

Antioxidant Activity of *Oxalis tuberosa* peel extracts

Actividad Antioxidante de extractos obtenidos de la cascara de *Oxalis tuberosa*

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Abstract:

Oxalis tuberosa it's an Andean crop that was introduced to Mexico by an intercultural exchange, it's a tuber rich in carbohydrates, calcium and iron, but also has antioxidants in the peel and pulp, making it ideal to reduce oxidative stress and apply it as an additive or preservative in food, however to increase the effectiveness it is necessary apply an extraction method of the bioactive compounds, this is why an extract based on *Oxalis tuberosa* peel flour and solvents was elaborated using: hexane (EH), acetone(EA), and ethanol(Ee) to evaluate the antioxidant capacity of this extracts. The principal methodology was the quantification of total phenolic compounds (TPC), DPPH scavenging assay, determination of the EC50 in DPPH and ABTS assay. In all the methods Ee showed significant differences ($p<0.05$) with 9.23 mEAG/g in TPC, a maximum inhibition percentage of 76.74% of DPPH radical and 789.6 mgEq. of Trolox at ABTS assay. Based on the results the use of ethanol is the most effective for extracting bioactive compounds as antioxidants, it will be possible to apply this compounds as additives to formulate functional foods or as preservatives to increase shelf life.

Keywords:

Oxalis tuberosa, extracts, antioxidant activity

Resumen:

La *Oxalis tuberosa* es un cultivo andino que fue introducido en México por un intercambio intercultural, es un tubérculo rico en carbohidratos, calcio y hierro, también posee antioxidantes en la cáscara y la pulpa, lo que lo hace ideal para reducir el estrés oxidativo y aplicarlo como aditivo o conservador en alimentos, para aumentar la efectividad es necesario aplicar un método de extracción de los compuestos bioactivos, es por eso que se elaboró un extracto a base de harina de cáscara de *Oxalis tuberosa* y solventes como: hexano (EH), acetona (EA) y etanol (Ee) para evaluar la capacidad antioxidante de estos extractos. La metodología principal fue la cuantificación de compuestos fenólicos totales (TPC), el ensayo de inhibición de DPPH, la determinación de EC50 y ABTS. En todos los métodos Ee mostró diferencias significativas ($p<0.05$) con 9.23 mEAG/g en TPC, un porcentaje de inhibición máximo de 76.74% de radical DPPH y 789.6 mgEq. de Trolox en el ensayo ABTS. Con base a los resultados, el uso de etanol es el más eficaz para extraer compuestos bioactivos como antioxidantes, será posible aplicar estos compuestos como aditivos para formular alimentos funcionales o como conservantes para aumentar la vida útil.

Palabras Clave:

Lorem, ipsum, dolor, sit, amet

Introduction

Red potato *Oxalis tuberosa* it's an andean, in Mexico the states that produce *Oxalis tuberosa* are Hidalgo, Puebla and Tlaxcala (Pérez, 2009). This variety it's source of

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carbohydrates, calcium and iron (Campos *et al.*, 2006). Another aspect of this tuber is the antioxidant content (polyphenols, anthocyanins, and flavonoids) in the peel as it gives a reddish coloration and in a minor concentration in the pulp, among the polyphenols we find out benzoic, caffeic, vanillic and cinnamic acids, in the case of anthocyanins we find out malvidins and flavonoids as flavonoles and flavones (Chirinos, 2008).

Antioxidants are important due to the ability to inhibit or stabilize free radicals (ROS) which can induce oxidative stress, it has been found that antioxidants can act as antimicrobials by the inhibition of protein synthesis, rupture in the cell membrane causing damage to the cell metabolism (Lamb, 2011). Although the benefits of these bioactive compounds can be acquired directly from the product, it's recommendable to obtain them by any extraction method which allowed to concentrate them and increase their capacity as additives and preservative in the formulation of functional foods. That's why extracts were obtained from *Oxalis tuberosa* peel flour using ethanol, hexane and acetone as solvents evaluating the content of phenols and inhibit capacity of free radicals DPPH and ABTS.

MATERIALS AND METHODS

1. PREPARATION OF FLOUR AND EXTRACT

Red potato *Oxalis tuberosa* was peeled and dried at 55°C, then grounded and sieved in mesh (Tyler No. 40-425). To make the extraction of the bio-compounds were used three solvents (ethanol, acetone and hexane 95%) in a ratio 1:1, the solution was stocked for 48h at 4°C after that it was filtered, the recuperated flour from the first extraction was macerated twice more, then the solvent of each extract was evaporated in rota-evaporator (Büchi. Modelo: R-215) and the extract was concentrated in heating grill at 35°C and stocked at 4°C until used.

1. TOTAL PHENOLS

The followed methodology from Singleton *et al.*, (1999) making changes in the quantities used of the samples, the results were reported as Gallic acid equivalents. Different concentrations of the extracts were used 0.5g of hexane extract (EH), 0.007g of acetone extract (EA) and 0.004g of ethanol extract (Ee) they were dissolved in 5mL of each solvent, 1.58mL were taken from the solution and added 100µL of Folin Ciocalteu reactive, after 20 minutes at 50°C the absorbance was measured at 765 nm in a spectrophotometer SP-300 nano.

2. DPPH, EC50

A modification was made to the methodology proposed by Brand-Williams (1995) making an adaptation in plant materials, performing a general sweep of the samples to determine the concentrations in the kinetics of radical inhibition, 0.05mL of the sample was taken then added 1.95mL of the radical at 0.02mMol, the absorbance was measured at 517 nm for 60 min in 10-minute intervals and the percentage of inhibition of the radical was determined.

3. ABTS

The ABTS reagent was mixed with potassium persulfate, the radical was standardized to absorption of 0.7 ± 0.02 nm (Re *et al.*, 2009). Were used 0.005g of each concentrate (EH, EA, Ee) which were dissolved in 1.5 mL of the corresponding solvents, for the reaction were used 0.50 mL of the concentrate and 1.450 mL of the ABTS solution, the absorbance was measured at 732 nm, the results are expressed in percentage of inhibition and TROLOX equivalents, so a standard curve was made.

4. STATISTICAL ANALYSIS

A completely randomized design was used, the extracts were evaluated by means of an analysis of variance (ANOVA) and the means were compared by the Tukey method using the IBM SPSS Statistics 20 software

RESULTS

1. FLOUR AND EXTRACT

After the drying of the potato flakes, 3 kg of flour were obtained, which represents 3.5% of yield, while for the concentrates, 6.08 g Ee, 0.96 g EA and 0.98 g of EH were obtained, with a yield of 3.04%, 0.49% and 0.48% respectively. Ee presents a higher yield, this may be due to the bioactive compounds that are extracted by ethanol, since it is able to drag compounds such as flavonoids, anthocyanins, phenols and saponins, while hexane and acetone extract fats, hydrocarbons and carboxylic compounds by what its performance is significantly lower than Ee. The results obtained are similar to those of Yung *et al.* in 2013, whom made extracts with blueberry leaves in different stages of maturation using methanol, ethanol and acetone as solvents, having a better yield in the ethanol extract.

1. TOTAL PHENOLS

The concentrations used for each extract correspond to 158 g/L for EH, 2.21 g/L for EA and 1.26 g/L for Ee, this due to the antioxidant capacity of each extract which corresponds to 0.0027 mgEAG/mg, 5.37 mgGAE/mg and 9.23 mgGAE/mg respectively, the concentrates show significant differences ($p < 0.05$), (Table I) with Ee being the highest phenol content, which may be due to the type of solvent used. The results obtained are comparable with those of Rjeibi *et al.* 2016 in terms of the extraction solvent since they used MeOH and EtOH in the extraction of bio-compounds of *Ficus microcarpa* showing greater amount of polyphenols in EtOH with 217 ± 9.03 mgGAE/g while that with MeOH they obtained 96 ± 8.87 mgGAE/g

1. DPPH, EC50

In the general scan of samples EA and Ee showed a 50% inhibition of the DPPH radical, and proceeded to look for the minimum inhibitory concentration of the radical using concentrations of 0.012, 0.016, 0.20 and 0.24 g/mL for both extracts. However, EH did not show 50% inhibition, reaching only the inhibition of 33.85% at a concentration of 0.12 g/mL (Table I). The minimum concentration for EA is 0.0227 g/mL achieving 55% inhibition of the DPPH radical at 10 min of reaction and a maximum inhibition of 71.24% at 60 min, on the other hand Ee has a concentration of 0.0129 g/mL inhibiting 55.41% of the radical at minute 10 and finding a maximum of 76.74% inhibition at 60 min (Figure 1).

2. ABTS

All the concentrates showed significant differences (p<0.05) (Table I) with Ee being the most effective in the inhibition of the ABTS radical, showing 62.48% inhibition and a concentration of 789.67 mgEq of Trolox, followed by EA that had an inhibition of 55.52% and 692.3 mgEq of Trolox while EH was the lowest inhibition percentage with 28.29% and 311 mgEq. of Trolox.

Regarding the DPPH and ABTS radicals, the percentage of EC50 inhibition is greater than that reported by Nono *et al.*, in 2014, using root *Dissotis thollonii*, they found a maximum inhibition of the DPPH radical of 62.85% and of ABTS of 51.36% in the extract with ethanol, this may be due to the extraction conditions and to the raw material used

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Table 1 Antioxidant activity

Extract	Total Phenols	DPPH, EC50	ABTS	%Inhibition
	mgGAE/mg	CMI(g/mL)	Eq. TROLOX (mg)	
EH	0.0027 ^a	0.1200 ^a	311.0 ^a	28.29 ^a
EA	5.37 ^b	0.0227 ^b	692.3 ^b	55.52 ^b
Ee	9.23 ^c	0.0129 ^c	789.6 ^c	62.48 ^c

^{a,b,c} represent significative differences p<0.05

CONCLUSIONS

The Ee concentrate shows significant differences in extraction performance and antioxidant activity compared to EA and EH, making it an excellent alternative for the addition of antioxidants in foods which can act in reducing the oxidation of the food itself or as a benefit to the consumer.

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