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EXTRACTION OF ANTIOXIDANT COMPOUNDS FROM DOVYALIS: MIXTURES OF ETHANOL AND WATER

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ABSTRACT - Ethanol/water 80/20 has been the most suitable solvent mixture for the extraction of antioxidant compounds from fruits. However, the efficiency of the extraction solvent varies with the polar affinity of the different chemical compounds found in plant species. The objective of this work was to determine the efficiency of extraction of antioxidant compounds from the pulp and peel of dovyalis using ethanol/water mixtures as extracting solvent. Dried and ground pulp and peel were extracted with mixtures of ethanol/water solvents 100/0, 75/25 and 50/50 (v/v). Antioxidant activity (DPPH, ABTS and FRAP), total phenolic compounds and total flavonoids of the extracts were determined. The results showed that the 50% and 75% ethanol/water mixtures showed more efficient extraction of total phenolic compounds from the pulp and peel than 100% ethanol. The same trend was observed in the determinations of the antioxidant activities DPPH in the peel, ABTS in the pulp and FRAP in both fractions of the fruits, where the solvents 50% and 75% ethanol were superior to the 100% ethanol. In the extraction of flavonoids from the pulp and skin of the fruits, 50% ethanol was more efficient compared to the other solvent mixtures. The same was verified in the determination of the DPPH antioxidant activity in the pulp and ABTS in the peel of dovyalis fruits, that is, 50% ethanol was also superior to the other solvents. The 50% ethanol/water mixture was the most efficient solvent in the extraction of antioxidant compounds from the pulp and bark of dovyalis.
Keywords: *Dovyalis hebecarpa* (Gardner) Warb., Phenolic compounds, antioxidant activity, post-harvest, aqueous solvent.

EXTRAÇÃO DE COMPOSTOS ANTIOXIDANTES DE DOVIÁLIS: MISTURAS DE ETANOL E ÁGUA

RESUMO - Etanol/água 80/20 tem sido a mistura de solventes mais indicada para a extração de compostos antioxidantes de frutos. Entretanto, a eficiência do solvente de extração varia com a afinidade polar dos diferentes compostos químicos encontrados nas espécies vegetais. O objetivo deste trabalho foi determinar a eficiência de extração de compostos antioxidantes da polpa e casca de doviális utilizando misturas de etanol/água como solvente extrator. Polpa e casca secas e moídas foram extraídas com misturas dos solventes etanol/água 100/0, 75/25 e 50/50 (v/v). Atividade antioxidante (DPPH, ABTS e FRAP), compostos fenólicos totais e flavonoides totais dos extratos foram determinados. Os resultados mostraram que as misturas etanol/água 50% e 75% mostraram extração de compostos fenólicos totais da polpa e da casca mais eficientes do que etanol 100%. A mesma tendência foi observada nas determinações das atividades antioxidantes DPPH na casca, ABTS na polpa e FRAP em ambas as frações dos frutos, onde os solventes etanol 50% e 75% foram superiores ao etanol 100%. Já na extração de flavonoides da polpa e da casca dos frutos, etanol 50% foi mais eficiente comparado às demais misturas de solventes. O mesmo foi verificado na determinação da atividade antioxidante DPPH na polpa e ABTS na casca dos frutos de doviális, ou seja, etanol 50% também foi superior aos demais solventes. A mistura etanol/água 50% foi o solvente mais eficiente na extração de compostos antioxidantes da polpa e casca de doviális.
Palavras-chaves: *Dovyalis hebecarpa* (Gardner) Warb., compostos fenólicos, atividade antioxidante, pós-colheita, solvente aquoso.

INTRODUCTION

Dovyalis (*Dovyalis hebecarpa* (Gardner) Warb. is a reddish berry-like fruit from the Asian continent. In Brazil, the southwest region stands out as the main producer (BOCHI et al., 2015a). Berry-type fruits are known for their high levels of bioactive compounds, and consequently, for being excellent sources of antioxidant compounds. Therefore, dovyalis fruits are also considered to be rich sources of phenolic compounds with high antioxidant activity, mainly due to the presence of anthocyanins (BOCHI et al., 2015b).

Bioactive compounds with high antioxidant activity present in dovyalis fruits can be found in their edible and non-edible parts, such as pulp, peel and seeds. However, the development of the extraction procedure is considered the most important step for the recovery of bioactive compounds present in any plant matrix, which can have its efficiency affected by factors such as temperature

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and extraction time, and solvent polarity (HIDALGO; ALMAJANO, 2017; XU et al., 2017; BARROS et al., 2019).

Solvents such as water, ethanol, methanol and acetone are commonly used in the extraction of bioactive compounds, pure or in mixtures, with the ethanol/water mixture being an efficient extractor of phenolic compounds when compared to pure solvents (HIDALGO; ALMAJANO, 2017). Water causes the plant material to swell while ethanol breaks the bonds between the solute and the plant matrix, favoring mass transfer to the solvent, facilitating the extraction of phenolic compounds (ĆUJIĆ et al., 2016).

Other studies have suggested the mixture of ethanol/water (80/20) solvents as the most suitable for the extraction of antioxidant compounds in different fruits (CELANT et al., 2016), but the solvent extraction efficiency may vary according to its affinity polar with the different chemical groups of compounds found in different plant species. Furthermore, adding ethanol to water can improve the solubility of hydrophobic compounds, while adding water can improve the solubility of hydrophilic compounds. Thus, the choice of the proportion of ethanol and water can be adjusted according to the properties of the desired antioxidant compounds. Therefore, optimizing this variable becomes important to ensure the efficiency and reproducibility of extracting antioxidant compounds. In view of the above, the objective of this work was to determine the efficiency of extraction of antioxidant compounds from the pulp and bark of dovyalis using ethanol/water mixtures as extractor solvent.

MATERIAL AND METHODS

Dovyalis fruits were harvested from an orchard located in Marechal Cândido Rondon, Paraná, Brazil. Soon after, they were selected for size uniformity, maturation pattern (mature), absence of mechanical damage or disease symptoms. Then the fruits were frozen at -18°C awaiting the extraction tests. Pulp and skin of the fruits were separated, dried in an oven with forced air circulation, at 50°C for 72 h and ground in liquid nitrogen. Subsequently, the samples were extracted with mixtures of ethanol and water solvents 100/0, 75/25 and 50/50 (ethanol/water), in the proportion 1/10 (m/v). The extract was vortexed for 30 sec., followed by agitation in an ultrasonic benchtop bath (Unique, USC-2850 a) for 30 min. Then the material was centrifuged at 20,000 g (MPW, 350-350R) for 5 min. and subsequently filtered through a qualitative paper filter and stored at -18°C until the time of analysis.

The experimental design used was completely randomized, containing 3 concentrations of ethanol (100%, 75% and 50%) and 10 repetitions for each part of the fruit (pulp and skin). Spectrophotometric analyzes of antioxidant activity (DPPH, ABTS and FRAP), total phenolic compounds (TFC) and total flavonoids (TF) of dried samples of pulp and peel of dovyalis fruits were performed in a UV-visible spectrophotometer (Shimadzu, UV-1800).

TFC were analyzed by the Folin-Ciocalteau spectrophotometric method according to Cheng et al. (2013). The results were expressed in mg EAG g^{-1} dry sample, Gallic Acid Equivalent (EAG), using the calibration curve for gallic acid in concentrations ranging from 0.158 to 0.940 mg EAG mL^{-1} ($R^2 = 0.9980$). FLT were determined according to Lin and Tang (2007). The results were expressed in mg EQ g^{-1} dry sample, Quercetin Equivalent (EQ), through the calibration curve for quercetin at concentrations ranging from 0.062 to 0.547 mg EQ mL^{-1} ($R^2 = 0.9992$).

The determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging was performed according to Siripatrawan and Harte (2010) and the results were expressed in mg ET g^{-1} dry sample, Trolox Equivalent (ET), using of the calibration curve for Trolox at concentrations ranging from 0.220 to 0.495 mg ET mL^{-1} ($R^2 = 0.9973$). The determination of free radical scavenging ABTS•+ [2,2'azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] was performed according to Rufino et al. (2007), with results expressed in mg ET g^{-1} dry sample, through the calibration curve for Trolox at concentrations ranging from 0.409 to 0.785 mg ET mL^{-1} ($R^2 = 0.9980$). The determination of the reduction of iron ions through the FRAP method was performed according to Rufino et al. (2006) with the results expressed in mg ESF g^{-1} dry sample, Ferrous Sulfate Equivalent (ESF), through the calibration curve for ferrous sulfate at concentrations ranging from 0.346 to 0.927 mg ESF mL^{-1} ($R^2 = 0.9958$).

The data were submitted to analysis of variance (ANOVA) and the parameters whose F test was significant, the Tukey test was applied ($P<0.05$ and $P>0.01$). For the tabulation and analysis of the data obtained, the statistical software Sisvar (FERREIRA, 2011) was used.

RESULTS AND DISCUSSION

Although water is the most polar solvent, its use as a pure extraction solvent is not recommended due to the high amount of polar components that can be extracted from a sample along with the compound of interest, such as sugars, soluble proteins and organic acids, impairing the quantification of phenolic compounds. However, water associated with other organic solvents, such as ethanol, contributes to the composition of a relatively polar solvent, favoring the extraction of polyphenols (MOKRANI; MADANI, 2016).

Veber et al. (2015) studied types of jambolan fruit and leaf extracts (*Syzygium cumini* L.) and found that 50% ethanol was the solvent that extracted the highest TFC contents, regardless of the plant material used, similar to what was observed in this work. Lim et al. (2019) also reported that aqueous ethanol (40% and 60%) were more efficient extractors of TFC compared to 100% ethanol in *Phaleria macrocarpa* (Scheff.) Boerl.

Table 2 presents the results for TF of dovyalis extracted with ethanol/water solvent mixtures. Ethanol 50% was the solvent mixture that showed the best extraction efficiency, both in the pulp and in the bark of the dovialis, whose extracted contents (0.58 and 2.16 mg EqQ g^{-1}, respectively) were significantly higher than when extracted with 75% ethanol (0.47 and 1.97 mg EqQ g^{-1}, respectively)

and with 100% ethanol for the peel (0.63 mg EqQ g^{-1}). This result for TF is similar to that for TFC, and reinforces the indication of the 50% ethanol/water mixture as the most efficient solvent for the extraction of these compounds. Corroborating these results, Garcia-Castello et al. (2015) mentioned that ethanol is efficient in the extraction of flavonoids, but its efficiency is conditioned to its concentration in a mixture with another solvent of high polarity, such as water.

TABLE 1 - Total phenolic compounds (TPC) in the pulp and peel of dovyalis fruits extracted with ethanol/water solvent mixtures.

Ethanol/water solvent (%)	TPC, mg EqAG g^{-1} (dry biomass)	
	Fruit pulp	Fruit peel
100/0	2.75 b*	3.01 b
75/25	6.29 a	18.61 a
50/50	7.23 a	19.48 a
CV(%)	21.80	14.02
DMS	0.95	1.55

*Means followed by the same lowercase letter in the column do not differ by Tukey's test (P<0.01), CV: coefficient of variation, DMS: least significant difference.

TABLE 2 - Total flavonoids (TF) in the pulp and peel of dovyalis fruits, extracted with ethanol/water solvent mixtures.

Ethanol/water solvent (%)	TF, mg EqQ g^{-1} (dry biomass)	
	Fruit pulp	Fruit peel
100/0	0.49 ab*	0.63 c
75/25	0.47 b	1.97 b
50/50	0.58 a	2.16 a
CV(%)	21.57	9.43
DMS	0.09	0.12

*Means followed by the same lowercase letter in the column do not differ by Tukey's test (P<0.01), CV: coefficient of variation, DMS: least significant difference.

Tables 2 and 3 also show that the TPC of the peel of the dovyalis fruits was 2.7 times greater than that of the pulp (19.48 and 7.23 mg EqAG g^{-1}, respectively) and, for the TF, 3.7 times concentrated also in the peel (2.16 and 0.58 mg EqQ g^{-1}, respectively), which makes it clear that dovialis bark is the best reservoir of phenolic compounds.

According to Table 3, the dovialis pulp extracted with the 50% ethanol mixture showed significantly higher DPPH antioxidant activity (11.17 mg EqT g^{-1}) than 75% ethanol (6.79 mg EqT g^{-1}) and 100% ethanol (2.32 mg EqT g^{-1}). However, for fruit peel extraction with 50% and 75% ethanol, there were no significant differences between the DPPH antioxidant activity results (43.09 and 42.50 mg EqT g^{-1}, respectively), and they were much higher than the extraction with 100% ethanol (6.57 mg EqT g^{-1}). These results are positively associated with those found for TPC (Table 1) and TF (Table 2), that is, the 50% ethanol mixture stood out as the most efficient solvent in the extraction of phenolic compounds and, consequently, also showed more DPPH antioxidant activity elevated.

In a similar study, Nour et al. (2013) found higher DPPH antioxidant activity when extraction of blackcurrant (*Ribes nigrum* L.) varieties was performed in 60% ethanol, when compared to 96%, corroborating the results found here.

TABLE 3 - DPPH antioxidant activity in pulp and peel of dovyalis fruits extracted with ethanol/water solvent mixtures.

Ethanol/water solvent (%)	DPPH, mg EqT g^{-1} (dry biomass)	
	Fruit pulp	Fruit peel
100/0	2.32 c*	6.57 b
75/25	6.79 b	42.50 a
50/50	11.17 a	43.09 a
CV(%)	10.50	22.22
DMS	0.57	5.52

*Means followed by the same lowercase letter in the column do not differ by Tukey's test (P<0.01), CV: coefficient of variation, DMS: least significant difference.

Table 4 shows the ABTS antioxidant activity data extracted with ethanol/water solvent mixtures. Ethanol 50% and 75% extracted 38.31 and 39.82 mg EqT g^{-1} dry mass, respectively, in the pulp, while ethanol 100% extracted an average of 17.80 mg EqT g^{-1} dry mass, which is the worst extractor compared to the first two (P>0.01). In the peel, 50% ethanol was the best extractor in the ABTS antioxidant activity analysis (89.33 mg EqT g^{-1} dry mass), followed by 75% ethanol (71.70 mg EqT g^{-1} dry mass) (P>0.01). As in the pulp, 100% ethanol was also the solvent with the lowest

extraction power in the peel (9.18 mg EqT g^{-1} dry mass) in relation to the other solvents.

Data from the FRAP antioxidant activity analysis of dovyalis pulp and bark are presented in Table 5. Ethanol 50% and 75% were superior extractors than ethanol 100% in the pulp (37.00, 33.15 and 16.63 mg EqSF g^{-1} dry mass, respectively) and in the peel (158.62, 161.87 and 26.75 mg EqSF g^{-1} dry biomass, respectively) ($P<0.01$) (Table 5), as well as observed in the peel for the analysis of DPPH antioxidant activity and in the pulp for the analysis of ABTS antioxidant activity, as per Tables 4 and 5, respectively.

TABLE 4 - ABTS antioxidant activity (mg EqT g^{-1} dry mass) in pulp and peel of dovyalis fruits extracted with ethanol/water solvent mixtures.

Ethanol/water solvent (%)	ABTS, mg EqT g^{-1} (dry biomass)	
	Fruit pulp	Fruit peel
100/0	17.80 b*	9.18 c
75/25	39.82 a	71.70 b
50/50	38.31 a	89.33 a
CV(%)	11.18	17.63
DMS	2.89	8.08

*Means followed by the same lowercase letter in the column do not differ by Tukey's test ($P<0.01$), CV: coefficient of variation, DMS: least significant difference.

TABLE 5 - Antioxidant activity FRAP (mg EqSF g^{-1} dry biomass) in pulp and peel of dovyalis fruits extracted with ethanol/water solvent mixtures.

Ethanol/water solvent (%)	FRAP, mg EqSF g^{-1} (dry biomass)	
	Fruit pulp	Fruit peel
100/0	16.63 b*	26.75 b
75/25	33.15 a	161.87 a
50/50	37.00 a	158.62 a
CV(%)	18.42	10.03
DMS	4.31	9.38

*Means followed by the same lowercase letter in the column do not differ by Tukey's test ($P<0.01$), CV: coefficient of variation, DMS: least significant difference.

In general, 50% ethanol was the most effective solvent in the extraction of antioxidant compounds by the DPPH, ABTS and FRAP methods, both in the pulp and in the peel of doviális fruits, followed by 75% ethanol. Similar to what was observed in this study, Bochi et al. (2014) also obtained better results for the extraction of TPC and anthocyanins from the pulp of doviális using mixtures of ethanol/acetone/water solvents, where water corresponded to the largest fraction used.

In general, there was considerable variation in the results of antioxidant activity in the different analysis methods (DPPH, ABTS and FRAP), with the DPPH method responsible for the lowest levels in relation to the others. Determining the antioxidant capacity in a complex matrix can be difficult, since it can be influenced by the chemical characteristics of the antioxidant compounds present in the extract, generating results possibly altered by the extraction conditions (BARROS et al., 2019).

Ethanol 50% and 75% were responsible for the best TPC extractions from the pulp and peel of doviális fruits. Ethanol 50% was also the solvent that best extracted TF from the pulp and peel of the fruits, followed by ethanol 75%, following the pattern observed in the analysis of antioxidant activity. The use of 50% ethanol proved to be an effective option in the extraction of antioxidant compounds from doviális, therefore, it is recommended to test this solvent in the extraction of antioxidant compounds from other plant matrices in order to verify its effectiveness as a solvent extractor of other compounds. antioxidants.

CONCLUSION

The 50% ethanol/water mixture was the most efficient solvent for extracting antioxidant compounds from dovialis pulp and bark.

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