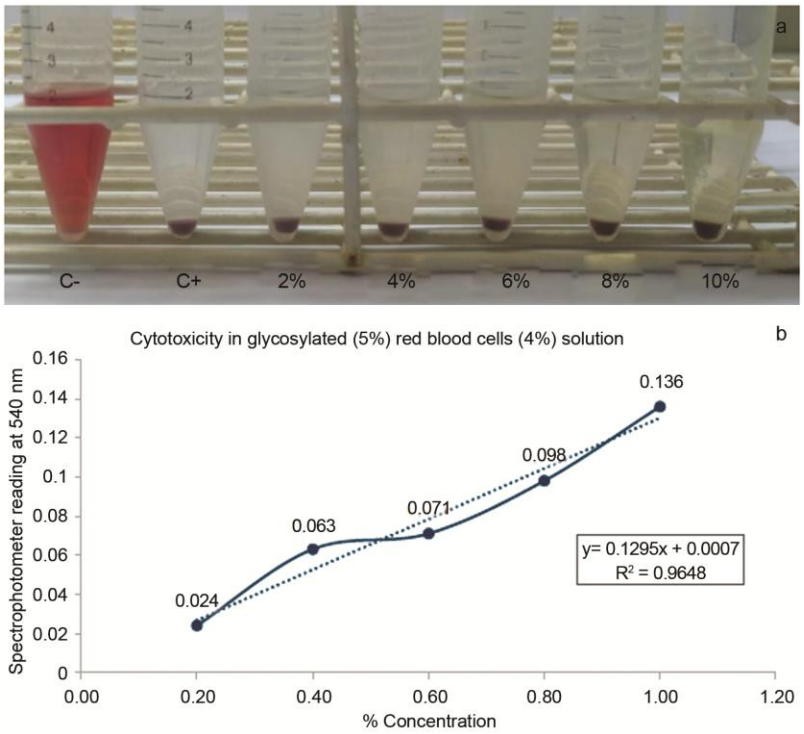

Fig. 3 — Hemolysis cytotoxicity test. a) Positive control (C+), negative control (C-), and samples at different concentrations; and b) Absorbance of the hemolysis caused in the samples.

development were observed in the embryo. However, at 100% concentration, the CE showed cytotoxicity, as expected (Fig. 4).

Discussion

Interest in new products of plant origin, rich in bioactive compounds with therapeutic properties, has increased in recent years. The fruits of *M. emarginata* are rich in nutrients and contain several benefits for human health due to their high antioxidant activity and the presence of bioactive compounds such as phenolic compounds, polyphenols, carotenoids, anthocyanins, and a high content of vitamin C[23,24].

The bioactive compounds identified in *M. emarginata* fruits have potent antioxidant, antitumor, anti-inflammatory, and anti-hyperglycemic activity[24,25]. However, there are few studies that address the search for relevant bioactive compounds in *M. emarginata* leaves.

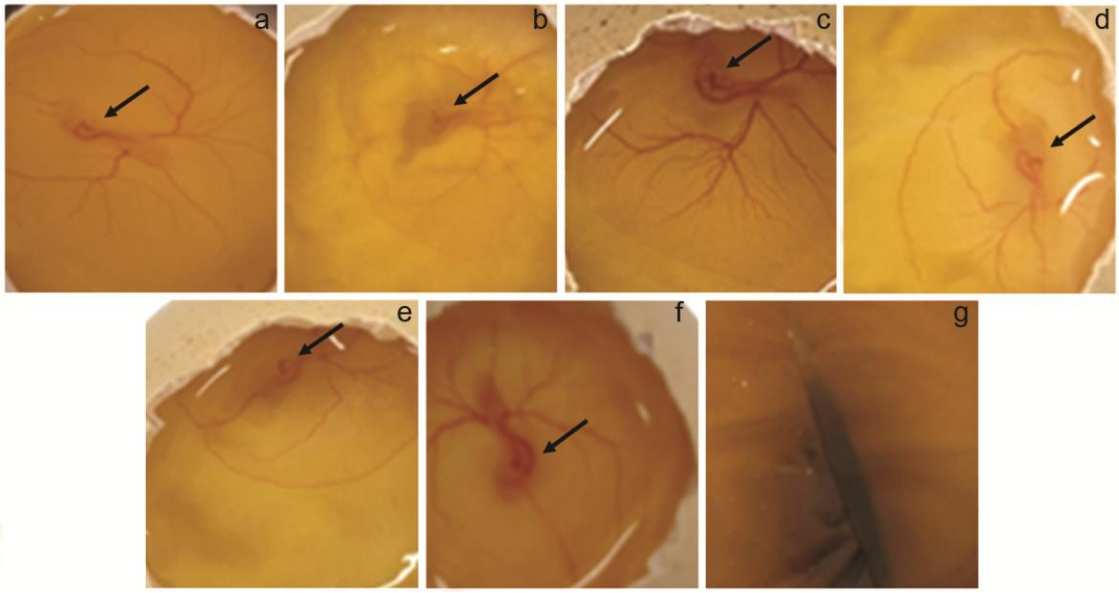

Fig. 4 — *In vivo* CAM cytotoxicity assay. a) control egg, where the saline solution was injected; b) egg that received 2% CE; c) egg that received 4% CE; d) egg that received 6% CE; e) egg that received 8% CE; f) egg that received 10% CE; and g) egg that received 100% CE. Arrows indicate embryo hearts.

Our study evaluated the crude extract of *M. emarginata* leaves. In qualitative and quantitative phytochemical analyses, we identified the presence of phenolic compounds, as has already been reported in phytochemical studies of the fruit[26-28]. The quantification of phenolic compounds in which the stable reaction that forms Aluminum Chloride (AlCl3) and quercetin was used to construct the standard curve in our study showed a large amount of total flavonoids, including catechin and gallic acid identified by HPTLC that have also been reported in studies with the fruit[2,29,30]. These findings highlighted the anti-inflammatory and antioxidant properties in the crude extract of *M. emarginata* leaves.

Although in lower quantities, total polyphenols and total alkaloids were also observed in the crude extract of *M. emarginata* leaves in this study. In an investigation that isolated three polyphenols from the fruit of *M. emarginata*, their antihyperglycemic effect was demonstrated, as these compounds strongly inhibited advanced glycation end product (AGE) formation[31]. In turn, alkaloids have important biological activities in the therapeutic and pharmacological areas, from antimicrobial to anticancer activities[32-34]. Therefore, the identification of these phytochemical compounds in our extract indicates that the *M. emarginata* leaves may have potential therapeutic use and benefits for human health.

The crude extract of *M. emarginata* leaves was also analysed by HPLC, and the presence of quercetin and myricetin was found. Quercetin is the most common flavonoid in nature and is also commonly found in species of the genus *Malpighia*[35–38]. This bioactive compound has several pharmacological activities such as anti-inflammatory, antidiarrheal and neuroprotective and is also capable of reducing levels of oxidative stress[39–42]. Studies highlight that ingesting acerola juice can induce antigenotoxic and antimutagenic effects[37,43,44].

Myricetin is another flavonoid that shows anti-inflammatory activity in several studies. It was reported that myricetin attenuated the deteriorative effects induced by cisplatin, a medication prescribed for chemotherapy, regulating levels of molecular markers of inflammation such as nuclear factor kappa B (NF-kB), nuclear factor erythroid 2-related factor 2 (Nrf-2), interleukin 6 (IL-6) and tumour Necrosis Factor α(TNF-α), in addition to improving the antioxidant status and protection of tissue damage[45]. Another study that investigated the protective effects of myricetin in a lung injury model reported that the compound alleviated acute lung injury by inhibiting the activation of macrophages, as well as inhibiting inflammation *in vitro* and *in vivo*, suggesting the possibility of a potential use therapeutic in the prevention of inflammatory diseases[46].

The antioxidant capacity of *M. emarginata* leaves crude extract measured in our research by DPPH was high, which corroborates studies on fruits. These investigations indicate that this capacity is due to the presence of vitamin C and phenolic compounds, which are highly antioxidant components[4,28,47]. Finally, hemolysis and CAM evaluations indicated low cytotoxicity up to a concentration of 10%, demonstrating that the extract up to at least this concentration does not significantly interfere with normal cellular functions. The safety related to the use of *M. emarginata* leaves extract corroborates a study that investigated the genotoxicity of pulp extract from ripe acerola fruits by comet assay in mice blood cells *in vitro*[5]. The authors observed that no acerola samples showed the potential to induce DNA damage. Furthermore, a recent study demonstrated the hepatoprotective effect of acerola leaf extract, which was superior to silymarin in reducing TNF-α induced cell death in rats' hepatocytes[48].

Conclusion

The results show that the ethanolic extract of the *M. emarginata* leaves presents important bioactive compounds, high antioxidant activity, and low cytotoxicity, making it a candidate for potential use as a herbal medicine and in future investigations of inflammatory and tumour processes.

Acknowledgement

The authors would like to thank the Analytical Center of the Chemistry Institute of the University of São Paulo (USP), in particular, the technician Vânia Blasques Bueno, for her suggestions and assistance with the HPLC.

This work was supported by the Research Program of Padre Albino University Center, grant number PESQPIN2021930 to Ana Paula Girol.

Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest. The authors declare no potential conflicts of interest.

References

1 Vieira A D S, Biscola V, de Albuquerque M A C, Bedani R and Saad S M I, Impact of acerola (*Malpighia emarginata* DC) byproduct and probiotic strains on technological and sensory features of fermented soy beverages, *J Food Sci*, 2019, **84**(12), 3726-3734, doi: 10.1111/1750-3841.14939.

2 Mezadri T, Fernández-Pachón M S, Villaño D, García-Parrilla M C and Troncoso A M, The acerola fruit: Composition, productive characteristics and economic importance, *Arch LatinoamNutr*, 2006, **56**(2), 101-109.

3 Vendramini A L and Trugo L C, Chemical composition of acerola fruit (*Malpighia punicifolia* L.) at three stages of maturity, *Food Chem*, 2000, **71**(2), 195-198, doi: 10.1016/S0308-8146(00)00152-7.

4 Mezadri T, Villaño D, Fernández-Pachón M S, García-Parrilla M C and Troncoso A M, Antioxidant compounds and antioxidant activity in acerola (*Malpighia emarginata* DC.) fruits and derivatives, *J Food Compos Anal*, 2008, **21**(4), 282-290, doi: 10.1016/j.jfca.2008.02.002.

5 Da Silva N R, Silva K V F, Da Silva S M, Richter M F, Abin-Carriquiry J A, *et al.*, Genotoxic and antigenotoxic activity of acerola (*Malpighia glabra* L.) extract in relation to the geographic origin, *Phytother Res*, 2013, **27**(10), 1495-1501, doi: 10.1002/ptr.4896.

6 Moghadasian M H, Alsaif M, Le K, Gangadaran S, Masisi K, *et al.*, Combination effects of wild rice and phytosterols on prevention of atherosclerosis in LDL receptor knockout mice, *J Nutr Biochem*, 2016, **33**, 128-135, doi: 10.1016/j.jnutbio. 2016.03.015.

7 Olaiya C O, Soetan K O and Esan A M, The role of nutraceuticals, functional foods and value added food products in the prevention and treatment of chronic diseases, *Afr J Food Sci*, 2016, **10**(10), 185-193, doi: 10.5897/ AJFS2015.1402.

8 Babbar N, Oberoi H S and Sandhu S K, Therapeutic and nutraceutical potential of bioactive compounds extracted from fruit residues, *Crit Rev Food Sci Nutr*, 2015, **55**(3), 319-337, doi: 10.1080/10408398.2011.653734.

9 Marques T R, Caetano A A, Rodrigues L M A, Simao A A, Machado G H A, *et al.*, Characterization of phenolic compounds, antioxidant and antibacterial potential the extract of acerola bagasse flour, *Acta Scientiarum Technol*, 2017, **39**(2), 143, doi: 10.4025/actascitechnol.v39i2.28410.

10 Barros B R S, Barboza B R, Ramos B A, Moura M C, Coelho L C B B, *et al*., Saline extract from *Malpighia emarginata* DC leaves showed higher polyphenol presence, antioxidant and antifungal activity andpromoted cell proliferation in mice splenocytes, *An Acad Bras Cienc*, 2019, **91**(1), doi: 10.1590/0001-3765201920180358.

11 Souza H R, Zucoloto A R, Francisco I T P, Rays H P, Tinti N P, *et al*., Evaluation of the healing properties of *Garcinia brasiliensis* extracts in a cutaneous wound model, *J Ethnopharmacol*, 2022, **295**, 115334, doi: 10.1016/ j.jep.2022.115334.

12 Dahanayake J M, Perera P K, Galappatty P, Melshandi P H D S and Arawwawala L D A M, Comparative phytochemical analysis and antioxidant activities of tamalakyadi decoction with its modified dosage forms, *Evid-Based Complement Altern Med*, 2019, **2019**, 1-9, doi: 10.1155/2019/6037137.

13 Lima R A and Silva A C, Identification class of secondary metabolites in ethanolic extract of fruits and leaves of *Eugenia uniflora* L., *Rev Eletrônica Gest Educ Tecnol Ambient*, 2016, **20**(1), 381, doi: 10.5902/2236117019537.

14 Abo-Dola M A and Lutfi M F, Anti-inflammatory activity of *Euphorbia aegyptiaca* extract in rats, *Int J Health Sci (Qassim)*, 2016, **10**(1), 69-75

15 Figueiredo S A, Vilela F M P, da Silva C A, Cunha T M, dos Santos M H, *et al.*, *In vitro* and *in vivo* photoprotective/ photochemopreventive potential of *Garcinia brasiliensis*

epicarp extract, *J Photochem Photobiol B*, 2014, **131**, 65-73, doi: 10.1016/j.jphotobiol.2014.01.004.

16 Saroni A P, Zeraik M L, Farias X V, da Fonseca L M, da Silva B, *et al.*, Redox-active biflavonoids from *Garcinia brasiliensis* as inhibitors of neutrophil oxidative burst and human erythrocyte membrane damage, *J Ethnopharmacol*, 2015, **174**, 410-418, doi: 10.1016/j.jep.2015.08.041.

17 Shamsa F, Monsef H, Ghamooshi R and Verdian-rizi M, Spectrophotometric determination of total alkaloids in some Iranian medicinal plants, *J Appl Hortic*, 2008, **12**, 69-70.

18 Jia B X, Huangfu Q Q, Ren F X, Jia L, Zhang Y B, *et al.*, Identification and quantification of flavonoids and chromes in *Baeckea frutescens* by using HPLC coupled with diode-array detection and quadruple time-of-flight mass spectrometry, *Nat Prod Res*, 2015, **29**(9), 800-806, doi: 10.1080/14786419.2014.987144.

19 Naves V M L, dos Santos M H, Ribeiro I S, da Silva C A, Silva N C, *et al.*, Antimicrobial and antioxidant activity of *Garcinia brasiliensis* extracts, *South Afr J Bot*, 2019, **124**, 244-250, doi: 10.1016/j.sajb.2019.05.021.

20 Martins F T, Doriguetto A C, de Souza T C, de Souza K R, Dos Santos M H, *et al*., Composition, and anti-inflammatory and antioxidant activities of the volatile oil from the fruit peel of *Garcinia brasiliensis*, *Chem Biodivers*, 2008, **5**(2), 251-258, doi: 10.1002/cbdv.200890022.

21 Ribatti D, The chick embryo chorioallantoic membrane (CAM) assay, *Reprod Toxicol*, 2017, **70**, 97-101, doi: 10.1016/j.reprotox.2016.11.004.

22 Kundeková B, Máčajová M, Meta M, Čavarga I and Bilčík B, Chorioallantoic membrane models of various avian species: differences and applications, *Biology (Basel)*, 2021, **10**(4), 301, doi: 10.3390/biology10040301.

23 Xu M, Shen C, Zheng H, Xu Y, Xue C, *et al*., Metabolomic analysis of acerola cherry (*Malpighia emarginata*) fruit during ripening development via UPLC-Q-TOF and contribution to the antioxidant activity, *Food Res Int*, 2020, **130**, 108915, doi: 10.1016/j.foodres.2019.108915.

24 Belwal T, Devkota H P, Hassan H A, Ahluwalia S, Ramadan M F, *et al.*, Phytopharmacology of Acerola (*Malpighia* spp.) and its potential as functional food, *Trends Food Sci Technol*, 2018, **74**, 99-106, doi: 10.1016/j.tifs.2018.01.014.

25 Dias F M, Leffa D D, Daumann F, de Oliveira M S, Luciano T F, *et al*., Acerola (*Malpighia emarginata* DC.) juice intake protects against alterations to proteins involved in inflammatory and lipolysis pathways in the adipose tissue of obese mice fed a cafeteria diet, *Lipids Health Dis*, 2014, **13**(1), 24, doi: 10.1186/1476-511X-13-24.

26 Silva P B, Duarte C R and Barrozo M A S, Dehydration of acerola (*Malpighia emarginata* D.C.) residue in a new designed rotary dryer: Effect of process variables on main bioactive compounds, *Food Bioprod Process*, 2016, **98**, 62-70, doi: 10.1016/j.fbp.2015.12.008.

27 Rezende Y R R S, Nogueira J P and Narain N, Microencapsulation of extracts of bioactive compounds obtained from acerola (*Malpighia emarginata* DC) pulp and residue by spray and freeze drying: Chemical, morphological and chemometric characterization, *Food Chem*, 2018, **254**, 281-291, doi: 10.1016/j.foodchem.2018.02.026.

28 Delva L and Schneider R G, Acerola (*Malpighia emarginata* DC): Production, postharvest handling, nutrition, and biological activity, *Food Rev Int*, 2013, **29**(2), 107-126, doi:10.1080/87559129.2012.714433.

29 Vendramini A L A and Trugo L C, Phenolic compounds in acerola fruit (Malpighia punicifolia, L.), *J Braz Chem Soc*, 2004, **15**(5), 664-668, doi: 10.1590/S0103-50532004000500009.

30 Bataglion G A, da Silva F M A, Eberlin M N and Koolen H H F, Determination of the phenolic composition from Brazilian tropical fruits by UHPLC–MS/MS, *Food Chem*, 2015, **180**, 280-287, doi: 10.1016/j.foodchem.2015.02.059.

31 Hanamura T, Hagiwara T and Kawagishi H, Structural and functional characterization of polyphenols isolated from acerola (*Malpighia emarginata* DC.) Fruit, *Biosci Biotechnol Biochem*, 2005, **69**(2), 280-286, doi: 10.1271/bbb.69.280.

32 Ettefagh K, Burns J, Junio H, Kaatz G and Cech N, Goldenseal (*Hydrastis canadensis* L.) extracts synergistically enhance the antibacterial activity of berberine via efflux pump inhibition, *Planta Med*, 2011, **77**(08), 835-840, doi: 10.1055/s-0030-1250606.

33 Qu Y, Easson M L A E, Froese J, Simionescu R, Hudlicky T, *et al.*, Completion of the seven-step pathway from tabersonine to the anticancer drug precursor vindoline and its assembly in yeast, *Proc Natl Acad Sci*, 2015, **112**(19), 6224-6229, doi: 10.1073/pnas.1501821112.

34 Bhambhani S, Kondhare K R and Giri A P, Diversity in chemical structures and biological properties of plant alkaloids, *Molecules*, 2021, **26**(11), 3374, doi: 10.3390/molecules26113374.

35 Marques T R, Cesar P H S, Braga M A, Marcussi S and Corrêa A D, Fruit bagasse phytochemicals from *Malpighia emarginata* rich in enzymatic inhibitor with modulatory action on hemostatic processes, *J Food Sci*, 2018, **83**(11), 2840-2849, doi: 10.1111/1750-3841.14330.

36 Alvarez-Suarez J M, Giampieri F, Gasparrini M, Mazzoni L, Santos-Buelga C, *et al.*, The protective effect of acerola (*Malpighia emarginata*) against oxidative damage in human dermal fibroblasts through the improvement of antioxidant enzyme activity and mitochondrial functionality, *Food Funct*, 2017, **8**(9), 3250-3258, doi: 10.1039/C7FO00859G.

37 Leffa D D, da Silva J, Daumann F, Dajori A L, Longaretti L M, *et al*., Corrective effects of acerola (*Malpighia emarginata* DC.) juice intake on biochemical and genotoxical parameters in mice fed on a high-fat diet, *Mutat Res/Fundam Mol Mech Mutagen*, 2014, **770**, 144-152, doi: 10.1016/j.mrfmmm.2013.11.005.

38 Nascimento E M M, Rodrigues F F G, Costa W D, Teixeira R N P, Boligon A A, *et al*., HPLC and *in vitro* evaluation of antioxidant properties of fruit from *Malpighia glabra* (Malpighiaceae) at different stages of maturation, *Food Chem Toxicol*, 2018, **119**, 457-463, doi: 10.1016/j.fct.2017.11.042.

39 Babujanarthanam R, Kavitha P, Mahadeva R U S and Pandian M R, Quercitrin a bioflavonoid improves the antioxidant status in streptozotocin: Induced diabetic rat tissues, *Mol Cell Biochem*, 2011, **358**(1-2), 121-129, doi: 10.1007/s11010-011-0927-x.

40 Gálvez J, Crespo M E, Jiménez J, Suárez A and Zarzuelo A, Antidiarrhoeic activity of quercitrin in mice and rats, *J Pharm Pharmacol*, 2011, **45**(2), 157-159, doi: 10.1111/j.2042-7158.1993.tb03706.x.

41 Taguchi K, Hagiwara Y, Kajiyama K and Suzuki Y, Pharmacological studies of houttuyniae herba: The anti-inflammatory effect of quercitrin, *Yakugaku Zasshi*, 1993, **113**(4), 327-333, doi: 10.1248/yakushi1947.113.4_327.

42 Hollman P C H and Katan M B, Dietary flavonoids: Intake, health effects and bioavailability, *Food Chem Toxicol*, 1999, **37**(9-10), 937-942, doi: 10.1016/S0278-6915(99)00079-4.

43 Nunes R S, Kahl V F, Sarmento M S, Richter M F, Abin-Carriquiry J A, *et al*., Genotoxic and antigenotoxic activity of acerola (*Malpighia glabra* L.) extract in relation to the geographic origin, *Phytother Res*, 2013, **27**(10), 1495-1501, doi: 10.1002/ptr.4896.

44 Nunes R S, Kahl V F, Sarmento M S, Richter M F, Costa-Lotufo L V, *et al.*, Antigenotoxicity and antioxidant activity of acerola fruit (*Malpighia glabra* L.) at two stages of ripeness, *Plant Foods Hum Nutr*, 2011, **66**(2), 129-135, doi: 10.1007/s11130-011-0223-7.

45 Rehman M U and Rather I A, Myricetin abrogates cisplatin-induced oxidative stress, inflammatory response, and goblet cell disintegration in colon of wistar rats, *Plants (Basel)*, 2019, **9**(1), doi: 10.3390/plants9010028.

46 Hou W, Hu S, Su Z, Wang Q, Meng G, *et al*., Myricetin attenuates LPS-induced inflammation in RAW 264.7 macrophages and mouse models, *Future Med Chem*, 2018, **10**(19), 2253-2264, doi: 10.4155/fmc-2018-0172.

47 Delva L and Goodrich R M, Anthocyanin identification, vitamin C content, and antioxidant capacity of acerola (*Malpighia emarginata* DC) juices, *Proc Florida State Hortic Soc*, 2010, **123**, 223-227.

48 El-Hawary S S, El-Fitiany R A, Mousa O M, Salama A A A and El Gedaily R A, Metabolic profiling and *in vivo* hepatoprotective activity of *Malpighia glabra* L. leaves, *J Food Biochem*, 2021, **45**(2), e13588, doi: 10.1111/jfbc.13588.