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Application of ethanol under pressurized conditions for the extraction of phytochemical compounds from pink pepper fruit

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ABSTRACT. The objective of this study was to extract phytochemical compounds from pink pepper (*Schinus terebinthifolius* Raddi) fruit by pressurized liquid extraction (PLE) using ethanol as solvent under dynamic conditions. The effects of temperature (40, 60, and 80°C) and extraction time (15, 30, and 45 min.) on global extraction yield and extract composition (total phenolic content and antioxidant activity) were evaluated. PLE results were compared with those obtained by Soxhlet extraction. The chemical composition and antibacterial activity of extracts were determined. Longer extraction times resulted in higher extraction yields (30.42 wt%). Higher temperatures also increased extraction yield, enhancing the recovery of phenolic compounds and, consequently, resulting in higher antioxidant activity. PLE demonstrated selectivity in phytochemical extraction compared with Soxhlet extraction. The PLE extract obtained under the best conditions for total phenolic content and antioxidant activity (80°C and 45 min.) and the Soxhlet extract consisted predominantly of 5-hydroxymethylfurfural and sesquiterpenes. The major components of the lipid fraction were linoleic and oleic acids. The extracts showed antibacterial activity, attributed to the presence of 5-hydroxymethylfurfural and phenolic compounds.

Keywords: Antibacterial activity; antioxidant activity; pressurized liquid extraction; *Schinus terebinthifolius* Raddi.

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Introduction

The fruits of *Schinus terebinthifolius* Raddi, a plant commonly known as pink pepper or Brazilian peppertree, contain phytochemicals with protective effects, such as tannins, alkaloids, flavonoids, anthocyanins, phenolic acids, biphenyl esters, bioflavonoids, and terpenes (Feuereisen et al., 2014; Ennigrou, Casabianca, Laarif, Hanchi, Hosni, 2017; Feuereisen, Barraza, Zimmermann, Schieber, Schulze-Kaysers, 2017). These compounds are capable of inhibiting the formation of free radicals, protecting DNA and bovine serum albumin proteins from oxidative stress (Ricordi, Garcia-Contreras, & Farnetti, 2015; Feriani et al., 2021). Some of the secondary metabolites found in pink pepper fruit have great therapeutic potential, having anti-inflammatory, antipyretic, analgesic (Johann et al., 2010; Carvalho, Melo, Aragão, Raffin, & Moura, 2013; Silva et al., 2017b), and antimicrobial properties (Silva et al., 2017a; Dannenberg, Funck, Silva, & Fiorentini, 2019).

It is possible to recover compounds from plant matrices by using pressurized liquid extraction (PLE), whereby the simultaneous action of temperature and pressure maintains the solvent in liquid state (Plaza & Turner, 2015). Under these conditions, it is possible to modify solvent density and promote its solvation (Teo, Tan, Yong, Hew, & Ong, 2010), resulting in increased compound recovery rates owing to better solvent diffusion inside pores of the matrix (Camel, 2001; Mustafa & Turner, 2011).

PLE stands out for its ability to extract compounds from complex matrices, showing good efficiency in extracting phytochemicals with low consumption of solvent and energy, high thermal stability, and short extraction times, providing high mass yields and high-quality products (Señoráns & Luna, 2012; Rifna, Misra, & Dwivedi, 2023). Another advantage of this technique is the possibility of using environmentally friendly solvents that can act selectively on a wide range of compound polarities (Lefebvre, Destandau, & Lesellier,

2021). For instance, ethanol, a renewable solvent, is capable of extracting polar compounds from plant matrices (Fadhil, Nayyef, & Sedeeq, 2020).

Feuereisen et al. (2017) applied PLE under static conditions to extract compounds from pink pepper fruit. The authors used ethanol acidified with acetic acid to selectively enhance the extraction of anthocyanins and biflavonoids. Rebelatto, Rodrigues, Rudke, Andrade, and Ferreira (2020) performed PLE with ethanol as solvent to extract secondary metabolites from pink pepper fruit. The authors fixed the extraction time at 30 min. and used a fruit-to-solvent ratio of 0.041 g mL^{-1}.

Based on the information presented above and aiming to foster the use of emerging extraction techniques for plant matrices, this study aimed to assess the extraction of phytochemical compounds from pink pepper fruit using ethanol as solvent under pressurized conditions. The effect of process variables (temperature and time) on overall extraction yield, total phenolic content, and antioxidant activity was evaluated, and the results were compared to those obtained by Soxhlet extraction. The extract obtained under the best conditions was characterized by gas chromatography coupled to mass spectrometry and assayed for antibacterial activity.

Materials and methods

For extract preparation, pink pepper fruits (Vida em Grãos, Produtos Naturais, moisture content of 8.89 ± 0.63 wt%) were purchased at a local market in Maringá, Paraná, Brazil. Ethanol (99.9%, Honeywell) and *n*-hexane (99.0%, Anidrol) were used as solvents.

The following reagents were used for determination of total phenolic compounds and antioxidant activity: n-hexane (98.5%, Synth), methanol (99.9%, PanReac), gallic acid (97.6%, Vetec), Folin–Ciocalteu reagent (Dinâmica), sodium carbonate (99.5%, Anidrol), ethanol (99.5%, Êxodo Científica; 99.5%, Anidrol), 2,2-diphenyl-1-picrylhydrazyl (DPPH•, 95.0%, Sigma–Aldrich), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97.0%, Sigma–Aldrich), 2,4,6-tris(2-piridil)-s-triazine (TPTZ, 99.0%, Sigma–Aldrich), ferrous sulfate heptahydrate (99.0%, Synth), and ferric chloride hexahydrate (97.0%, Synth).

For determination of chemical profile, ethanol (99.5%, Honeywell) was used as dilution solvent and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 1% TMCS, Fluka) as derivatization agent. Fatty acid composition was determined using methanol (99.9%, PanReac), potassium hydroxide (85.0%, Synth), sulfuric acid (H_2SO_4, 95.0%, Anidrol), and heptane (99.0%, Anidrol).

The following materials were used for antibacterial assays: brain heart infusion (BHI) broth (Kasvi), Tween 80 (Sigma–Aldrich), 2,3,5-triphenyltetrazolium chloride (TTC, Sigma–Aldrich), *Staphylococcus aureus* (ATCC 12026), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 25922), and *Salmonella enterica* subspecies *enterica* (CCCD S016).

Prior to extraction, fruits were separated from peels. Each fraction was ground separately and sieved (Bertel, Caieiras, São Paulo, Brazil) to a particle size of about 2.3 mm. The samples used for extraction consisted of a mixture of fruit and peel at a 3:1 (w/w) ratio.

Extraction assays

Figure 1 shows the experimental apparatus used in extraction assays, which is similar to that previously used by Mello, Stevanato, Cardozo Filho, and Silva (2021), except for the lack of a heated extraction bed, which was replaced by a heating bath (Quimis®, Q334M, Diadema, São Paulo, Brazil). In each run, the extractor was fed with sample (~2 g), and the extraction process was carried out as described in detail by Raspe, Silva, and Costa (2023), with 30 min. of static extraction. The effect of temperature (40, 60, and 80°C) was evaluated at 15, 30, and 45 min. of extraction, corresponding to sample-to-solvent ratios of 0.033, 0.05, and 0.10 g mL^{-1}, respectively. The pressure was kept constant at 10 MPa, based on the studies of Feuereisen et al. (2017) and Rebelatto et al. (2020).

Soxhlet extraction was performed in triplicate using 5 g of sample and 150 mL of ethanol under continuous reflux for 8h (Stevanato & Silva, 2019). The solvent was removed using a vacuum rotary evaporator (Marconi, MA 120, Piracicaba, São Paulo, Brazil). The global extraction yield (GEY) was calculated as the ratio of dry extract weight to sample weight.

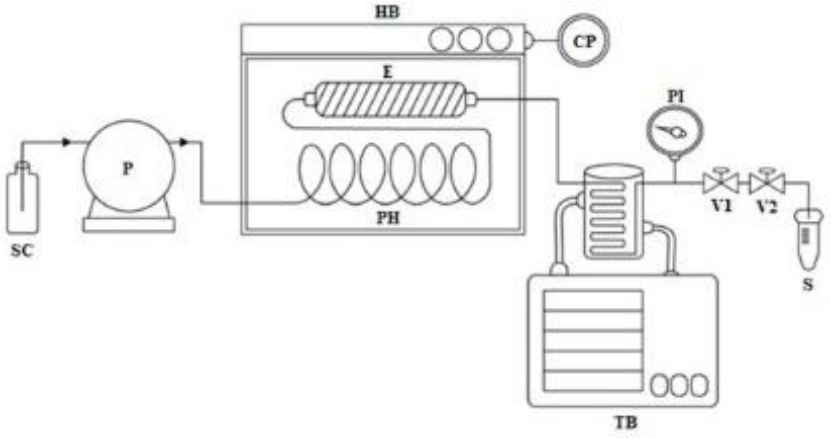

Figure 1. Schematic diagram of the extraction apparatus: solvent container (SC), high-pressure pump for liquids (P), pre-heating system (PH), extraction bed (E), heating bath (HB), control panel (CP), pressure indicator (PI), needle valve (V1), pressure reduction valve (V2), and sampling outlet (S).

Extract analysis

Phenolics were extracted (Haiyan, Bedgood, Bishop, Prenzler, & Robards, 2007), and total phenolic content (TPC) was determined by the Folin–Ciocalteu method, as described by Singleton, Orthofer, and Lamuela-Raventós (1999). Absorbance was determined at 760 nm (Shimadzu, UV 1900, Tokyo, Japan), and the data were compared against a standard curve of gallic acid.

Antioxidant activity was determined using the DPPH• radical scavenging and ferric reducing antioxidant power (FRAP) assays, performed as described by Brand-Williams, Cuvelier, and Berset (1995) and Benzie and Strain (1996), respectively. For this, ethanolic extract solutions were prepared and mixed with an ethanolic solution of DPPH• or FRAP reagent. After incubation, absorbance values were determined at 517 and 595 nm for DPPH• or FRAP assays, respectively. Data were compared against standard curves of Trolox.

The chemical profile of extracts was determined by gas chromatography coupled to mass spectrometry (GC-MS) (Shimadzu, model CGMS-QP2010 SE, Tokyo, Japan). Samples were derivatized with BSTFA for 60 min. at 60°C. The GC-MS system was equipped with a SH-Rtx-5MS™ capillary column (30 m × 0.25 mm i.d. × 0.25 µm, Shimadzu, Tokyo, Japan). The analysis was performed using helium (1 mL $min.^{-1}$) as carrier gas. A temperature gradient was applied, as follows: initial temperature of 50°C, ramp of 6.0°C $min.^{-1}$ to 300°C, held for 12 min. The injector and interface were kept at 250°C, and 10 µL of sample diluted in ethanol was injected in split mode (30:1). For fatty acid composition analysis, the compounds were previously converted into their corresponding fatty acid methyl esters (FAME), and the resulting sample was analyzed as described by Mello et al. (2021). Individual components were identified by comparison with standard mass fragments included in the NIST14 mass spectral library. Quantification was performed from relative peak areas.

Antibacterial activity was analyzed in independent triplicates by the serial microdilution method (Clinical and Laboratory Standards Institute [CLSI], 2009), and results are presented as minimum inhibitory concentrations (MIC). For inoculum preparation, bacterial cells (3.7×10^6 CFU mL^{-1}) cultured for 24h at 36°C in BHI broth were suspended in sterile saline (0.9% NaCl). The concentration was adjusted to 1.5×10^8 CFU mL^{-1}.

The extract obtained under the best conditions (PLE at 80°C for 45 min.) was dissolved in 1% (v/v) Tween 80 and tested at final concentrations ranging from 1.25 to 125 mg mL^{-1} in a total volume of 100 µL (BHI broth and sample). After serial dilutions were performed, 5 µL of inoculum was added to each well, and the plates were incubated at 36°C for 24h. Then, 20 µL of TTC solution (2%) was added to the wells, and the plates were incubated at 36°C for a further 2h. The MIC was defined as the lowest concentration without visible growth.

The results are expressed as mean ± standard deviation. Extractions and analyses were performed in duplicate ($n = 4$). Mean values were compared using analysis of variance (ANOVA) and Tukey's test at the 5% significance level (Statistica 8.0 software). Data were subjected to principal component analysis (PCA) using Paleontological Statistics software (Past version 4.03).

Results and discussion

Figure 2 shows the effect of temperature (40, 60, and 80°C) on GEY at different extraction times under dynamic extraction and compares the results with those obtained by Soxhlet extraction. Temperature is the

main parameter influencing solvent properties, thereby affecting the extraction rate and efficiency of PLE (Martínez & Aguiar, 2015). As can be seen in Figure 2, increasing the extraction temperature from 40 to 60 and 80°C promoted an increase in GEY of 41.0% and ~69.0%, respectively, after 15 min. of extraction. This increase was greater after 30 min., with increments of ~35.0% and ~48.0% at 60 and 80°C, respectively. After 45 min. of extraction, GEY increased by ~27.0 and 53.0% for the same temperature conditions.

Application of temperature disrupts van der Waals interactions, hydrogen bonds, and dipole–dipole forces between compounds and the plant matrix, reducing the mechanical work required for desorption (Viganó et al., 2016). Additionally, it produces an increase in the internal vapor pressure of the matrix, promoting fragmentation of cell walls and membranes, which results in increased porosity and surface area (Xu et al., 2018), favoring the diffusivity of the analyte to the solvent. Pereira, Zabot, Reyes, Iglesias, and Martínez (2021) obtained high mass yields when conducting PLE (10 MPa) in the temperature range of 60 to 90°C and attributed this result to the lower activation energy required for the desorption of extractable compounds.

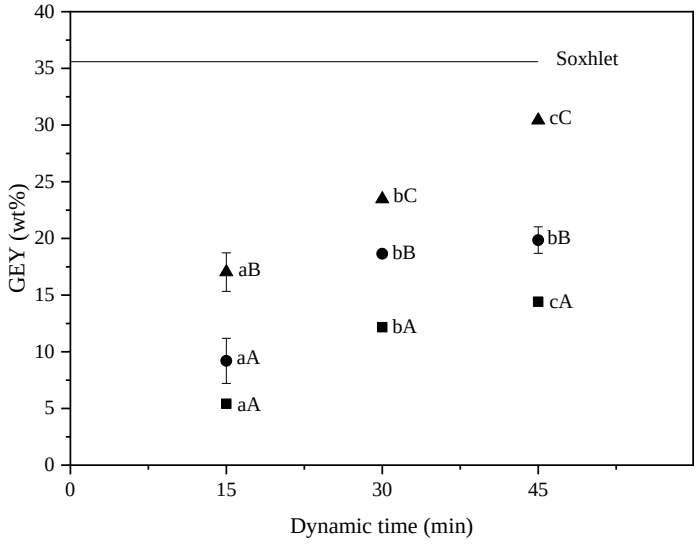

Figure 2. Global extraction yield (GEY) of pink pepper fruit at (■) 40°C, (●) 60°C, and (▲) 80°C by pressurized liquid extraction as compared with Soxhlet extraction. Means followed by the same lowercase letter (within temperature treatments) or uppercase letter (within time periods) do not differ statistically ($p > 0.05$).

GEY values increased with increasing dynamic extraction time at the different temperatures (Figure 2), attributed to the greater contact between solvent and matrix in the extractive system. Such contact is favorable for solute removal from the inner layers of the matrix (Santos, Ribeiro, Micke, Vitali, & Hense, 2019). This factor, coupled with higher processing temperatures, promoted an increase in the response variable. The higher GEY values resulting from the longer extraction time are due, in part, to the increased solubility of compounds during the reaction (Pereira, Hamerski, Andrade, Scheer, & Corazza, 2017; Benito-Román, Rodríguez-Perrino, Sanz, Melgosa, & Beltrán, 2018). Additionally, a greater amount of solvent comes into contact with the matrix from 15 to 45 min., thereby increasing the extraction mass.

The initial extraction step (15 to 30 min.) reached 55, 50, and 27% of the total GEY at 40, 60, and 80°C, respectively. This step corresponds to washing for solute removal from the sample surface, governed by convection and a constant extraction rate. Commonly, in PLE, this step occurs in the first 40 min. (Colivet, Oliveira, & Carvalho, 2016; Santos, Aguiar, Silva, & Silva, 2021; Secco et al., 2022). After 30 min., extraction was governed by diffusion, given the strong solute–solvent interaction (Mezzomo, Martínez, & Ferreira, 2009; Li et al., 2014). At this stage, it was possible to obtain 62, 54, and 44% of the total GEY at 40, 60 and 80°C, respectively. PLE (80°C, 45 min., 0.033 g mL^{-1}) achieved a ~14% lower GEY than the Soxhlet method (70°C, 480 min., 0.033 g mL^{-1}), but a shorter time was required for PLE.

Table 1 shows the TPC and antioxidant activity of pink pepper fruit extracts obtained by PLE at 60 or 80°C and Soxhlet extraction. The extraction of active compounds was influenced by experimental conditions: TPC and antioxidant activity increased with increasing temperature and time. Rudke, Mazzutti, Andrade, Vitali, and Ferreira (2019) suggested that the effect of temperature (35 to 71°C) on phenolic extraction is due to the resulting increase in compound solubility and solvent molecular agitation. Garcia-Mendoza et al. (2017) and

Pereira et al. (2021) demonstrated that TPC extraction was directly proportional to an increase in temperature (from 40 to 80°C and from 60 to 90°C, respectively), indicating higher compound solubility in the solvent solution.

Table 1. Phenolic content and antioxidant activity of pink pepper fruit extracts obtained under different operating conditions by pressurized liquid extraction (PLE) or Soxhlet extraction.

Extraction technique	T (°C)	Dynamic time (min.)	Sample/ solvent ratio (g mL^{-1})	Phenolic content (mg GAE g^{-1} extract)	Antioxidant activity	
					DPPH• (μmol TEAC g^{-1} extract)	FRAP (μmol TEAC g^{-1} extract)
PLE	60	15	0.100	23.26 ± 0.03^{aA}	126.76 ± 0.29^{aA}	118.33 ± 0.54^{aA}
		30	0.050	23.08 ± 0.08^{bA}	114.35 ± 0.24^{bA}	151.48 ± 0.01^{bA}
		45	0.033	46.40 ± 0.03^{cA}	263.45 ± 0.27^{cA}	320.04 ± 0.50^{cA}
	80	15	0.100	27.61 ± 0.01^{aB}	108.33 ± 0.01^{aB}	152.32 ± 1.54^{aB}
		30	0.050	30.85 ± 0.03^{bB}	141.08 ± 0.01^{bB}	163.73 ± 0.52^{bB}
		45	0.033	54.86 ± 0.03^{cB}	317.14 ± 0.26^{cB}	392.26 ± 0.56^{cB}
Soxhlet	70	180	0.033	30.21 ± 0.02^{d}	140.24 ± 0.25^{d}	215.43 ± 0.76^{d}

GAE, gallic acid equivalent; TEAC, Trolox equivalent antioxidant capacity; T, temperature. Means followed by the same lowercase letter (within temperature conditions) and uppercase letter (within time periods) do not differ statistically ($p > 0.05$). The results of Soxhlet extraction were compared with those of the best PLE condition (80°C, 45 min.) ($p > 0.05$).

PLE (80°C, 45 min.) afforded extracts with about 45% higher TPC than Soxhlet extraction. As a result, antioxidant activity was about 56% (DPPH•) and 45% (FRAP) higher in PLE extracts than in the Soxhlet extract. According to Santos, Kammers, Silva, Oliveira, and Hense (2021), an increase in phenolic recovery is due to the relevant effect of pressure in PLE.

Martins, Guedes, Brito, and Ferreira (2022), in assessing the PLE of tamarind seeds with ethanol at 80°C (10 MPa), obtained an increase of 10.64% in phenolic content compared with the Soxhlet method. The authors argued that PLE is efficient because of the greater interaction of solvent with the compounds of interest. Garcia-Mendoza et al. (2017) and Rudke et al. (2019) showed an increase in phenolic compound recovery from buriti bark extracts and juçara residues by PLE, as compared with Soxhlet extraction. The shorter reaction time in PLE compared with Soxhlet extraction translates into lower energy consumption and faster component extraction.

PCA was applied to the data to better understand the variability of the dataset (Table 1). For this, a 7 × 3 matrix, consisting of 7 rows and 3 columns corresponding to the tests (PLE and Soxhlet) and response variables (TPC, DPPH•, and FRAP), was plotted. The results are expressed on a score graph, as shown in Figure 3.

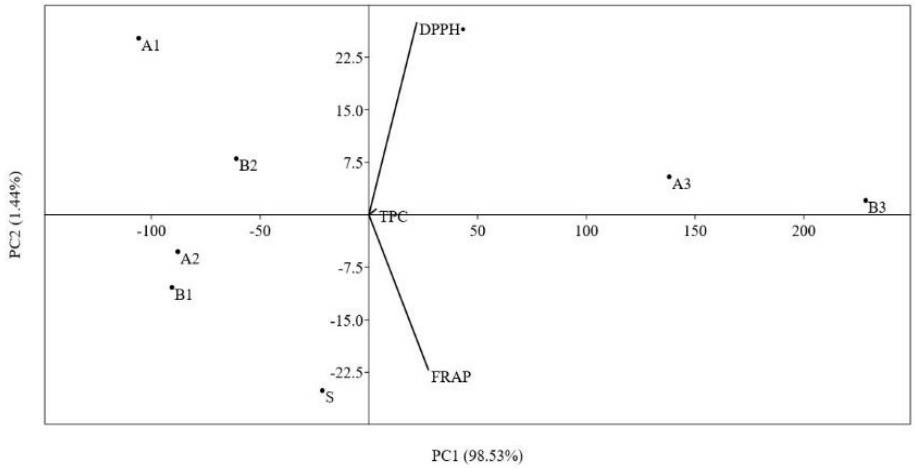

Figure 3. Principal component analysis of active compound recovery (TPC, DPPH•, and FRAP) by pressurized liquid extraction at 60°C (A1, 15 min.; A2, 30 min.; and A3, 45 min.) or 80°C (B1, 15 min.; B2, 30 min.; and B3, 45 min.) and Soxhlet extraction (S).

Principal components PC1 (98.53%) and PC2 (1.44%) explained 99.97% of the total variability of the PCA dataset. The vectors corresponding to TPC, DPPH•, and FRAP were directed toward A3 and B3, explained by the higher levels of these variables in the respective samples. On the other hand, A1 and A2, located on the quadrant opposite to FRAP and TPC vectors, had a negative and absent correlation, respectively, with the mentioned vectors. This finding is explained by the data reported in Table 2. The proximity of B1 and B2 demonstrates the similarity of data, mainly in relation to TPC. However, because S was associated with a

higher FRAP value, this sample was closer to the vectors corresponding to TPC and FRAP. B1 and B2 showed no correlation with DPPH•, as they were plotted on the quadrant opposite to the vector. In general, the grouping of A1 to A3, B1 to B3, and S in the upper and lower quadrants of the PCA biplot, respectively, was consistent with the results shown in Table 2. The highest TPC, DPPH•, and FRAP values were observed in the PLE sample obtained under the highest temperature condition. The antioxidant assays had high correlation rates (Pearson): DPPH•/FRAP, $r = 0.97$, $p \leqslant 0.001$; DPPH•/TPC, $r = 0.98$, $p \leqslant 0.001$; and FRAP/TPC, $r = 0.98$, $p \leqslant 0.001$.

Table 2 presents the chemical profile and fatty acid composition of PLE (80°C and 45 min.) and Soxhlet extracts. GC-MS revealed the major compound to be 5-hydroxymethylfurfural. Its presence is explained by ethanol's ability to extract polar compounds such as sugars, which are converted into this compound. Furthermore, mass transfer rate and sugar extraction yield are favored by high temperatures (Baümler, Carrín, & Carelli, 2017), as occurs in PLE. α-Eudesmol has anti-Alzheimer properties (Aoyama, Araki, & Konoike, 2001), and β-eudesmol has potential antitumor and antiangiogenic activities (Acharya, Chaijaroenkul, & Na-Bangchang, 2021).

Table 2. Chemical profile and fatty acid composition of extracts obtained by pressurized liquid extraction (PLE) and Soxhlet extraction.

Item	PLE	Soxhlet
Chemical compound (% normative area)		
Elemol	3.31	13.53
γ-Eudesmol	5.74	8.04
β-Eudesmol	14.53	11.84
α-Eudesmol	8.59	10.22
5-Hydroxymethylfurfural	29.96	10.76
Fatty acid (%)[1]		
Myristic	1.15 ± 0.27	1.09 ± 0.07
Palmitic	15.86 ± 0.60	19.55 ± 0.85
Estearic	9.11 ± 0.76	8.75 ± 0.22
Oleic	29.89 ± 0.89	28.76 ± 0.10
Linoleic	43.99 ± 0.74	42.42 ± 0.43

[1]In relation to the lipid fraction of the extract.

The major fatty acid in the extract was linoleic acid (~43% to 44%), suggesting a potential for food application, as the fatty acid contributes to the maintenance of blood cholesterol levels (European Commission, 2012). The lipid fraction of pink pepper fruit extract is mainly composed of unsaturated fatty acids, which can promote health benefits such as improved insulin sensitivity and reduced diabetes risk (Riserus, Willett, & Hu, 2009). The main fatty acids observed here are in line with those reported by Sassi, Elayeb, Karaman, Marzouk, and Mastouri (2020).

The in vitro antibacterial activity of PLE and Soxhlet extracts is presented in Table 3. Both samples had an MBC greater than 125 mg mL^{-1}.

Table 3. Minimum inhibitory concentration (MIC) of pink pepper fruit extract obtained by pressurized liquid extraction (PLE) against strains of Gram-positive and Gram-negative bacteria.

Bacteria	MIC (mg mL^{-1})	
	PLE[1]	Soxhlet
Escherichia coli	15.625	125.00
Staphylococcus aureus	62.5	31.25
Pseudomonas aeruginosa	125.00	125.00

[1]Extracted at 80°C for 45 min.

The antibacterial properties of pink pepper fruits are frequently attributed to lipophilic essential oils (Dannenberg et al., 2019; El-Nashar, Mostafa, El-Badry, Eldahshan, & Singab, 2019; Simões et al., 2020). However, pink pepper fruits are also rich in hydrophilic antioxidants, such as polyphenols, many of which possess excellent antioxidant activity and good antibacterial activity (Degaspari, Waszczynsky, & Prado, 2005); Pereira et al., 2011; Gomes et al., 2020). Pereira et al. (2011) evaluated the susceptibility of oral pathogenic microorganisms to an ethanolic extract. The extract may be an efficient alternative for the treatment of oral cavity infections caused by *Staphylococcus aureus*. Degaspari, Waszczynsky, and Prado (2005) showed that alcoholic extracts have an inhibitory effect on *Staphylococcus aureus* growth; however, no inhibitory effect on *Escherichia coli* or *Pseudomonas aeruginosa* growth was observed. Of note, no study has reported on the antibacterial activity of pink pepper fruit extracts obtained by PLE (Feuereisen et al., 2017, Rebelatto et al., 2020).

The antibacterial activity observed in this study can be attributed to the phenolic composition of extracts. The phenol group can damage cell membranes, activate enzymes, and denature proteins, resulting in low membrane permeability (Purwantiningsih, Suranindyah, & Widodo, 2014). The sample with the highest phenolic content also had a better growth inhibitory action. 5-Hydroxymethylfurfural, the major compound of the extracts, is known for its strong bactericidal potential (Manganyi, Regnier, Tchatchouang, Bezuidenhout, & Ateba, 2019). Roy and Lingampeta (2014) reported that plant extracts containing higher levels of phenolic compounds had superior antimicrobial effects, and that the combined presence of these compounds seems to have a vital role in antimicrobial activity. Dias et al. (2022) investigated the antimicrobial properties of isolated α-eudesmol and stated that this terpene has high potential as a natural antimicrobial agent. Thus, the extract's antimicrobial activity likely derives from the variety of antibacterial compounds or from their synergistic effect. Furthermore, the lipid fraction might have compromised antibacterial activity, given that fatty acids have low antimicrobial potential. A direct comparison with values for essential oils was thus not possible.

Conclusion

PLE proved to be effective for the rapid recovery of compounds from pink pepper fruit. The combination of high temperature and time conditions provided an increase in GEY, directly reflecting on TPC and antioxidant activity. The method was superior to Soxhlet extraction in terms of TPC and antioxidant activity. The extract's good antibacterial activity, mainly against *Escherichia coli*, added to its nutraceutical composition, demonstrates its potential applications in food. The emerging technique allowed obtaining phytochemicals in a quantitative and qualitative manner, in a reduced operational time, making use of a renewable solvent with easy separation, supporting the concept of green chemistry. The findings of this study may serve as a basis for future investigations assessing other PLE variables, as well as different extraction solvents.

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