Journal of Applied Pharmaceutical Science 2025. Article in Press
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2025.243158
ISSN 2231-3354

Agitation-assisted extraction of total phenolic and flavonoid compounds from lanche leaves [*Myrcianthes discolor* (Kunth) McVaugh]: Influence of solvent ratio and its impact on antioxidant activity

Juan Ernesto Valdiviezo-Campos[1*], Cinthya Stephany Neglia Cermeño[1], Segundo Guillermo Ruiz-Reyes[2]

[1]Escuela de Nutrición, Universidad César Vallejo, Campus Trujillo, Perú.
[2]Departamento de Farmacotecnia, Escuela de Farmacia y Bioquímica, Universidad Nacional de Trujillo, Trujillo, Perú.

ARTICLE HISTORY

Received on: 17/02/2025
Accepted on: 18/06/2025
Available Online: XX

Key words:
Antioxidants, correlation, flavonoids, PCA, polyphenols, solvents.

ABSTRACT

Myrcianthes discolor, commonly known as "lanche," is a native species of the Peruvian highlands that has been traditionally used for its medicinal properties, particularly its antioxidant potential in counteracting oxidative stress. However, there is limited information regarding the influence of solvent composition on the extraction efficiency of its bioactive compounds. This study investigated how different solvent systems affect the extraction yield of total phenolics and flavonoids, as well as their associated antioxidant activity. Leaf samples were collected in Santa Úrsula, Baños del Inca (Cajamarca, Peru) and extracted using six solvents (acetonitrile, water, and ethanol at 30%, 50%, 70%, and 96%) under agitated and temperature-controlled conditions. The resulting extracts were analyzed for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl, 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid, and ferric reducing power assays. The highest concentrations of phenolic compounds and flavonoids were observed in the 30% and 96% ethanol extracts, reaching 59.31 mg GAE/g and 5.58 mg QCE/g of dry sample, respectively. A strong positive correlation was found between antioxidant activity and TPC, indicating that the extraction protocol effectively preserved antioxidant compounds. These results emphasize the importance of selecting the right solvent to maximize the recovery of bioactive metabolites from *M. discolor*, which supports its potential as a valuable natural source for antioxidant-rich formulations.

1. INTRODUCTION

In Latin America, traditional medicine constitutes an established and highly significant healthcare system. In Peru, medicinal plants play a fundamental role in therapeutic interventions [1]. Globally, approximately 422,000 plant species have been reported, with over 50,000 classified as medicinal; however, only a small fraction has been scientifically studied for therapeutic purposes [2]. Peru, characterized by its vast biodiversity and deep-rooted cultural tradition of medicinal plant use, is home to approximately 1,408 plant species utilized for medicinal purposes [3,4].

Today, it is crucial to identify and study new sources of bioactives with potential therapeutic activities, as well as to reveal their safe and effective use in order to establish the optimal consumption for positive outcomes [5]. Without scientific validation, traditional medicine would not persist, leading to the disappearance of traditional practices and, consequently, the loss of associated knowledge and culture [6]. Based on the sustainable use of biodiversity and the rational utilization of medicinal plants, phytotherapy can pave the way to improve the quality of life for the population and contribute to the economic and technological development of our country [7,8].

Polyphenolic compounds, including phenols, flavonoids, and anthocyanins, have been extensively studied in medicinal plants due to their diverse pharmacological properties, with antioxidant activity being the most prominent and well-researched [9]. It has been found that the majority of

*Corresponding Author
Juan Ernesto Valdiviezo-Campos, Escuela de Nutrición, Universidad César Vallejo, Campus Trujillo, Perú. E-mail: jvaldiviezo @ ucv.edu.pe

diseases are linked to oxidative stress and the accumulation of free radicals. These compounds have shown efficacy in neutralizing free radicals and halting cellular oxidative stress [10,11]. In recent years, interest has grown due to the crucial role of medicinal plants in addressing chronic diseases by counteracting oxidative stress [12,13].

Myrcianthes discolor (Kunth) McVaugh, commonly known as "lanche," "mirto," or "uñico," is a shrub that belongs to the Myrtaceae family. It can reach up to 3 m in height, with brown-grayish stems and branches; the leaves are simple, entire, opposite, coriaceous, and aromatic. The flowers are simple cymes ranging from pink to red, and the fruit is smooth, blue–black, and an ovoid drupe [14–16].

The entire fresh plant is used, with the leaves commonly consumed as an infusion, while the fruits are edible, sweet-tasting, and consumed fresh [14,16]. The consumption of the leaves as an infusion after meals aids digestion due to their high concentration of phenolic compounds, flavonoids, essential oils, and tannins, which may help alleviate stomach discomfort by reducing the effects of fermented foods. Additionally, the bark is boiled and consumed to treat kidney conditions and inflammation [16,17].

Essential oils, such as E-caryophyllene, bicyclogermacrene, β-elemen, α-cubebene, δ-cadinene, α-humulene, and limonene, have been identified in *M. discolor*; however, a complete chemical profile of this species has yet to be reported [18].

Traditionally, *M. discolor* is used as an energizing food, memory enhancer, and for treating colds, inflammation, rheumatic pain, as well as for stomach and menstrual regulation [14–16]. Furthermore, studies have highlighted pharmacological and biological properties such as antibacterial, antioxidant, and anticholinesterase activities [18]. Given the limited information on this plant species, further research will expand scientific knowledge, and by thoroughly establishing these characteristics, it will be possible to formulate effective, safe, and high-quality phytopharmaceuticals as a natural therapeutic alternative. The aim is to validate traditional usage by incorporating it as a promising functional food with medicinal potential.

The growing interest in native plants as sources of bioactive compounds has revealed a scarce characterization of extraction conditions. In the case of *M. discolor*, we investigate how solvent polarity affects extraction efficiency and antioxidant activity. This research aims to systematically evaluate the effect of different solvent systems on the extraction of phenolic and flavonoid compounds, as well as on their antioxidant capacity, by establishing correlations between phytochemical contents and antioxidant activity. The aim is to contribute to the understanding of the potential of this species for nutraceutical or pharmaceutical applications.

This research aims to determine the influence of solvent ratio and its impact on antioxidant activity in the agitation-assisted extraction of total phenolic and flavonoid compounds from *M. discolor* leaves.

2. MATERIALS AND METHODS

2.1. Reagents and solvents

Ethanol 96° GL (CKF®), distilled water (Dropaksa®), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Folin–Ciocalteu reagent (Sigma-Aldrich), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), iron(III) chloride hexahydrate, sodium bicarbonate, aluminum chloride, sodium acetate, and hydrochloric acid (Merck) were used. Gallic acid (Merck), quercetin (Sigma-Aldrich), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma-Aldrich) were used as standards.

2.2. Botanical material

The species used was *M. discolor* (Kunth) McVaugh, which was collected in the village of Santa Úrsula, Baños del Inca district, Cajamarca province, Cajamarca region, at geographic coordinates of latitude: 7.154531, and longitude: −78.40375, in UTM format (Zone: 17S East: 320951.65 meters, North: 790944.73 meters). A complete specimen was pressed and prepared according to the standard protocols of the *Herbarium Truxillense* and taxonomically identified with voucher number N° 65651.

2.3. Preparation and extraction

The *M. discolor* species acquired had its leaves selected as plant material, ensuring they were intact, free from inert material or decomposition. The plant material was dried in a Memmert oven at 40°C, followed by mechanical milling with an electric grinder to obtain fine particles. The pulverized *M. discolor* sample was extracted using six solvents: 96% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, water, and acetonitrile. A 5 g sample was used for each solvent, in a sample-to-solvent ratio of 1:20. The mixture was then placed on a heating plate with magnetic stirring (500 rpm) for 1 hour at a temperature of 100°C. The filtered extracts were stored at 6°C for further analysis [19].

2.4. Total phenols content

This was carried out using the Folin–Ciocalteu method with some modifications. In 10 ml volumetric flasks, 0.1 ml of extract was mixed with 2 ml of Folin–Ciocalteu reagent (1:10), 4.4 ml of a 7.5% sodium bicarbonate solution, and distilled water (to a final volume of 10 ml). A blank was prepared by omitting the sample. Total phenolic content (TPC) was estimated using an external standard calibration curve of gallic acid (1.26–10.08 µg/ml). After incubating the mixture for 60 minutes in the dark, the absorbance was measured at 765 nm using a UV-visible spectrophotometer (Peak Instruments C7000V). Each measurement was performed in triplicate. The total phenol concentration was expressed as gallic acid equivalents per gram of dry sample (mg GAE/g DS) [20,21].

2.5. Total flavonoid content

The total flavonoid content (TFC) was measured using the aluminum chloride colorimetric method with some modifications. In 10 ml volumetric flasks, 0.2 ml of extract was diluted with 1.5 ml of distilled water, and 0.4 ml of 10% (w/v) aluminum chloride, 0.4 ml of 1 M sodium acetate, and distilled water (to a final volume of 10 ml) were added to the mixture. TFC was estimated using an external standard calibration curve of quercetin (1–16 µg/ml). After incubating the mixture for 30 minutes in the dark, the absorbance was measured at 430

nm using a UV-visible spectrophotometer (Peak Instruments C7000V). Each measurement was performed in triplicate. The total flavonoid concentration was expressed as quercetin equivalents per gram of dry sample [22,23].

2.6. Antioxidant activity

2.6.1. 2,2-diphenyl-1-picrylhydrazyl

The free radical scavenging activity was measured using the DPPH method with some modifications. The stock solution was prepared at 0.1 mM in 96°GL ethanol. In 10 ml volumetric flasks, 300 µl of the extract was mixed with the 0.1 mM DPPH solution, gently shaken, and incubated for 30 minutes in the dark. Trolox was used as the standard for the calibration curve at concentrations ranging from 3 to 30 µM/ml. Finally, the absorbance was measured using a Peak Instruments C7000V spectrophotometer at 517 nm, with each measurement performed in triplicate. The results were expressed as Trolox equivalents (mg/100 g dry sample) [24,25].

2.6.2. 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid

The free radical scavenging activity was measured using the ABTS method with some modifications. The stock solution was prepared by mixing equal volumes of ABTS (7 mM) and $K_2S_2O_8$ (2.45 mM) and then incubated in the dark for 16 hours, followed by dilution with 50% ethanol. In 10 ml volumetric flasks, 300 µl of the extract was mixed with the ABTS solution, gently shaken, and incubated for 30 minutes in the dark. Trolox was used as the standard for the calibration curve at concentrations ranging from 3 to 20 µM/ml. Absorbance was then measured using a Peak Instruments C7000V spectrophotometer at 734 nm, with each measurement performed in triplicate. The results were expressed as Trolox equivalents (mg/100 g dry sample) [19,23].

2.6.3. Ferric reducing power

The antioxidant ferric reducing power (FRAP) was measured with some modifications. The FRAP reagent was freshly prepared before each measurement by mixing acetate buffer (300 mM), TPTZ [10 mM in HCl (40 mM)], and $FeCl_3$ (20 mM) in a ratio of 10:1:1 (v/v/v), and incubated at 37°C for 10 minutes before use. In 10 ml volumetric flasks, 300 µl of the extract was mixed with the FRAP solution, gently shaken, and incubated at 37°C for 30 minutes in the dark. Trolox was used as the standard for the calibration curve at concentrations ranging from 5 to 30 µM/ml. Absorbance was then measured using a Peak Instruments C7000V spectrophotometer at 593 nm, with each measurement performed in triplicate. The results were expressed as Trolox equivalents (mg/100 g dry sample) [26,27].

2.7. Statistical analysis

The TPC, TFC, and antioxidant activity (using different assays) were determined in three replicates. The results, presented as mean ± SD, were subjected to analysis of variance (followed by Tukey's test). The differences were considered statistically significant at $p < 0.05$. Principal component analysis (PCA) was conducted to correlate the TPC, TFC, DPPH, ABTS, and FRAP results using Jamovi v.17.05 software [28].

3. RESULTS AND DISCUSSION

The results of this study show that the type of solvent used has a significant influence on the extraction of phenolic and flavonoid compounds, as well as on the antioxidant capacity of *M. discolor* leaf extracts (Table 1).

Several studies have reported differences in polyphenol and flavonoid content depending on the polarity and composition of the extracting solvent. Lalremruati *et al*. [29] found methanol to be the most efficient solvent, while El Oihabi *et al*. [30] reported that acetone yielded the highest TPC and TFC values. Purba and Paengkoum [31] also found that ethanol was effective in extracting phytoconstituents from plant matrices.

Specifically with regard to ethanol, it has been described that higher concentrations enhance flavonoid extraction [32,33]. However, moderate ethanol-water mixtures may improve the solubility of both hydrophilic and moderately lipophilic compounds. For example, a study using a pressurized liquid extraction system with a 1:1 water:ethanol ratio revealed a greater variety of extracted compounds [34]. Similarly, TPC, TFC, and antioxidant activities have been reported to increase when ethanol concentrations range from 60% to 100% [35]. In contrast, Linhares *et al*. [36] found that a mixture of ethanol and water in a ratio of 2:8 (v/v) performed best overall in the extraction of bioactives. This supports the idea that adding

Table 1. TPC, TFC, and antioxidant activity by different methods for the leaf extract of *M. discolor* extracted by agitation using different solvents.

Dissolvents	TPC[1*]	TFC[2*]	DPPH assay[3*]	ABTS assay[3*]	FRAP assay[3*]
Acetonitrile	18.99 ± 0.0^{d}	3.33 ± 0.02^{e}	0.18 ± 0.02^{e}	0.25 ± 0.01^{f}	0.14 ± 0.02^{f}
Water	48.63 ± 0.0^{b}	3.67 ± 0.02^{d}	0.49 ± 0.06^{c}	0.65 ± 0.17^{d}	0.55 ± 0.03^{d}
30% ethanolic	58.79 ± 0.07^{a}	3.96 ± 0.02^{d}	0.71 ± 0.21^{a}	0.74 ± 0.24^{b}	0.82 ± 0.21^{a}
50% ethanolic	59.31 ± 0.01^{a}	4.30 ± 0.02^{c}	0.68 ± 0.16^{a}	0.77 ± 0.27^{a}	0.74 ± 0.09^{b}
70% ethanolic	54.53 ± 0.03^{a}	4.83 ± 0.03^{b}	0.58 ± 0.10^{b}	0.71 ± 0.23^{c}	0.69 ± 0.07^{c}
96% ethanolic	35.40 ± 0.01^{c}	5.58 ± 0.02^{a}	0.34 ± 0.01^{d}	0.46 ± 0.05^{e}	0.43 ± 0.07^{e}

*X ± DE (n = 3), 1mg of gallic acid equivalent (GAE) g−1, 2mg of quercetin equivalent (QCE) g−1, 3mg of Trolox equivalent (TE) g−1. Statistical analysis: ANOVA followed by Tukey's test. Different letters within the same column indicate statistically significant differences among treatments according to Tukey's test ($p < 0.05$).

water to organic solvents creates a more polar medium that enhances the desorption of polyphenols from the plant matrix [37]. Furthermore, the solubility of phenolic compounds in alcohols such as methanol and ethanol is increased due to their lack of sugar moieties and relatively low molecular weight.

As shown in Table 1, the substantial variation in the concentration of bioactive compounds between different solvents is strongly linked to the chemical nature and polarity of the extraction solvent. Polar-protic alcoholic solvents such as ethanol and methanol facilitate the solubilization of low-molecular-weight phenolic compounds, including glycosylated and aglycone forms, due to their ability to act as hydrogen bond donors [38].

Regarding total phenol content (Fig. 1A), values of 59.31, 58.79, and 54.53 mg gallic acid equivalents per gram of dry material (mg GAE/g DM) were obtained for the 50%, 30%, and 70% ethanolic extracts, respectively; the 50% ethanol extract was the most effective. These results are consistent with the idea that hydroalcoholic mixtures optimize the extraction of medium-polarity polyphenols.

In contrast, studies on *M. pungens* fruits revealed significantly higher TPC values. Seraglio *et al*. [39] reported values of 2,061.35 ± 51.26 and 1,739.28 ± 8.12 mg GAE/100 g for immature and mature fruits, respectively. In a comprehensive review, Peixoto *et al.* [40] reported a wide range of 28.41–59.34 mg GAE/g dry weight in *Myrtaceae* fruits. Similarly, Schulz *et*

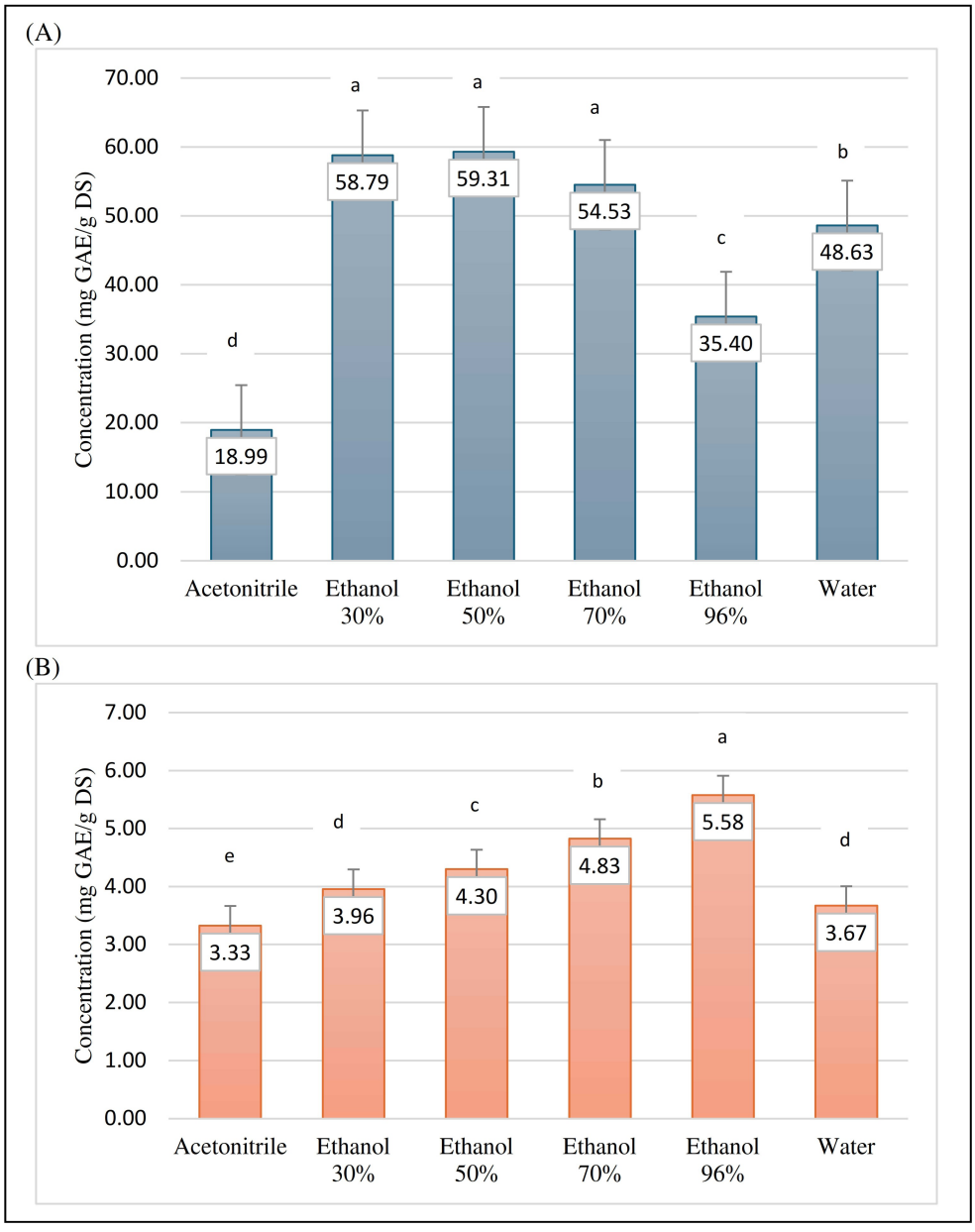

Figure 1. (A) Total phenols content and (B) total flavonoid content in *M. discolor* extracts obtained by agitation using different solvents. DS = dry sample; GAE = gallic acid equivalent; QCE = quercetin equivalent.

al. [41] found values between 1,739 and 4,613 mg GAE/100 g dry drug. These higher values are likely related to the use of different plant organs (fruits *vs.* leaves), extraction techniques, and environmental factors affecting phytochemical profiles.

Regarding TFC (see Fig. 1B), the values obtained for the 96%, 70%, and 50% ethanol extracts were 5.58 mg, 4.83 mg, and 4.30 mg quercetin equivalents per gram of dry material (mg QE/g DM), respectively. The superior performance of the 96% ethanol extract may be attributed to its effectiveness in extracting less polar flavonoid aglycones. By comparison, Andrade *et al*. [42] reported TFC values between 79.8 and 154 mg/100 g dry weight in *Myrcianthes pungens* fruit. Bombana *et al*. [32] also found very high concentrations in ethanolic *M. pungens* extracts obtained by ultrasound (10,544.04 and 1,621.78 mg QE/100 g). Spinelli *et al*. [43] further supported these findings, analyzing *M. pungens* fruit by LC-MS/MS and obtaining values of 6230 µg GAE/g and 91.61 mg QE/100 g.

Similarly, other *Myrtaceae* species exhibited diverse values. For instance, *Eugenia myrcianthes* exhibited values of 102.87 ± 1.80 µg GAE/g and 8.83 ± 0.08 µg QE/g in its extract [44], whereas the fruits of *Myrciaria cauliflora* and *Myrciaria jaboticaba* showed values of 1,443–3,160 mg and up to 6,000 mg GAE/100 g dry weight, respectively [41].

It is evident that agitation and moderate heating facilitate the release of secondary metabolites by disrupting plant cell walls, thereby enhancing compound diffusion into the solvent. Environmental factors such as temperature, rainfall, soil composition, plant maturity stage, and collection location can also contribute to phytochemical variability [45,46]. Additionally, the plant part used and the specific extraction methodology can markedly impact final yields [47,48].

Figure 2 shows the antioxidant activity of extracts prepared using different solvents. The extracts prepared using 30% and 50% ethanol exhibited the highest antioxidant capacities. Notably, the extract prepared with 30% ethanol yielded 0.82 mM TE (FRAP) and 0.71 mM TE (DPPH) per gram of dry material, whereas the extract prepared with 50% ethanol reached 0.77 mM TE in the ABTS assay. These values support the observation that intermediate ethanol concentrations favor the extraction of hydrophilic antioxidants.

Romero *et al*. [18] reported markedly higher antioxidant capacities for *M. discolor* essential oil (144.93 and 3,599.6 µM TE for ABTS and DPPH, respectively). Antonelo *et al*. [49] evaluated three *Myrtaceae* species (*M. gigantea*, *M. oblongata*, and *M. tenella*), reporting values of 26.00–139.51 µM TE/g for DPPH and 29.35–88.08 µM TE/g for ABTS. However, other studies have reported even higher values, for example, in the peel of the fruit of *M. cauliflora* (6,834.5 ± 77.9 µM TE, ABTS; 316.2 ± 211.03 µM TE, FRAP) [50] and in the leaves of *E. myrcianthes* (1,052.32 ± 3.61 µM TE, DPPH; 6,132.94 ± 429.07 µM TE, ABTS) [44].

Bombana *et al*. [32] found an ABTS scavenging capacity of 337.35 µM TE/g DS and FRAP values ranging from 28.4 to 42.6 µmol TE/g DW in *M. pungens* [51]. This data suggests that, although *M. discolor* exhibits moderate

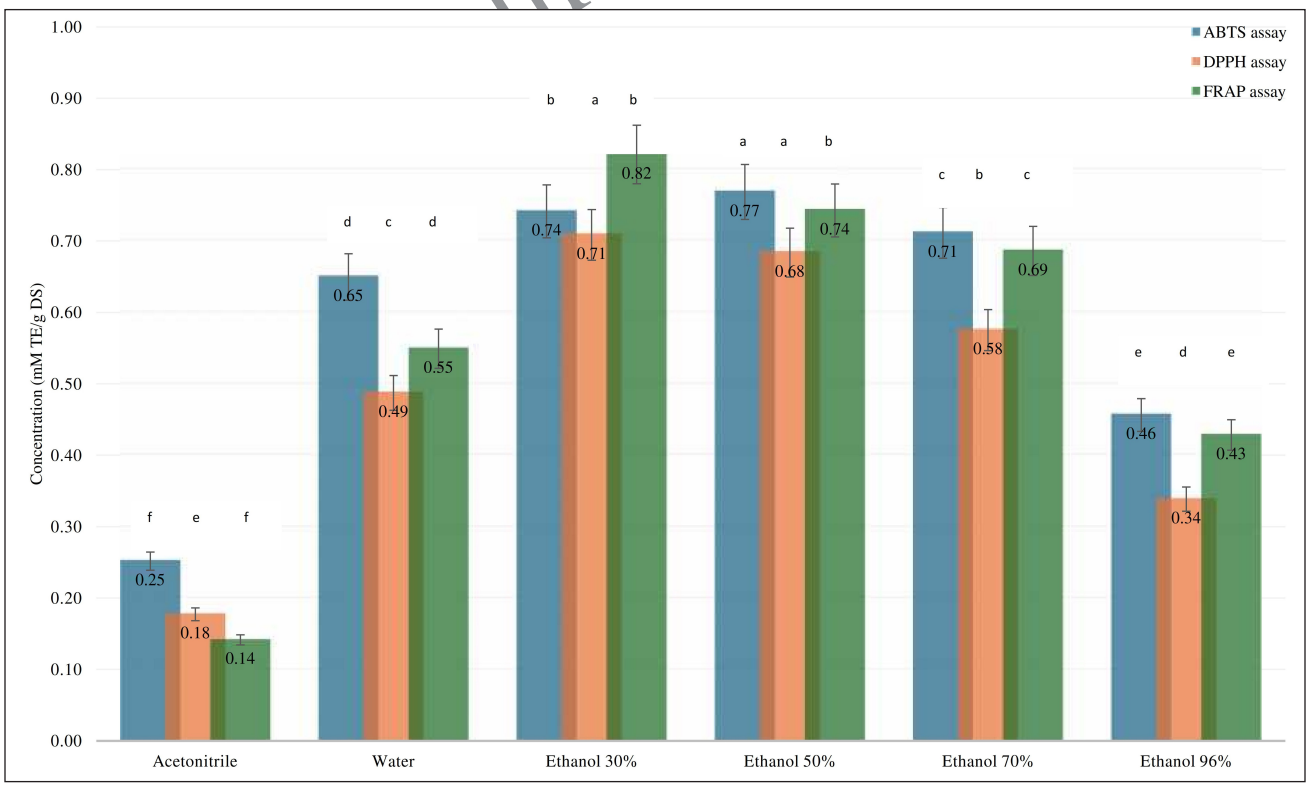

Figure 2. Antioxidant activity in *M. discolor* extracts obtained by agitation using different solvents. DS = dry sample; mM TE = millimolar Trolox equivalent.

antioxidant capacity, its efficacy is notable given the simplicity of the extraction method and solvent range used.

Of the methods applied (ABTS, DPPH, and FRAP), the highest antioxidant values were observed in the FRAP and ABTS assays. Pacheco *et al*. [52] suggested that these differences could be due to the physicochemical nature of each assay and the solubility of the active constituents. The antioxidant mechanism is primarily driven by hydrogen atom transfer (HAT), single electron transfer (SET), or mixed-mode processes [53,54]. High values in SET-based assays (such as the FRAP assay) suggest that electron donation is dominant, whereas lower responses in HAT assays may imply limited proton-donating capacity or suboptimal solvent extraction [49].

The results of this study highlight the promising antioxidant potential of *M. discolor* and support its inclusion in nutraceuticals as a means of mitigating oxidative stress-related pathologies. Furthermore, Romero *et al*. [18] emphasized the role of sesquiterpenes in *M. discolor* essential oil in inhibiting acetylcholinesterase, thereby enhancing its pharmacological relevance.

Nevertheless, data on *M. discolor* remain scarce, indicating the need for further research into its chemical profile and biological activities. A better understanding could

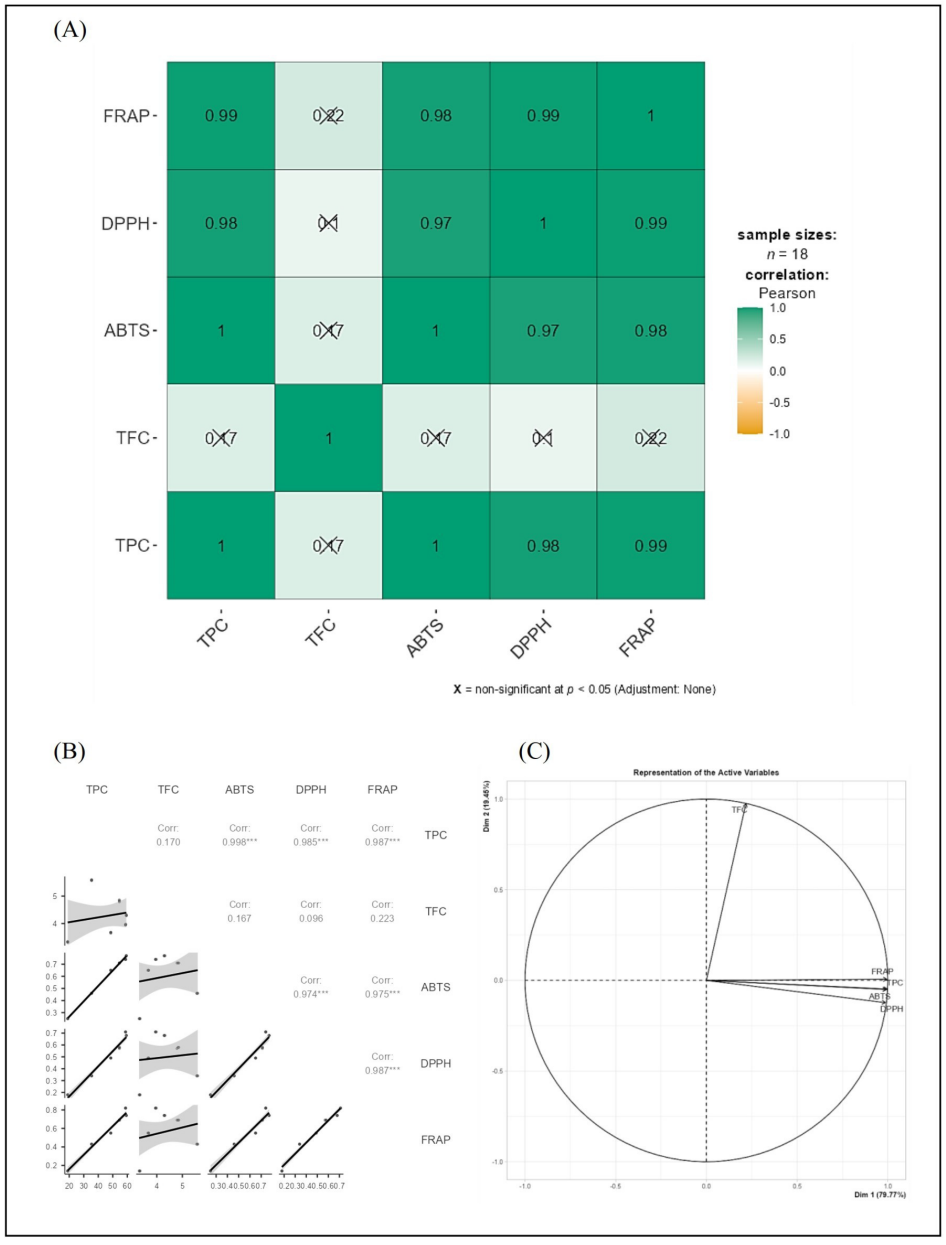

Figure 3. Correlation and PCA of TPC, TFC, and antioxidant activity by different methods in *M. discolor* extracts obtained by agitation using different solvents.

lead to applications in the food, pharmaceutical, and cosmetic industries.

Figure 3A shows the correlations and PCA of TPC, TFC, and antioxidant activity. Strong correlations were observed between TPC and the antioxidant assays: ABTS (R^2 = 0.998), FRAP (R^2 = 0.987), and DPPH (R^2 = 0.985). Similarly, FRAP showed a strong correlation with both DPPH (R^2 = 0.987) and ABTS (R^2 = 0.975) (Fig. 3B). However, no significant correlation was found with TFC, suggesting that phenolic compounds, rather than flavonoids, are the main contributors to antioxidant capacity.

PCA revealed that TPC and the antioxidant assays formed a principal cluster, while TFC was less associated (Fig 3C). The first two components explained 79.77% and 19.45% of the variance, respectively, totaling 99.22%. This aligns with prior studies on *M. pungens*, in which phenolic compounds were identified as the main contributors to antioxidant function [55], and with evidence from other *Myrtaceae* species, which link TPC more strongly than TFC to radical-scavenging potential [26,56,57].

This study is one of the few experimental assessments of *M. discolor*. However, it is limited by the absence of metabolite identification through advanced chromatographic or spectrometric methods. The solvent range was restricted to polar systems, which may have excluded bioactive lipophilic constituents. Future research should, therefore, incorporate broader solvent systems (e.g., acetone and ethyl acetate), in-depth metabolomic profiling (e.g., LC-MS and GC-MS), and bioactivity-guided fractionation, in order to better understand the full therapeutic potential of this species and promote its use in health-related applications.

4. CONCLUSION

The highest TPC was obtained with a 50% ethanol solvent, while the highest TFC was achieved with a 96% ethanol solvent. The extracts demonstrated excellent antioxidant activity, particularly in the 30% and 50% ethanolic extracts. Strong positive correlations ($R^2 > 0.95$) were found between the FRAP, ABTS, and DPPH assays and TPC, whereas TFC showed no correlation. However, the lack of correlation between TFC and these methods does not imply that TFC is unsuitable for measurement in *M. discolor* extracts. It is recommended to continue research on this species, as there is limited information available, which could enhance the potential for therapeutic applications.

5. ACKNOWLEDGMENTS

The authors would like to express their gratitude to the technical support staff from the Laboratory of Nutrition at the School of Nutrition at Universidad César Vallejo and the Laboratory of Pharmacognosy at Universidad Nacional de Trujillo for their valuable assistance.

6. AUTHORS CONTRIBUTION

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. FINANCIAL SUPPORT

This research was funded by the Vice-Rectorate for Research of Universidad César Vallejo through Resolution No. 194-2023-VI-UCV, with project code P-2023-198, entitled "Potential source of polyphenols and antioxidants from the species *Myrcianthes discolor* (Kunth) McVaugh."

8. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This project was approved by the Ethics Committee of the School of Nutrition at César Vallejo University with registration code PI-CEI-NUTRICIÓN-002.

10. DATA AVAILABILITY

All data generated and analyzed are included in this research article.

11. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Tácuna-Calderón A, Moncada–Mapelli E, Lens-Sardón L, Huaccho-Rojas J, Gamarra-Castillo F, Salazar-Granara A. Estrategias de la organización mundial de la salud en medicina tradicional y reconocimiento de sistemas de medicina tradicional. Rev Cuerpo Med HNAAA. 2020;13(1):101–2. doi: https://doi.org/10.35434/rcmhnaaa.2020.131.633
2. Astutik S, Pretzsch J, Ndzifon Kimengsi J. Asian medicinal plants' production and utilization potentials: a review. Sustainability 2019;11(19):5483. doi: https://doi.org/10.3390/su11195483
3. Aguirre LG, Pereyra-Aguilar P, Silva-Arrieta-Ontaneda I, Alarcón-Urbina M, Medina-Salazar H. Consumo de plantas medicinales en usuarios del "Centro Integral del Adulto Mayor" de La Punta-Callao (Perú). Rev Fitoter. 2016;16(2):165–75.
4. Bermúdez del Sol A, Bravo SLR, Abreu NR, Kanga EF. Traditional use of medicinal plants by the population of the municipality of Santa Clara, Cuba. J Pharm Pharmacogn Res. 2018;6(1):374–85. doi: https://doi.org/10.56499/jppres18.395_6.5.374
5. Moncada–Mapelli E, Salazar-Granara A. Medicina tradicional y COVID-19, oportunidad para la revaloración de las plantas medicinales peruanas. Rev Cuerpo Med HNAAA. 2020;13(1):103–4. doi: https://doi.org/10.35434/rcmhnaaa.2020.131.634

6. Bahmanzadegan JM, Mehdizadeha A, Tafazoli V. The conceptual incommensurability of inference between evidence-based and traditional medical paradigms. J Islam Iran Tradit Med. 2021;12(3):209–19.
7. Leite PM, Camargos LM, Castilho RO. Recent progess in phytotherapy: a Brazilian perspective. Eur J Integr Med. 202;41:101270. doi: https://doi.org/10.1016/j.eujim.2020.101270
8. Leonida AMG, Caballero AR. Aleaf: an android-based phytotherapy leaf recognition using custom vision machine learning. 2022 7th International Conference on Business and Industrial Research (ICBIR); Piscataway, NJ: IEEE; 2022. pp 488–93. doi: https://doi.org/10.1109/icbir54589.2022.9786509
9. Yan Y, Castellarin SD. Blueberry water loss is related to both cuticular wax composition and stem scar size. Postharvest Biol Technol. 2022;188:111907. doi: https://doi.org/10.1016/j.postharvbio.2022.111907
10. Jain C, Khatana S, Vijayvergia R. Bioactivity of secondary metabolites of various plants: a review. Int J Pharm Sci Res. 2019;10(2):494–504. doi: https://doi.org/10.13040/IJPSR.0975-8232.10(2).494-04
11. Koop BL, da Silva MN, da Silva FD, dos Santos LKT, Santos SL, de Andrade JC, *et al.* Flavonoids, anthocyanins, betalains, curcumin, and carotenoids: sources, classification and enhanced stabilization by encapsulation and adsorption. Food Res Int. 2022;153:110929. doi: https://doi.org/10.1016/j.foodres.2021.110929
12. de Arruda NE, de Lima CL, da Silva CJ, de Lima VLAG, dos Santos AJ. *In vitro* anticancer properties of anthocyanins: a systematic review. Biochim Biophys Acta Rev Cancer 2022;1877(4):188748. doi: https://doi.org/10.1016/j.bbcan.2022.188748
13. Lamdan H, Garcia-Lazaro RS, Lorenzo N, Caligiuri LG, Alonso DF, Farina HG. Anti-proliferative effects of a blueberry extract on a panel of tumor cell lines of different origin. Exp Oncol. 2023;42(2):101–8. doi: https://doi.org/10.32471/exp-oncology.2312-8852.vol-42-no-2.14766
14. Paniagua-Zambrana NY, Bussmann RW. *Myrcianthes discolor* (Kunth) McVaugh *Myrcianthes fragrans* (Kunth) McVaugh *Myrcianthes hallii* (O. Berg.) McVaugh Myrtaceae. In: Paniagua-Zambrana N, Bussmann R, editors. Ethnobotany of mountain regions. Cham, Switzerland: Springer International Publishing; 2020. pp. 1–4.
15. Bussmann RW, Ashley G, Sharon D, Chait G, Diaz D, Pourmand K, *et al.* Proving that traditional knowledge works: the antibacterial activity of northern Peruvian medicinal plants. Ethnobot Res App. 2011;9:67–96.
16. Alva EJM. Etnobotánica y características morfológicas de la vegetación leñosa en un remanente de bosque de la microcuenca río grande, La Encañada-Cajamarca. Cajamarca, Peru: Universidad Nacional de Cajamarca; 2017. Available from: http://hdl.handle.net/20.500.14074/1694
17. Vera IC. Importancia cultural de la flora silvestre utilizada por los pobladores del caserío de Cabrero en la microcuenca Quebrada Honda (Cajabamba, Cajamarca, Perú). Lima, Peru: Universidad Nacional Mayor de San Marcos; 2018. Available from: https://hdl.handle.net/20.500.12672/10051
18. Romero D, Cartuche L, Valarezo E, Cumbicus N, Morocho V. Chemical profiling, anticholinesterase, antioxidant, and antibacterial potential of the essential oil from *Myrcianthes discolor* (Kunth) McVaugh, an aromatic tree from southern Ecuador. Antibiotics 2023;12(4):677. doi: https://doi.org/10.3390/antibiotics12040677
19. Florian VNE, Sisniegas CGM, Valdiviezo-Campos JE. Effect of different extraction solvents on the total phenolic content and antioxidant activity of *Brassica oleracea* var. *italica*. Pharmacogn J. 2025;17(1):58–62. doi: https://doi.org/10.5530/pj.2025.17.7
20. Al Hashemi MY, Al Maktoumi H, Akhtar MdJ, Khan SA. Antioxidant activity and in silico anticholinesterase studies of major phenolic constituents of three commercial olive oils: a comparative study. Pharmacol Res - Nat Prod. 2024;2:100012. doi: https://doi.org/10.1016/j.prenap.2023.100012
21. Valdiviezo-Campos JE, Olascuaga-Castillo KA, Ruiz-Reyes SG. Ethnobotany, total phenolic and flavonoid content in the species *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson. J Appl Pharm Sci. 2024;14(7):82–9. doi: https://doi.org/10.7324/japs.2024.172838
22. Sari KRP, Ikawati Z, Danarti R, Hertiani T. Micro-titer plate assay for measurement of total phenolic and total flavonoid contents in medicinal plant extracts. Arab J Chem. 2023;16(9):105003. doi: https://doi.org/10.1016/j.arabjc.2023.105003
23. Costea L, Chițescu CL, Boscencu R, Ghica M, Lupuliasa D, Mihai DP, *et al.* The polyphenolic profile and antioxidant activity of five vegetal extracts with hepatoprotective potential. Plants 2022;11(13):1680. doi: https://doi.org/10.3390/plants11131680
24. Bibi N, Shah MH, Khan N, Al-Hashimi A, Elshikh MS, Iqbal A, *et al.* Variations in total phenolic, total flavonoid contents, and free radicals' scavenging potential of onion varieties planted under diverse environmental conditions. Plants 2022;11(7):950. doi: https://doi.org/10.3390/plants11070950
25. Olascuaga-Castillo K, Castillo-Medina O, Villacorta-Zavaleta M, Altamirano-Sarmiento D, Cáceres-Andonaire E, Valdiviezo-Campos JE, Blanco-Olano C. *Muehlenbeckia volcanica* (Benth.) Endl.: contenido fenólico y actividad antioxidante de un fruto andino peruano. Interciencia 49(3):187–91.
26. Rumpf J, Burger R, Schulze M. Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins. Int J Biol Macromol. 2023;233:123470. doi: https://doi.org/10.1016/j.ijbiomac.2023.123470
27. Belew AA, Gebre SH. Comparative assessment of phenolic and flavonoid contents and antioxidant activities in methanol extracts of spices from Jigjiga market, Ethiopia. Pharmacol Res - Nat Prod. 2025;6:100168. doi: https://doi.org/10.1016/j.prenap.2025.100168
28. The jamovi project. jamovi. (Version 2.6) [Computer Software]. 2024. Available from https://www.jamovi.org.
29. Lalremruati M, Lalmuansangi C, Siama Z. Free radical scavenging activity and antioxidative potential of various solvent extracts of *Mussaenda macrophylla* Wall: an *in vitro* and *ex vivo* study. J Appl Pharm Sci. 2019;9(12):94–102. doi: https://doi.org/10.7324/JAPS.2019.91213
30. El Oihabi M, Soultana M, El Fellah I, Fakih Lanjri H, Ben Allal L, Ammari M, *et al.* Optimized extraction of phenolic compounds and antioxidant activity from cannabis co-products via a combination of solvent-ultrasound-assisted extraction, response surface methodology, and sensitivity analysis. Case Stud Chem Environ Eng. 2024;10:100906. doi: https://doi.org/10.1016/j.cscee.2024.100906
31. Purba RAP, Paengkoum P. Bioanalytical HPLC method of *Piper betle* L. for quantifying phenolic compound, watersoluble vitamin, and essential oil in five different solvent extracts. J Appl Pharm Sci. 2019;9(05):33–9. doi: https://doi.org/10.7324/JAPS.2019.90504
32. Bombana VB, do Nascimento LH, Rigo D, Fischer B, Colet R, Paroul N, *et al.* Extraction by maceration, ultrasound, and pressurized liquid methods for the recovery of anthocyanins present in the peel of guabiju (*Myrcianthes pungens*). Sustain Chem Pharm. 2023;36:101264. doi: https://doi.org/10.1016/j.scp.2023.101264
33. Ursu MGS, Milea Ștefania A, Păcularu-Burada B, Dumitrașcu L, Râpeanu G, Stanciu S, *et al.* Optimizing of the extraction conditions for anthocyanin's from purple corn flour (*Zea mays* L): evidences on selected properties of optimized extract. Food Chem X. 2023;17:100521. doi: https://doi.org/10.1016/j.fochx.2022.100521
34. Zandoná GP, Bagatini L, Woloszyn N, de Souza Cardoso J, Hoffmann JF, Moroni LS, *et al.* Extraction and characterization of phytochemical compounds from araçazeiro (*Psidium cattleianum*) leaf: Putative antioxidant and antimicrobial properties. Food Res Int. 2020;137:109573. doi: https://doi.org/10.1016/j.foodres.2020.109573
35. Arya OP, Bhatt ID, Mohanty K. Effect of different extraction solvents on bioactive phenolics and antioxidant potential of *Illicium griffithii*

fruit. J Appl Res Med Aromat Plants 2024;40:100547. doi: https://doi.org/10.1016/j.jarmap.2024.100547
36. Linhares SN, Maziero FH, Murillo-Franco SL, Oliviera BM, Vicente ML, da Silva DV, *et al.* Investigating the influence of solvents and extraction methods on the efficacy of phenolic compound recovery from spent coffee grounds. Sep Purif Technol. 2025;362:131793. doi: https://doi.org/10.1016/j.seppur.2025.131793
37. Solomakou N, Loukri A, Tsafrakidou P, Michaelidou AM, Mourtzinos I, Goula AM. Recovery of phenolic compounds from spent coffee grounds through optimized extraction processes. Sustain Chem Pharm. 2022;25:100592. doi: https://doi.org/10.1016/j.scp.2021.100592
38. Martínez-Ramos T, Benedito-Fort J, Watson NJ, Ruiz-López II, Che-Galicia G, Corona-Jiménez E. Effect of solvent composition and its interaction with ultrasonic energy on the ultrasound-assisted extraction of phenolic compounds from Mango peels (*Mangifera indica* L.). Food Bioprod Process 2020;122:41–54. doi: https://doi.org/10.1016/j.fbp.2020.03.011
39. Seraglio SKT, Schulz M, Nehring P, Della Betta F, Valese AC, Daguer H, *et al.* Nutritional and bioactive potential of Myrtaceae fruits during ripening. Food Chem. 2018;239:649–56. doi: https://doi.org/10.1016/j.foodchem.2017.06.118
40. Peixoto Araujo NM, Berni P, Zandoná LR, Toledo NMV de, Silva PPM da, Toledo AA de, *et al.* Potential of Brazilian berries in developing innovative, healthy, and sustainable food products. Sustain Food Technol. 2024;2(3):506–30. doi: https://doi.org/10.1039/d3fb00130j
41. Schulz M, Seraglio SKT, Brugnerotto P, Gonzaga LV, Costa ACO, Fett R. Composition and potential health effects of dark-colored underutilized Brazilian fruits – a review. Food Res Int. 2020;137:109744. doi: https://doi.org/10.1016/j.foodres.2020.109744
42. Andrade JMM, Aboy AL, Apel MA, Raseira MCB, Pereira JFM, Henriques AT. Phenolic composition in different genotypes of guabiju fruits (*Myrcianthes pungens*) and their potential as antioxidant and antichemotactic agents. J Food Sci. 2011;76(8):C1181–7. doi: https://doi.org/10.1111/j.1750-3841.2011.02375.x
43. Spinelli LV, Anzanello MJ, Areze da Silva SR, Carbom MC, Freo SJ, Aparecida SSM, *et al.* Uncovering the phenolic diversity of Guabiju fruit: LC-MS/MS-based targeted metabolomics approach. Food Res Int. 2023;173:113236. doi: https://doi.org/10.1016/j.foodres.2023.113236
44. Dalmagro M, Donadel G, Moraes Pinc M, Becker Viana AP, Klein EJ, da Silva EA, *et al.* Exploring antioxidant and α-glucosidase inhibition in *Eugenia* L. extracts: a comprehensive phytochemical study. Nat Prod Res. 2024;38:1–7. doi: https://doi.org/10.1080/14786419.2024.2352868
45. Paludo M, Colombo R, Teixeira J, Hermosín-Gutiérrez I, Ballus C, Godoy H. Optimizing the extraction of anthocyanins from the skin and phenolic compounds from the seed of jabuticaba fruits (*Myrciaria jabuticaba* (Vell.) O. Berg) with ternary mixture experimental designs. J Braz Chem Soc. 2019;30(7):1506–14. doi: https://doi.org/10.21577/0103-5053.20190047
46. Rienth M, Vigneron N, Darriet P, Sweetman C, Burbidge C, Bonghi C, *et al.* Grape berry secondary metabolites and their modulation by abiotic factors in a climate change scenario–a review. Front Plant Sci. 2021;12:643258. doi: https://doi.org/10.3389/fpls.2021.643258
47. Hosseinzadeh L, Mirzaei S, Hajialyani M, Ahmadi F, Emami SA, Mojarrab M. The protective effect of different extracts of aerial parts of *Artemisia ciniformis* against H2O2-induced oxidative stress and apoptosis in PC12 pheochromocytoma cells. J Appl Pharm Sci. 2019;9(4):16–23. doi: https://doi.org/10.7324/JAPS.2019.90403
48. Halim MA, Kanan KA, Nahar T, Rahman MJ, Ahmed KS, Hossain H, *et al.* Metabolic profiling of phenolics of the extracts from the various parts of blackberry plant (*Syzygium cumini* L.) and their antioxidant activities. LWT. 2022;167:113813. doi: https://doi.org/10.1016/j.lwt.2022.113813
49. Antonelo FA, Rodrigues MS, Cruz LC, Pagnoncelli MG, Cunha MAA da, Bonatto SJR, *et al.* Bioactive compounds derived from Brazilian Myrtaceae species: chemical composition and antioxidant, antimicrobial and cytotoxic activities. Biocatal Agric Biotechnol. 2023;48:102629. doi: https://doi.org/10.1016/j.bcab.2023.102629
50. Lamas CA, Lenquiste SA, Baseggio AM, Cuquetto-Leite L, Kido LA, Aguiar AC, *et al.* Jaboticaba extract prevents prediabetes and liver steatosis in high-fat-fed aging mice. J Funct Foods. 2018;47:434–46. doi: https://doi.org/10.1016/j.jff.2018.06.005
51. Betta FD, Nehring P, Seraglio SKT, Schulz M, Valese AC, Daguer H, *et al.* Phenolic compounds determined by LC-MS/MS and *in vitro* antioxidant capacity of Brazilian fruits in two edible ripening stages. Plant Foods Hum Nutr. 2018;73(4):302–7. doi. https://doi.org/10.1007/s11130-018-0690-1
52. Pacheco AFC, de Souza LB, Paiva PHC, Lelis CA, Vieira ENR, Tribst AAL, *et al.* Impact of ultrasound on pumpkin seed protein concentrate hydrolysis: effects on alcalase, protein, and assisted reaction. Appl Food Res. 2023;3(1):100281. doi: https://doi.org/10.1016/j.afres.2023.100281
53. Jerônimo LB, da Costa JS, Pinto LC, Montenegro RC, Setzer WN, Mourão RHV, *et al.* Antioxidant and cytotoxic activities of Myrtaceae essential oils rich in terpenoids from Brazil. Nat Prod Commun. 2021;16(2):1–13. doi: https://doi.org/10.1177/1934578x21996156
54. Sharopov FS, Wink M, Setzer WN. Radical scavenging and antioxidant activities of essential oil components – an experimental and computational investigation. Nat Prod Commun. 2015;10(1). doi: https://doi.org/10.1177/1934578x1501000135
55. Machado PG, Londero DS, Farias CAA, Pudenzi MA, Barcia MT, Ballus CA. Guabijú (*Myrcianthes pungens*): a comprehensive evaluation of anthocyanins and free, esterified, glycosylated, and insoluble phenolic compounds in its peel, pulp, and seeds. Food Chem. 2024;432:137296:1–13. doi: https://doi.org/10.1016/j.foodchem.2023.137296
56. Astyka R, Zaitun HPA, Sumaiyah S, Juwita NA, Lubis MF. Optimization of microwave-assisted extraction of total flavonoid content from red betel leaf (*Piper crocatum* Ruiz and Pav) and its correlation with antioxidant and antibacterial activities using response surface methodology. J Appl Pharm Sci. 2024;14(8):150–9. doi: https://doi.org/10.7324/japs.2024.170411
57. Yuliana N, Nurainy F, Sari GW, Sumardi, Widiastuti EL. Total microbe, physicochemical property, and antioxidative activity during fermentation of cocoa honey into kombucha functional drink. Appl Food Res. 2023;3(1):100297. doi: https://doi.org/10.1016/j.afres.2023.100297

How to cite this article:
Valdiviezo-Campos JE, Cermeño CSN, Ruiz-Reyes SG. Agitation-assisted extraction of total phenolic and flavonoid compounds from lanche leaves [*Myrcianthes discolor* (Kunth) McVaugh]: Influence of solvent ratio and its impact on antioxidant activity. J Appl Pharm Sci. 2025. Article in Press.
http://doi.org/10.7324/JAPS.2025.243158