UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE
ALIMENTOS

AVALIAÇÃO DO POTENCIAL NUTRICIONAL E DE COMPOSTOS BIOATIVOS EM PLANTAS ALIMENTÍCIAS NÃO CONVENCIONAIS DO RIO GRANDE DO SUL

GICELE SBARDELOTTO DE BONA

PORTO ALEGRE, 2014

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AVALIAÇÃO DO POTENCIAL NUTRICIONAL E DE COMPOSTOS BIOATIVOS EM PLANTAS ALIMENTÍCIAS NÃO CONVENCIONAIS DO RIO GRANDE DO SUL

Dissertação apresentada ao Programa de Pós Graduação em Ciência e Tecnologia de Alimentos da Universidade Federal do Rio Grande do Sul, como requisito à obtenção do GRAU DE MESTRE em Ciência e Tecnologia de Alimentos.

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"Não tentes ser bem sucedido, tenta antes ser um homem de valor."

(Albert Einstein)

AGRADECIMENTOS

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RESUMO

A região Sul aparece com destaque na biodiversidade vegetal devido à sua gama única de clima e relevo. Além disso, a falta de pesquisa dessas plantas as torna subutilizadas e conhecidas como "plantas do mato". Embora algumas plantas nativas estão sendo domesticadas, a utilização potencial de muitas espécies não foi ainda explorada e caracterizada. O objetivo deste estudo foi avaliar as características nutricionais de três espécies do Sul do Brasil através da composição nutricional, determinação dos compostos fenólicos totais e pigmentos totais, quantificação de compostos bioativos individuais e avaliação da atividade antioxidante utilizando métodos diferentes. Além, dos compostos que contribuem para a atividade antioxidante em dois diferentes extratos. A polpa *Opuntia monacantha* (Willd) Haw foi promissora, especialmente em comparação com outras espécies do mesmo gênero *Opuntia*. A *O. monacantha* apresentou altos níveis de carotenoides (luteína $133,04 \pm 0,84$ mg/ g peso seco (ps) e β -caroteno $102,42 \pm 0,94$ mg/ g ps), flavonoides (luteolina $440 \pm 3,87$ mg/ g ps), compostos fenólicos totais ($6,61 \pm 0,01$ mg equivalente de gálico ácido EAG)/ g de (ps), clorofila total ($0,98 \pm 0,15$ mg/ g de ps) e apresentou atividade antioxidante, pelos métodos DPPH e ABTS ($577 \pm 0,56$ g ps/ g de DPPH e $149,03 \pm 1,50$ uM trolox/ g ps). No entanto, a reatividade do extrato foi baixa, medida pelo método TAR. A *Urera Aurantiaca* Wedd tem potencial no setor agrícola pelas suas altas quantidades de luteína ($808,09 \pm 5,95$ mg/g ps), zeaxantina ($24,89 \pm 0,18$ mg/ g ps), (β-caroteno $613,25 \pm 4,74$ mg/ g ps), quercetina ($1970 \pm 6,98$ mg/ g ps), clorofila total ($12,39 \pm 0,95$ mg/g ps) e proteína ($20,98 \pm 0,71$ g/ 100g ps). Este estudo também demonstrou alta atividade antioxidante utilizando o método DPPH ($119,87 \pm 0,84$ g ps/ g DPPH) e alta quantidade e qualidade do extrato usando os métodos TRAP e TAR. O tubérculo, as folhas e as flores do *Tropaeolum pentaphyllum* Lam revelaram diferença significativa ($p < 0,05$) em todos os parâmetros, com alto teor de luteína ($642,50 \pm 4,56$ mg/ g ps e $243,23 \pm 0,89$ mg/ g ps) nas folhas e flores, respectivamente. Alto conteúdo de luteolina ($4450 \pm 12,50$ mg/ g ps) e quercetina ($3750 \pm 8,09$ mg/ g ps) foram observados nas folhas. Além disso, as qualidades antioxidantes dos extratos das folhas e flores foram reveladas utilizando o método TAR. O estudo apresentou o potencial da espécie *pentaphyllum*, particularmente em comparação com as folhas e flores do *Tropaeolum majus*, que têm sido investigados em outros estudos.

Palavras-chave: *Opuntia monacantha* (Willd) Haw, *Urera aurantiaca* Wedd, *Tropaeolum pentaphyllum* Lam, plantas nativas comestíveis, compostos fenólicos, carotenoides, flavonoides, ácido ascórbico, tocoferóis, atividade antioxidante.

ABSTRACT

The southern region features prominently in plant biodiversity because of its unique range of climate and topography. Moreover, a lack of research of these plants leaves them underutilized and they are known as "plants in the jungle." Although some native plants are being domesticated, the potential use of many species has not yet been explored and characterized. The objective of this study is to assess the nutritional characteristics of three species in South Brazil by determining the total phenolics and total pigments, quantifying individual bioactive compounds, and evaluating the antioxidant activity suing different methods. Moreover, the compounds that contribute to the activity of two different extracts will be identified. The *Opuntia monacantha* (Willd.) Haw pulp was promising, particularly compared to other species of the same genus *Opuntia*. The *O. monacantha* exhibited high levels of carotenoids (lutein 133.04 ± 0.84 µg/g dry weight (dw) and β-carotene 102.42 ± 0.94 µg/g dw), flavonoids (luteolin 440 ± 3.87 µg/g dw), total phenolics (6.61 ± 0.01 mg gallic acid equivalent GAE)/ g dw, and total chlorophyll (0.98 ± 0.15 mg/g dw) and was presented with antioxidant activity, as measured by DPPH and ABTS (577 ± 0.56 g/g DPPH dw and 149.03 ± 1.50 µM trolox/g dw). However, the reactivity of the extract was low as measured by the TAR method. The *Urera aurantiaca Wedd* has potential in the agricultural industry because of its high amounts of lutein (808.09 ± 5.95 µg/g dw), zeaxanthin (24.89 ± 0.18 µg/g dw), (β-carotene 613.25 ± 4.74 µg/g dw), quercetin (1970 ± 6.98 µg/g dw), total chlorophyll (12.39 ± 0.95 mg/g dw), and protein (20.98 ± 0.71 g/100g dw). This study also demonstrated high antioxidant activity using the DPPH (119.87 ± 0.84 g dw/ g DPPH) and TRAP methods and high quantity and extract quality using the TRAP and TAR methods. The tuber, leaves and flowers of *Tropaeolum pentaphyllum* Lam displayed significant differences (p < 0.05) in all parameters, with high lutein contents (642.50 ± 4.56 µg/g dw and 243.23 ± 0.89 µg/g dw) in the leaves and flowers, respectively. High luteolin (4450 ± 12.50 µg/g dw) and quercetin (3750 ± 8.09 µg/g dw) contents were observed in the leaves. In addition, the antioxidant qualities of leaf and flower extracts were revealed using the TAR method. The study revealed the potential of the species *pentaphyllum*, particularly in comparison with the leaves and flowers of *Tropaeolum majus*, which have been investigated in other studies.

Keywords: *Opuntia monacantha* (Willd) Haw, *Urera aurantiaca* Wedd, *Tropaeolum pentaphyllum* Lam, characterization, edible native plants, phenolic compounds, carotenoids, flavonoids, ascorbic acid, tocopherols, antioxidant activity.

1. INTRODUÇÃO

As plantas têm sido usadas há anos na medicina tradicional para o tratamento de várias doenças. Muitas destas plantas medicinais também são excelentes substâncias isoladas, com potente atividade antioxidante (RAZALI, *et al.* 2008). O Brasil detém a maior biodiversidade do mundo, sendo considerado o país da megadiversidade, com 15 a 20% das espécies do planeta. Contém, portanto, em seu território a maior riqueza de espécies da flora, além dos maiores remanescentes de ecossistemas tropicais (MYERS *et al.* 2000). Na lista da flora do Brasil publicada recentemente, estão relacionadas 40.989 espécies (FORZZA *et al.* 2010).

Os recursos vegetais nativos, além de fontes de alimentos, também podem ser utilizados para a obtenção de pigmentos, condimentos, aromas e de princípios ativos s3para produção de medicamentos. A exploração do potencial de uso dos recursos fitogenéticos nativos depende de um maior conhecimento das espécies e de seus usos pelos agricultores familiares e pelos povos e comunidades tradicionais, como caiçaras, ribeirinhos, quilombolas, indígenas, entre outros. (LEITE & CORADIN, 2011). Na região Sul do Brasil, como em outras partes do mundo, algumas dessas plantas são subutilizadas por desinformação e divulgação deficiente, sendo que em sua maioria são tidas como inços ou plantas do mato (KINUPP, 2007).

Segundo DIAZ-BETANCOURT *et al.* 1999 as chamadas plantas "daninhas" (ruderais) ou "plantas do mato" (silvestres) podem ser fontes complementares de alimentos interessantes para assentamentos humanos de pequeno a médio porte, e nas grandes cidades, as populações da periferia e dos arredores também poderiam fazer uso destas plantas espontâneas comestíveis.

Coradin (2011) afirma que apesar da existência no Brasil, de um número considerável de espécies nativas já domesticadas, ou em processo de domesticação, que remontam aos primeiros povos americanos, a utilização de recursos genéticos nativos em escala comercial é ainda incipiente quando comparada ao seu notório potencial. Existem, por exemplo, centenas de espécies comestíveis, porém poucas disponíveis nos mercados. Neste contexto, a domesticação de plantas nativas, conhecidas e já utilizadas por populações locais ou regionais, mas sem penetração no mercado nacional ou internacional, é uma grande oportunidade que se oferece aos países com amplos recursos genéticos.

Deste modo, a utilização adequada destas espécies, bem como a informação quanto aos seus componentes bioativos propiciaria aumento da diversidade alimentar, além de contribuir para conservação ambiental pela valorização real destas espécies e de seus habitats até então negligenciados e, provavelmente, pela redução do uso de agrotóxicos (KINUPP, 2007). Também o uso de extratos e componentes de plantas tornou-se cada vez mais importante para a investigação científica e aplicações industriais nos últimos anos (CHOU *et al.* 2012).

Plantas e seus produtos também são fontes potenciais de substâncias químicas capazes de neutralizar radicais livres, devido a sua atividade antioxidante (KHALAFALLA*, et al.* 2010). Os radicais livres são moléculas que causam estresse oxidativo, como resultado do desequilíbrio entre o sistema de defesa antioxidante e as espécies reativas de oxigênio (ROS). As ROS induzem o dano oxidativo das biomoléculas essenciais, como proteínas, DNA, lipoproteínas e lipídios (YAZDANPARAST & ARDESTANI, 2007). Este dano é um fator crucial etiológico implicado em várias doenças humanas, incluindo câncer, diabetes, cirrose hepática e aterosclerose (ADEDAPO *et al.* 2008).

Estudos epidemiológicos demonstraram que o consumo de alimentos contendo antioxidantes e fitonutrientes podem reduzir este processo degenerativo (HALLIWELL, 1977; RAPISARDA *et al.* 1999)

Para neutralizar os radicais livres, os antioxidantes nutricionais agem como doadores, de hidrogênio ou de elétrons, ou ainda podem reagir diretamente com eles (OLIVEIRA *et al.* 2009). Entre os compostos mais estudados estão os carotenoides, tais como carotenos, xantofilas, os polifenóis, como os ácidos fenólicos, flavonoides e as vitaminas A, C e E.

Plantas nativas do Rio Grande do Sul tais como, urumbeba (*Opuntia monacantha* Willd. Haw.) urtiga cansanção (*Urera aurantiaca* Wedd.) e o crem (*Tropaeolum pentaphyllum* Lam.) são consideradas inexploradas pela insuficiente divulgação e de pesquisa de caracterização botânica e nutricional dessas espécies. Sendo que, estas plantas podem representar significativa fonte de compostos antioxidantes e de outros constituintes benéficos para a saúde humana. Dessa forma, este trabalho teve como objetivo: (i) caracterizar partes das plantas (tubérculo, folha e flor da espécie *T. pentaphyllum*, folha da espécie *U. aurantiaca* e a polpa da fruta da espécie *O. monacantha*) com análises físico-químicas e composição centesimal. (ii) qualificar e quantificar os compostos bioativos presentes nos extratos das três espécies de plantas

citadas anteriormente. (iii) avaliar a atividade antioxidante por três diferentes métodos nos extratos das três espécies de plantas e os compostos que contribuem para sua atividade em dois diferentes extratos (50:50 v/v metanol:água e 70:30 v/v acetona:água) para os ensaios antioxidantes DPPH (2,2-difenil-1-picrilhidrazila) e ABTS(Ácido 2,2'-azino-bis-(3-etilbenzotiazolina-6-sulfônico) e extrato alcóolico (álcool etílico 95% P.A) para o ensaio antioxidante TRAP (atividade antioxidante total).

1.2. OBJETIVO GERAL

Caracterizar o potencial nutricional, qualificar e quantificar os compostos bioativos, a atividade antioxidante e correlacionar atividade antioxidante com os compostos bioativos das plantas alimentícias não convencionais do Rio Grande do Sul, tais como: Urumbeba (*Opuntia monacantha* Willd Haw.), Crem (*Tropaeolum pentaphyllum* Lam.) e Urtiga cansanção (*Urera aurantiaca* Wedd.).

1.2.1 Objetivos Específicos

- Analisar físico-quimicamente as plantas alimentícias não convencionais através de análises de pH, acidez titulável, sólidos solúveis totais (°Brix) e açúcares totais e redutores;

- Analisar a composição centesimal (carboidratos, proteínas, lipídios, cinzas, fibra dietética total, insolúvel, solúvel e umidade).

- Determinar o conteúdo de clorofila a, b e clorofila total, carotenoides totais e compostos fenólicos totais com metodologias em espectrofotômetro;

- Determinar a atividade antioxidante pelos métodos DPPH e ABTS, com mecanismos de atividade sequestradora e o TRAP com mecanismo de atividade antioxidante;

- Identificar o perfil de compostos bioativos, tais como: carotenoides, tocoferóis, flavonoides e ácido ascórbico por cromatografia líquida de alta eficiência;

- Determinar quantitativamente os compostos bioativos, previamente identificados;

- Correlacionar os resultados de atividade antioxidante com a quantificação dos compostos bioativos.

CAPÍTULO 1: REVISÃO DA LITERATURA

2. REVISÃO

2.1 Plantas Alimentícias Não Convencionais

Conceitualmente, plantas alimentícias são aquelas que possuem uma ou mais partes ou produtos que podem ser utilizados na alimentação humana, tais como: raízes tuberosas, tubérculos, bulbos, rizomas, cormos, ramos tenros ("talos"), folhas, brotos, flores, frutos e sementes ou ainda látex, resina e goma, ou que são usadas para obtenção de óleos e gorduras comestíveis. Inclui-se neste conceito também as especiarias, substâncias condimentares e aromáticas, assim como plantas que são utilizadas como substitutos do sal, como edulcorantes, amaciantes de carnes, corantes alimentares e na produção de bebidas, de tonificantes e infusões (KUNKEL, 1984; FAO, 1992; KINUPP, 2007).

Segundo (KINUPP & BARROS, 2008) poucas informações a respeito da composição nutricional das plantas alimentícias nativas no Brasil estão disponíveis e, na maioria, quando encontradas, são pesquisas realizadas em outros países para espécies de ampla distribuição, mas em condições de adaptação de solo e clima muito distintos. Assim, torna-se necessário o aprofundamento de pesquisa científica, em plantas nativas do Brasil ainda negligenciadas.

A domesticação de espécies nativas representa uma grande oportunidade a ser explorada, incluindo aquelas já conhecidas e utilizadas em pequena escala por populações locais e regionais, porém com pouca penetração no mercado nacional ou internacional (NASS *et al.* 2009). A Região Sul do Brasil é detentora de grande biodiversidade vegetal devido à sua privilegiada amplitude de clima e relevo. Dentre as plantas da região, muitas possuem raízes tuberosas, caules, folhas, flores, frutos e/ou sementes potencialmente alimentícios (KINUPP, 2011).

No entanto, algumas espécies nativas ainda continuam praticamente inexploradas, tais como: Urumbeba (*Opuntia monacantha* (Willd) Haw.), Batata-crem, folha e flor (*Tropaeolum pentaphyllum* Lam) e Urtiga *(Urera aurantiaca* Wedd.).

2.1.1 Urumbeba ((*Opuntia monacantha* (Willd) Haw.))

A fruta urumbeba *Opuntia monacantha* (Willd) Haw. pode ser encontrada na literatura pelos sinônimos *Cactus urumbeba* Vell., *Opuntia urumbeba* (Vell.) Steud., *Opuntia arechavaletae* Speg. É popularmente conhecida por monducuru e palmatória, pertence à família Cactaceae, é uma planta frutífera não cultivada, porém relativamente comum na natureza, nativa na costa litorânea desde Sergipe até o Rio Grande do Sul, principalmente em restingas abertas (LORENZI *et al*. 2006).

A planta é um subarbusto suculento, ereto ou decumbente, de 1-3 metros de altura. O tronco principal é muito curto e achatado, como filocládios grossos e com segmentos de 10-20 cm de comprimento, com auréolas espaçadas de 2-4 cm, portanto cada uma 1-2 espinhos pungentes de 1-5 cm. As flores são solitárias, diurnas, andróginas e dispostas no ápice do segmento terminal, formadas na primavera. Os frutos (Figura 1) são do tipo baga, alongados, contendo polpa suculenta globosa de cor verde no ápice e placenta amarelada na base, de sabor levemente doce e pode ser consumida no estado natural (LORENZI *et al*. 2006).

Figura 1: Fruto do cactus *Opuntia monacantha* Willd Haw.
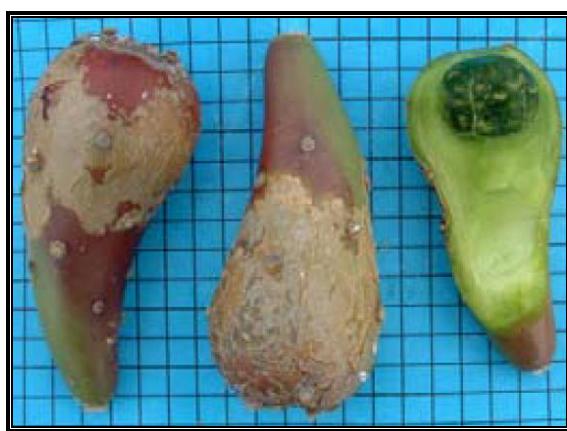
Fonte: LORENZI *et al*. (2006)

Kinupp & Barros (2008) avaliaram o teor de proteína dos cladódios da espécie *Opuntia monacantha* e encontram 3,91 g/ 100g de proteína em base seca. VALENTE *et al*. (2010) avaliaram o teor de nutrientes dos cladódios da mesma espécie e encontraram 91,1 g/ 100g de umidade, 15,0 g/ 100g de cinzas, 18,5 g/ 100g de fibras (fibras em detergente neutro - FDN), 5,4 g/ 100g de proteína e 1,4 g/ 100g de lipídios em base seca. OLIVEIRA *et al*., (1992) avaliaram as características físico-químicas da polpa do

fruto *O. monacantha* e encontraram pH 5,3, sólidos solúveis (°Brix) 12 g/ 100g, acidez titulável 0,20 g/ 100g, açúcares redutores 7,9 g/ 100g, cinzas 0,28 g/ 100g e umidade 95,23 g/ 100g em base úmida.

2.1.2 Batata – crem (*Tropaeolum pentaphyllum* Lam.)

A batata – crem *Tropaeolum pentaphyllum* Lam. também pode ser encontrada na literatura pelos sinônimos *Tropaeolum quinatum* Hellenius, *Tropaeolum chymocarpus* Morong, *Chymocarpuspentaphyllus* (Lam.) D. Don. É popularmente conhecida por crem, batata-crem, crem-de-baraço e crem-trepador, pertence à família Tropaeolaceae, e é a única subespécie encontrada em cultivo nos produtores tradicionais dos Estados de Santa Catarina e Rio Grande do Sul (KINUPP; LISBÔA; BARROS, 2011).

O consumo dos tubérculos é realizado em especial no Sul do Brasil, principalmente em cidades com influência da imigração italiana. O principal uso é dos tubérculos frescos ralados e condimentados no vinagre tinto ou branco. O hábito do consumo é juntamente com sopas e carnes.

De acordo com as características botânicas essa planta é uma trepadeira herbácea que fixa ao suporte pela torção dos pecíolos, sendo a parte aérea anual, mas os tubérculos subterrâneos rebrotando (perenes) na estação seguinte. Essa planta produz diversos tubérculos separados por segmentos intersticiais (Figura 2) que podem chegar até 1,6kg aproximadamente (KINUPP; LISBÔA; BARROS, 2011).

Figura 2 - Tubérculos de *Tropaeolum pentaphyllum* Lam.

Fonte: KINUPP (2007)

Os frutos maduros caem rapidamente da planta-mãe dificultando a colheita para propagação, sendo assim, deve ser feita com os frutos desenvolvidos, mas ainda verdes e com o pericarpo duro (FABBRI & VALLA, 1998). Além do uso alimentício, a espécie é bastante utilizada medicinalmente. No estudo realizado por RITTER *et al*. (2002) avaliaram que a população do município de Ipê – RS, faz uso popular do tubérculo na proteção contra o vírus da gripe. Segundo MORS; RIZZINI; PEREIRA (2000) os tubérculos são considerados antiescorbúticos e depurativos. Há indicação popular dos tubérculos também para redução e controle do colesterol, o que faz a demanda pelo produto aumentar (KINUPP, 2007). No entanto, frisa-se a inexistência de caracterização nutricional e de compostos bioativos desta espécie.

2.1.3 Folhas e Flores (*Tropaeolum pentaphyllum* Lam.)

As folhas da espécie *Tropaeolum pentaphyllum* Lam., são consumidas em saladas e as flores comestíveis são utilizadas para ornamentação de pratos (Figura 3). Devido aos longos pedicelos e à durabilidade, as flores podem ser comercializadas em pequenos molhos, o que já vem sendo realizado por produtora agroecológica, na cidade de Porto Alegre, tanto para alimentação direta quanto para decoração. As flores do crem são mais duradouras e possuem aroma e consistência diferentes da capuchinha ou nastúrcio (*Tropaeolum majus* L.) já comercializadas em diversas cidades brasileiras e do mundo (KINUPP; LISBÔA; BARROS 2011). Segundo RITTER *et al*. (2002) as flores são utilizadas na medicina popular no município de Ipê – RS como antidiabético.

Figura 3 – Folhas e Flores de *Tropaeolum pentaphyllum* Lam.

Fonte: KINUPP (2007); KINUPP; LISBÔA; BARROS (2011).

2.1.4 Urtiga (*Urera aurantiaca* Wedd.)

A urtiga da espécie *Urera aurantiaca* Wedd. é popularmente conhecida como cansanção (KINUPP, 2007) ou urtigão (BRACK, 1989), pertencente à família Urticaceae. De acordo com as características botânicas essa planta apresenta arbustos, com aproximadamente 1,5 metros, sendo que as estípulas possuem 1-1,2 cm. As folhas com pecíolos de 3–4 cm e lâminas ovadas com 8–5 cm × 4,5–7,5 cm e bordo regularmente dentado (Figura 4). As flores pistiladas com tépalas e máculas arredondadas claras, estiletes curtos e retos (PEDERNEIRAS *et. al.* 2011).

Segundo KINUPP (2007) esta espécie não possui acúleos (espinhos) nos ramos e nas folhas, o que facilita o manejo, além disso, é uma hortaliça saborosa, com grande potencial de uso direto e dos derivados, tanto para autoconsumo na propriedade quanto para comercialização. A urticância de suas folhas desaparece após mantê-las secando a sombra por 12 horas ou rapidamente se exposta ao sol ou estufa (calor). Seus perigônios carnosos e adocicados possuem coloração fortemente alaranjada e também são comestíveis. Segundo BRACK (1989) a etimologia da palavra latina "*aurantiacus*", significa cor alaranjada, relativo à cor do perigônio frutífero. As folhas merecem destaque pelo teor considerável de proteína, sendo as Urticaceae *Boehmeria caudata* e *Phenax uliginosus* (ambas com 24,15 g/ 100 g) e *Urera aurantiaca* (20,7 g/ 100 g) (KINUPP & BARROS, 2008).

Figura 4: *Urera aurantiaca* Wedd.

Fonte: KINUPP (2007)

2.2. Atividade antioxidante em plantas alimentícias não convencionais

Segundo BIANCHI & ANTUNES (1999) compostos antioxidantes são aqueles capazes de inibir a oxidação de moléculas simples a polímeros e biossistemas complexos. Estes compostos agem por meio da inibição da formação de radicais livres que possibilitam a etapa de iniciação, bem como, através da eliminação de radicais importantes (alcoxila e peroxila), impedindo a etapa de propagação da oxidação através da doação de átomos de hidrogênio a estas moléculas, interrompendo a reação em cadeia (SOARES, 2002).

Alguns compostos antioxidantes impedem a formação dos radicais livres e são capazes de interceptar os radicais gerados pelo metabolismo celular ou por fontes exógenas, impedindo o ataque sobre os lipídios, aminoácidos das proteínas, a dupla ligação dos ácidos graxos poliinsaturados e as bases do DNA, evitando as lesões e recuperando as membranas celulares e moléculas do DNA (BIANCHI & ANTUNES, 1999).

Sob o prisma terapêutico, alimentos ricos em antioxidantes também têm sido reconhecidos como essenciais na prevenção de doenças cardiovasculares, cânceres, problemas causados pelo envelhecimento das células e doenças neurodegenerativas como a doença de Parkinson (BANDONIENE *et al.* 2002). Segundo AVIGNON *et al.* (2012) alimentos derivados de plantas contêm centenas de compostos antioxidantes ativos, incluindo ácido ascórbico, tocoferóis, carotenoides, e uma vasta gama de outros constituintes como os ácidos fenólicos e flavonoides. Tais substâncias modulam o estresse oxidativo e protegem contra o dano oxidativo e suas complicações.

Valente *et al.* (2010) avaliaram a atividade antioxidante dos cladódios de cactus da espécie *Opuntia monacantha* através do ensaio com DPPH (2,2-difenil-1-picril-hidrazil). Segundo os autores, a atividade do extrato estudado (IC$_{50}$ 833,3 µg/mL) com as frações acetato de etila e n-butanol foram mais ativos (53,2 e 278 µg/mL respectivamente) e mostraram a presença de substâncias fenólicas por cromatografia em coluna (CC). A análise complementar por CLAE-DAD e técnicas de RMN dessas sub-frações reunidas, revelaram a presença de mistura de flavonoides glicosilados. A hidrólise ácida dessa mistura gerou os flavonóis campferol e isoramnetina, como únicos produtos desta correlação entre atividade antioxidante e presença de compostos bioativos.

2.2.1 Compostos Fenólicos

Os compostos fenólicos são metabolitos secundários de plantas e estão envolvidos no crescimento normal e desenvolvimento das plantas, atuando ainda como mecanismos de defesa contra parasitas patógenos, infecção e geração de radicais livres (MAISUTHISAKUL; SUTTAJIT; PONGSAWATMANIT, 2007). Para muitos compostos fenólicos têm sido demonstrada a atividade antioxidante (RICE-EVANS ; MILLER; PAGANGA, 1997).

As plantas sintetizam centenas de compostos fenólicos e polifenólicos que possuem variadas estruturas e funções. Entre estes compostos, os mais estudados como antioxidantes são os flavonóides que têm em comum a estrutura C6-C3-C6, consistindo de dois anéis aromáticos ligados por um heterocíclico oxigenado (CERQUEIRA; MEDEIROS; AUGUSTO, 2007). HURTADO-FERNÁNDEZ *et al*. (2010) apresenta uma classificação simplificada de compostos fenólicos e estruturas representativas pertencentes aos ácidos benzóicos, ácidos hidroxicinâmicos, flavonas, isoflavonas, flavanonas, flavonóis, flavonoides, antocianinas e taninos. Apenas os esqueletos de base, a partir dos quais os compostos fenólicos de origem vegetal são derivados são mostrados na (Figura 5).

FIGURA 5: Classificação simplificada dos compostos fenólicos.
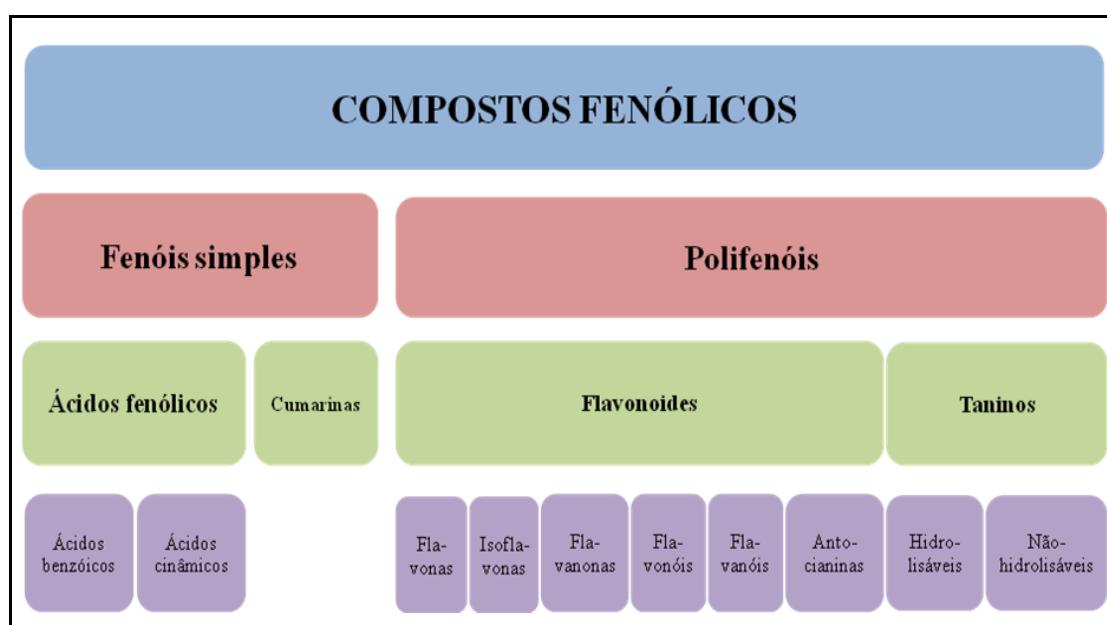
Adaptado de: HURTADO-FERNÁNDEZ *et al.* (2010)

Os antioxidantes fenólicos podem atuar como sequestradores de radicais livres e/ou quelantes de íons metálicos, sendo capazes de catalisar a peroxidação lipídica (BRAVO, 1998). Segundo CERQUEIRA; MEDEIROS & AUGUSTO (2007) os polifenóis são capazes de captar radicais alcoxila (RO•), alquilperoxila (ROO•), superóxido (O2 •-), radical hidroxila (HO•), óxido nítrico (NO•), além do oxidante peroxinitrito (ONOO-/ ONOOH).

Os componentes polifenólicos de plantas superiores podem atuar como antioxidantes ou como agentes por meio de mecanismos que contribuem para a ação anticarcinogênica ou cardioprotetora (RICE-EVANS ; MILLER & PAGANGA, 1997).

Kirka & Arslan (2008) avaliaram os teores de compostos fenólicos totais presentes em um extrato compostos de caules, folhas e flores da urtiga (*Urtica dioica* L.) e encontraram 9,25 mg EAG/ g em amostra seca, apresentando valores superiores quando comparado com os cladódios da *Opuntia fícus indica* f. *inermis* com 8,25 mg EAG/ g em amostra seca (AYADI *et al*. 2009). Garzón & Wrolstad (2009) encontraram nas flores alaranjadas da capuchinha (*Tropaeolum majus*) 4,06 mg EAG/ g em amostra úmida, sendo maior quando comparado com as folhas da batata-doce (*Ipomoea batatas* L.) com 3,97 mg EAG/ g em amostra úmida (ISABELLE, *et al*. 2010).

2.2.1.1 Flavonas e Flavonóis

Flavonoides são metabólitos secundários sintetizados pelas plantas e pertencem ao grupo dos compostos fenólicos. São formados pela combinação de derivados sintetizados a partir da fenilalanina (via metabólica do ácido shiquímico) e ácido acético (HUBER & RODRIGUEZ-AMAYA, 2008).

A estrutura dos flavonoides é baseada no núcleo que consiste de dois anéis fenólicos A e B e um anel C, que pode ser um pirano heterocíclico, como no caso de flavanóis (catequinas) e antocianidinas, ou pirona, como nos flavonóis, flavonas, isoflavonas e flavanonas, que possuem um grupo carbonila na posição C-4 do anel C, compreendendo as principais classes dos flavonoides (Figura 6) (HUBER & RODRIGUEZ-AMAYA, 2008).

Os flavonoides (exceto as catequinas) são encontrados em plantas principalmente na forma glicosilada, ou seja, ligados a moléculas de açúcares, sendo normalmente *O*-glicosídeos, com a molécula de açúcar ligada ao grupo hidroxila na posição C3 ou C7 (HERMANN, 1988 & ERLUND, 2004).

Figura 6: Quatro das principais classes de flavonas e flavonóis

Flavonóis			
	R_1	R_2	R_3
Quercetina	OH	OH	OH
Kaempferol	OH	H	OH
Flavonas			
Luteolina	H	OH	OH
Apigenina	H	H	OH
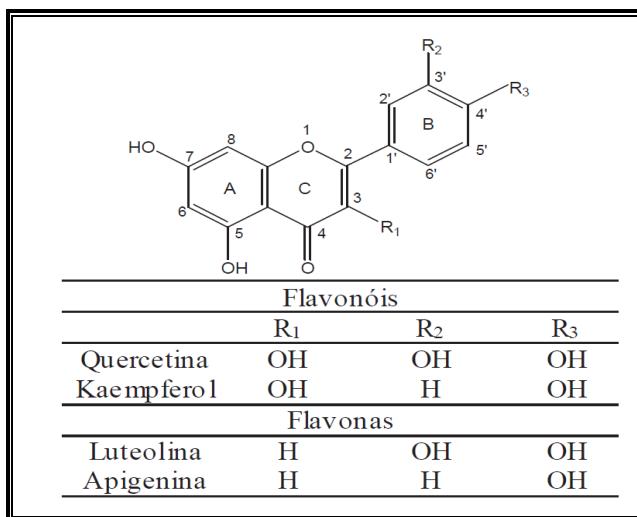
Fonte: HUBER & RODRIGUEZ-AMAYA (2008).

Os açúcares mais comuns são D-glicose e L-ramnose, porém, pelo menos 8 monossacarídeos diferentes ou combinações destes podem ligar-se aos diferentes grupos hidroxilas do flavonoide, resultando em um grande número de glicosídeos conhecidos. As moléculas desprovidas de açúcares são denominadas agliconas (HUBER & RODRIGUEZ-AMAYA, 2008). Entretanto, das principais classes, os flavonóis glicosídicos predominam em frutas e verduras (KÜHNAU, 1976; MARKHAM, 1989).

Das seis classes principais de flavonoides, flavonóis (por exemplo, miricetina, quercetina e campferol) e flavonas (por exemplo, apigenina, luteolina) são os mais amplamente distribuídos em alimentos vegetais, em que se encontram sob a forma glicosídica. Na análise de flavonoides, a hidrólise é normalmente realizada e as agliconas resultantes identificadas e quantificadas (HUBER; HOFFMANN-RIBANI; RODRIGUEZ-AMAYA, 2008).

Estudos demonstram que a quercetina possui um excelente potencial antioxidante *in vitro*, sendo o flavonoide com o maior poder sequestrador de espécies reativas de oxigênio. Este elevado poder antioxidante se deve à presença do grupo catecol no anel B e do grupo hidroxila na posição 3 do anel C (HEIJNEN, *et al*. 2002).

Estudos epidemiológicos indicam que uma dieta rica em flavonoides está associada à redução do risco de doenças cardiovasculares (KNEKT, *et al*. 1996; HERTOG; FESKENS; KROMHOUT, 1997; YOCHUM, et al. 1999) e certos tipos de cânceres (KNEKT *et al*. 1997; YANG, *et al*. 2001; NEUHOUSER, 2004).

Huber; Hoffmann-Ribani; Rodriguez-Amaya (2009) avaliaram o conteúdo de flavonas e flavonóis, tais como miricetina, quercetina, campferol, apigenina e luteolina na couve (*Brassica oleraceae*) e rúcula (*Eruca sativa*) cultivadas e comercializadas no Brasil. Na couve foram apresentados 327 µg/ g de quercetina e 336 µg/ g de campferol em base úmida, sendo superior quando comparado com o conteúdo de quercetina na rúcula, apresentando 140 µg/ g e inferior quando comparado com o conteúdo de campferol na rúcula 452 µg/ g em base úmida. Para os demais compostos (miricetina, apigenina e luteolina) não foram determinados em ambas às amostras.

2.2.2. Clorofilas

As clorofilas são os pigmentos naturais mais abundantes presentes nas plantas e ocorrem nos cloroplastos das folhas e em outros tecidos vegetais. Estudos em uma grande variedade de plantas caracterizaram que os pigmentos clorofilados são os mesmos. As diferenças aparentes na cor do vegetal são devidas à presença e distribuição variável de outros pigmentos associados, como os carotenoides, os quais sempre acompanham as clorofilas (VON ELBE, 2000).

As clorofilas *a* e *b* encontram-se na natureza numa proporção de 3:1, respectivamente, e diferem nos substituintes de carbono C-3. Na clorofila *a*, o anel de porfirina contém um grupo metil (-CH3) no C-3 e a clorofila *b* (considerada um pigmento acessório) contém um grupo aldeído (-CHO), que substitui o grupo metil-CH3 (Figura 7). A estabilidade da clorofila *b* deve-se ao efeito atrativo de elétrons de seu grupo aldeído no C-3 (VON ELBE, 2000).

As clorofilas localizam-se nos cloroplastos, sendo esta organela a responsável pela fotossíntese, onde ocorrem as duas reações importantes: a fotoquímica, nas membranas dos tilacóides e a bioquímica, no estroma do cloroplasto. Tais organelas, além das clorofilas, contêm outros pigmentos, como os carotenoides (STREIT, *et al*. 2005).

Os pigmentos verdes muito comuns em legumes e em várias frutas são também usados como aditivos para produtos alimentícios. Entretanto, são quimicamente instáveis e podem ser alterados ou destruídos facilmente, modificando a percepção e a qualidade dos produtos. Em geral, as clorofilas são relativamente instáveis e sensíveis à luz, aquecimento, oxigênio e a degradação química (SCHOEFS, 2002).

Figura 7: Estrutura da Clorofila *a* e *b*
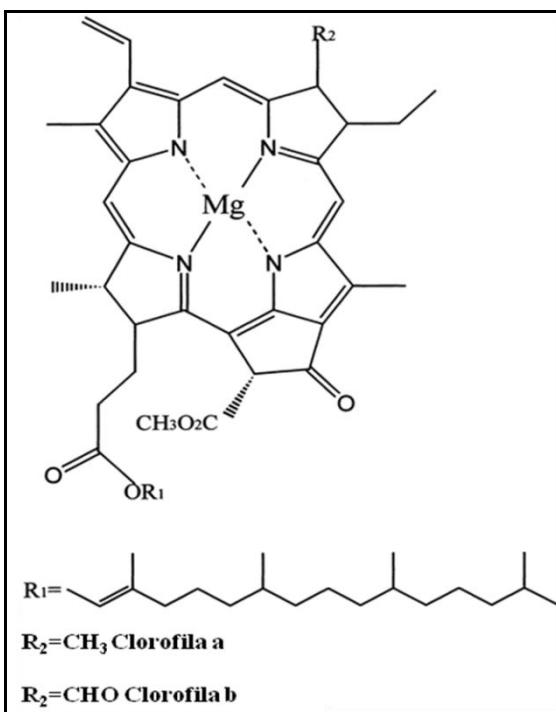
Adaptado de: TAN *et al*. 2000

As clorofilas tendem a ser foto-oxidadas sob alta irradiação; os carotenoides por sua vez podem prevenir a foto-oxidação das clorofilas. As relações entre as clorofilas e os carotenoides podem ser usadas como um indicador potencial de perdas foto-oxidativas causadas por fortes irradiações (HENDRY & PRICE, 1993). Contudo, os pigmentos clorofilados são de grande importância comercial, podendo ser utilizados tanto como pigmentos quanto como antioxidantes (STREIT, *et al*., 2005).

Kukrić *et al*. (2012) avaliaram o conteúdo de clorofila a, b e clorofila total nas folhas de urtiga (*Urtica dioica* L.) e foram encontrados 0,88 mg/ g de clorofila a, 0,28 mg/ g de clorofila b e 1,17 mg/ g de clorofila total em base úmida, apresentado valores maiores quando comparado com a polpa da fruta amarela da *Opuntia megacantha* com 0.017, 0.016 e 0.037 mg/ g em base úmida respectivamente, no estágio zero (semana) de amadurecimento.

2.2.3. Carotenoides

Os carotenoides são pigmentos naturais responsáveis pela gama de cores, de amarelo a laranja ou vermelha de muitas frutas, hortaliças, gema de ovo, crustáceos

cozidos e alguns peixes. São também substâncias bioativas, com efeitos benéficos à saúde, e alguns deles apresentam atividade pró-vitamínica A (RODRIGUEZ-AMAYA; KIMURA; AMAYA-FARFAN, 2008).

Frutas e vegetais constituem as principais fontes de carotenoides na dieta humana (MANGELS *et al*. 1993; AGARWAL & RAO, 2000). Embora eles estejam presentes como microcomponentes em frutas e vegetais (RAO & RAO, 2007), os carotenoides estão entre os constituintes alimentícios mais importantes (RODRIGUEZ-AMAYA; KIMURA; AMAYA-FARFAN, 2008). Segundo RODRIGUEZ-AMAYA; KIMURA; AMAYA-FARFAN, (2008) os carotenoides mais pesquisados por seu envolvimento na saúde humana são o β-caroteno (β,β- caroteno), α-caroteno (β,ε-caroteno), β-criptoxantina (β, β-caroten-3-ol), licopeno (ψ,ψ-caroteno), luteína (β,ε-caroteno-3,3'-diol) e zeaxantina (β,β- caroteno-3,3'-diol) (Figura 8).

FIGURA 8: Estrutura de alguns dos principais carotenoides na dieta.
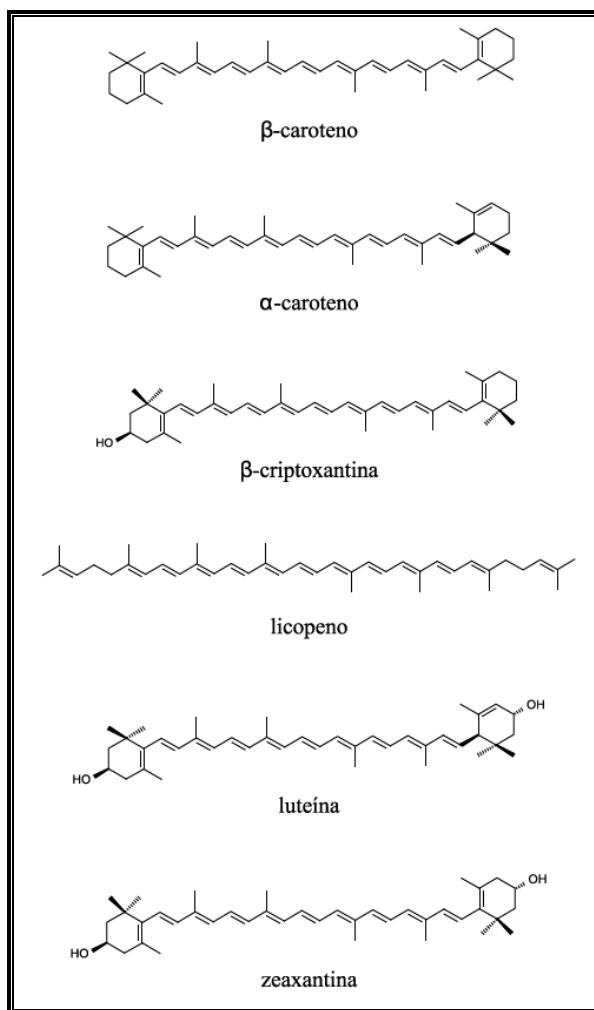
Fonte: RODRIGUEZ-AMAYA; KIMURA; AMAYA-FARFAN (2008).

O β-caroteno, o α-caroteno e a β-criptoxantina são pró-vitaminas A. Basicamente, a estrutura da vitamina A (retinol) é a metade da molécula do β-caroteno, com uma molécula de água adicionada no final da cadeia poliênica. Conseqüentemente, o β-caroteno é o carotenoide de maior potência vitamínica A e ao qual se atribui, teoricamente, 100% de atividade (RODRIGUEZ-AMAYA; KIMURA; AMAYA-FARFAN, 2008).

Efeitos promotores da daúde humana têm sido atribuídos aos carotenoides, como redução do risco de contrair doenças crônicas degenerativas, câncer, doenças cardiovasculares, catarata e degeneração macular relacionada à idade (GAZIANO & HENNEKENS, 1993; KRINSKY, 1993; ASTORG, 1997; OLSON, 1999). Tais atividades fisiológicas não possuem relação com a atividade vitamínica A e têm sido atribuídos às suas propriedades antioxidantes, especificamente, à capacidade de seqüestrar o oxigênio singleto e interagir com os radicais livres (PALOZZA E KRINSKY, 1992).

Eles podem atuar na proteção de células contra radicais livres como sequestrar espécies reativas de oxigênio (EDGE; MCGARVEY; TRUSCOTT, 1997). A propriedade antioxidante dos carotenoides se deve pela presença de um sistema de duplas ligações conjugadas, que confere a estes a capacidade de capturar os radicais livres (YOUNG & LOWE, 2001).

Estudos em humanos também têm demonstrado que o consumo de frutas e vegetais ricos em carotenoides, aumenta a resistência à oxidação de lipoproteínas de baixa densidade (LDL) e diminui o dano ao DNA (CHOPRA *et al.* 2000).

Rodriguez-Amaya, Kimura & Amaya-Farfan (2008) avaliaram o conteúdo de carotenoides das folhas e das flores amarelas e alaranjadas da espécie *Tropaeolum majus* e encontraram 136 μg/ g de luteína e 69 μg/ g de β-caroteno nas folhas. Nas flores foi encontrado alto teor de luteína, sendo 450 μg/ g nas flores amarelas e 350 μg/ g nas flores alaranjadas.

2.2.4. Ácido ascórbico

A vitamina C é uma das mais importantes vitaminas solúveis em água, naturalmente presentes em alimentos, especialmente em frutas e legumes, e é amplamente utilizada como um aditivo alimentar e como antioxidante (PÉNICAUD *et al.* 2010).

Apesar de ser amplamente conhecido por sua atividade antioxidante, o ácido ascórbico também pode atuar como pró-oxidante, pois ao doar os dois hidrogênios redutores ficam susceptíveis a receber elétrons, devido ao radical ascorbila formado, que é um agente oxidante (HASSIMOTO *et al*. 2005). Assim, baixas concentrações de ascorbato aumentam a atividade dos radicais de oxigênio, enquanto que altas concentrações de ascorbato atuam sequestrando radical hidroxila, oxigênio singlete e peróxidos (SAKAGAMI *et al*. 2000).

Estudos epidemiológicos mostram que os indivíduos com maior ingestão de vitamina C, têm menor risco de serem acometidos por várias doenças crônicas, ou seja, doenças cardíacas, diabetes, câncer ou doenças neurodegenerativas (JACOB & SOTOUDEH, 2002).

Pereira *et al*., (2013) avaliaram o conteúdo de vitamina C em frutos da família Cactaceae, conhecido popularmente como mandacaru-de-três-quinas (*Cereus hildmannianus* K. Schum) e encontraram 0,25 mg/ g de ácido ascórbico em base úmida, apresentando valor superior quando comparado com o espinafre, com 0,089 mg/ g de ácido ascórbico em base seca (FRANKE et al. 2004).

2.2.5. Tocoferol

Tocoferóis fazem parte de um grupo de quatro (α, β, γ e δ) antioxidantes lipofílicos sintetizado por organismos fotossintéticos, ocorre principalmente nas folhas e sementes (MUNNE-BOSCH & ALEGRE, 2002). Sua função antioxidante é atribuída à inibição da peroxidação lipídica de membranas e eliminação de espécies reativas de oxigênio (KRUK *et al*. 2005; MUNNE-BOSCH & ALEGRE (2002); TREBST; DEPKA; HOLLÄNDER-CZYTKO (2002), mas também outras funções foram mostradas no metabolismo vegetal, como papel na exportação de açúcar a partir do floema localizados nas folhas (PORFIROVA *et al*. 2002; HOFIUS *et al*., 2004).

Devido ao seu papel como varredor de radicais livres, acredita-se que a vitamina E também protege contra processos degenerativos, como o câncer e doenças cardiovasculares (BURTON & TRABER, 1990).

Os dados da literatura sobre o conteúdo de tocoferóis foram principalmente dedicados aos óleos de sementes, devido à importância nutricional da vitamina E (SZYMANSKA & KRUK, 2007). Entretanto, a maioria dos alimentos de origem vegetal, especialmente frutas e verduras, contêm níveis de vitamina E de baixa a moderada quantidade, mas devido à abundância de alimentos derivados de plantas em

nossa dieta, eles fornecem uma fonte significativa e consistente dessa vitamina. (EITENMILLER & LEE, 2004).

Contudo, DELLAPENNA (2005) mostra dados de níveis de tocoferol em vegetais, como folha de alface apresentando 7 µg/ g em peso úmido e na folha de espinafre apresentando 30 µg/ g em peso úmido no total de tocoferol. Deste total de tocoferol apresentado, a folha de alface apresenta 55 % de α-tocoferol e 45 % de γ-tocoferol e a folha de espinafre, apresenta 63 % de α-tocoferol, 5 % de γ-tocoferol e 33 % de δ-tocoferol (DELLAPENNA, 2005).

2.2.6. Atividade antioxidante *in vitro* pelos métodos ABTS, DPPH e TRAP

Halliwell & Gutteridge (1995) definiram antioxidantes como "qualquer substância que, quando presentes em baixas concentrações em comparação com a de um substrato oxidável, atrasa significativamente ou inibe a oxidação do referido substrato". Mais tarde definiu como "qualquer substância que retarda, evita ou remove o dano oxidativo a uma molécula alvo" (HALLIWELL, 2007).

A atividade antioxidante pode ser eficaz por meio de várias formas, como inibidores de reações de oxidação de radicais livres (oxidantes preventivos) inibindo a formação de radicais livres de lípides; interrompendo a propagação da reação em cadeia de auto-oxidação (antioxidantes de ruptura de cadeia); como supressores de oxigênio singlete; através de sinergia com outros antioxidantes; como agentes que convertem hidroperóxidos reduzindo em compostos estáveis; como quelantes de metais que se convertem de metal pro-oxidantes (derivados de ferro e cobre) em produtos estáveis; e finalmente, como inibidores de enzimas pró-oxidativas (lipooxigenases) (DARMANYAN *et al*., 1998; HEIM *et al*., 2002; MIN & BOFF, 2002; POKORNÝ, 2007; KANCHEVA, 2009).

Até o momento existem vários ensaios de atividade antioxidante, cada um tendo seu alvo específico dentro da matriz alimentar e todas elas com vantagens e desvantagens. Não há um método que pode fornecer resultados inequívocos e a melhor solução é a utilização de vários métodos, ao invés de uma única abordagem metodológica (CAROCHO & FERREIRA, 2013).

Os ensaios baseados em transferência de elétrons analisados por espectrofotometria medem a capacidade de um antioxidante na redução de um oxidante, que muda de cor quando reduzido. O grau de mudança de cor (um aumento ou

diminuição da absorvância a um determinado comprimento de onda) é correlacionado com a concentração de antioxidantes na amostra (APAK *et al.*, 2013).

Os ensaios antioxidantes ABTS (Ácido 2,2'-azino-bis-(3-etilbenzotiazolina-6-sulfônico) e DPPH (2,2-difenil-1-picrilhidrazila) são baseados pelos mecanismos de atividade sequestradora do radical (ABTS ou DPPH) por antioxidantes (ANTOLOVICH *et al.*, 2002; MOON & SHIBAMOTO, 2009). O método ABTS é um ensaio colorimétrico, em que o radical ABTS descolore na presença de antioxidantes, como por exemplo, carotenóides, compostos fenólicos, etc. O ensaio DPPH baseia-se na premissa de que um hidrogênio doador é um antioxidante. Este ensaio colorimétrico usa o radical DPPH, que muda de roxo para amarelo na presença de antioxidantes, e é amplamente usado como um estudo preliminar (MOON & SHIBAMOTO, 2009).

O potencial reativo antioxidante total (TRAP) é um ensaio utilizado para determinar a atividade antioxidante total, através do método desenvolvido por (WAYNER *et al.,*1985) e adaptado por (LISSI; PASCUAL; DEL CASTILLO, 1992).

Resumidamente, este método baseia-se na utilização de um composto azo solúvel em água AAPH 2,2'-azobis (2-amidinopropano) dihidrocloro como fonte de radical livre. A decomposição térmica deste composto na presença de luminol produz quimiluminescência, a qual pode ser diretamente relacionada à taxa de oxidação do luminol. A adição de substâncias sequestrantes de radicais livres reduz esta intensidade. O tempo para recuperar a luminescência é relacionado à quantidade total de antioxidantes presentes na amostra adicionada e é chamado de tempo de indução (VISIOLI & GALLI, 1997) que pode ser comparada com o tempo de indução do padrão trolox, obtendo-se o valor de TRAP para amostras expressos em equivalentes de trolox (DE PEREZ *et al.*, 2000).

O TRAP mede o tempo que a amostra ou padrão mantém o sequestro do radical livre, sendo o parâmetro medido chamado de área sob a curva (AUC), enquanto que o TAR (reatividade antioxidante total) indica a qualidade (dado pela reatividade) na amostra com atividade antioxidante (ROSSATO *et al.*, 2009).

No estudo realizado por (PEREIRA *et al.*, 2013) foi avaliada a atividade antioxidante pelos métodos ABTS e DPPH do fruto de cactus *Cereus hildmannianus* K. Schum. O valor encontrado para ABTS foi de 19,61 µM de trolox/ g de fruta fresca e para DPPH foi de 3249,77 g de fruta fresca/ g de DPPH. Apresentando maior atividade antioxidante, quando comparado com o umbu (6,3 µM de trolox/ g de fruta) para ABTS e para o DPPH (7074 g de fruta/ g DPPH).

Rossato *et al*., (2009) avaliaram a atividade antioxidante pelo método TRAP da polpa de três cultivares de pêssegos (Leonense, Maciel e Eldorado). As cultivares Leonense e Maciel apresentaram menores valores de AUC (maior potencial antioxidante) quando comparada com a cultivar Eldorado. Com relação ao índice TAR, a cultivar Leonense apresentou maior reatividade e a Eldorado a menor.

Este trabalho teve como proposta, buscar informações a respeito da composição nutricional de três plantas alimentícias nativas do Rio Grande do Sul, que ainda são consideradas negligenciadas pela população e na pesquisa científica. Algumas espécies nativas continuam completamente inexploradas, como é o caso do crem (*Tropaeolum pentaphyllum* Lam.), sendo este, o primeiro estudo a pesquisar o potencial nutricional desta espécie. A partir deste trabalho, torna-se necessário o aprofundamento de pesquisas também em outros setores, como nutracêutico e farmacológico.

CAPÍTULO 2: ARTIGOS

BIOACTIVE COMPOUNDS AND NUTRITIONAL AND ANTIOXIDANT ACTIVITY OF *Opuntia monacantha* (Willd.) Haw PULP AND *Urera aurantiaca* Wedd. LEAVES

Bioactive compounds and nutritional and antioxidant activity of *Opuntia monacantha* (Willd.) Haw pulp and *Urera aurantiaca* Wedd. leaves

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Abstract

The objectives of this study were to evaluate the nutritional characteristics of Brazilian plant species, determine the total phenolics, total carotenoids and chlorophyll a and b levels, quantify the individual bioactive compounds, and assess the antioxidant activity using different methods. Furthermore, the compounds that contribute the activity of two different extracts were identified. *Opuntia monacantha* (Willd.) Haw. (*Om*) pulp was promising, particularly compared to other species of the same genus *Opuntia*. *Om* exhibited high levels of carotenoids (lutein 133.04 ± 0.84 µg/ g dry weight (dw) and β-carotene 102.42 ± 0.94 µg/ g dw), flavonoids (luteolin 440 ± 3.87 µg/ g dw), total phenolics (6.61 ± 0.01 mg gallic acid equivalent GAE)/ g dw, and total chlorophyll (0.98 ± 0.15 mg/ g dw) and was presented with antioxidant activity, as measured by DPPH and ABTS (577 ± 0.56 g dw/ g DPPH and 149.03 ± 1.50 µM trolox/g dw). However, the reactivity of the extract was low as measured by the TAR method. *Urera aurantiaca Wedd.* (*Ua*) has potential in the agricultural industry because of its high amounts of lutein (808.09 ± 5.95 µg/ g dw), zeaxanthin (24.89 ± 0.18 µg/ g dw), β-carotene (613.25 ± 4.74 µg/ g dw), quercetin (1970 ± 6.98 µg/ g dw), total chlorophyll (12.39 ± 0.95 mg/ g dw), and protein (20.98 ± 0.71 g/ 100g dw). This study also demonstrated high antioxidant activity using the DPPH (119.87 ± 0.84 g dw/ g dpph) and TRAP methods and high quantity and extract quality using the TRAP/TAR methods.

Keywords: *Opuntia monacantha* (Willd) Haw, *Urera aurantiaca* Wedd, native food plants, chemical composition, bioactive compounds, antioxidant, food potential and alternative

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1. Introduction

Brazil has a rich diversity of plant species with an estimated number of almost 56,000 species, nearly 19% of the world's flora and 54.2% are endemic (Giulietti et al. 2005; Scariot, 2010).

The search for native fruits and vegetables has become a worldwide endeavor. Several native plant resources are of great current and potential importance that can be used as food and alternative income sources (Leite & Coradin, 2011).

Opuntia monacantha (Willd.) Haw. (Cactaceae) is native to Brazil, Argentina, Paraguay and Uruguay. A study of the cladodes of *O. monacantha* revealed antiradical activity, a high levels of flavonoids and nutritional potential (Valente et al. 2010). *Urera aurantiaca* Wedd. (Urticaceae) is native to Brazil, Argentina and Paraguay. A study of the content protein of *U. aurantiaca* leaves and *O. monacantha* fruit (Kinupp & Barros, 2008) identified high protein levels compared to other common vegetables and fruits.

The antioxidant capacity of fruits and vegetables is directly related to the presence of bioactive compounds. However, it is important to estimate the amount of bioactive compounds that provide antioxidant activity, are several methods are used to determine this activity. The chemical diversity of natural antioxidants makes it difficult to separate, detect and quantify individual antioxidants from a complex food matrix. Currently, there is no single antioxidant assay for food because of the lack of standard quantification methods (Apak et al. 2013). Assays, such as ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (Antolovich et al. 2002; Moon and Shibamoto 2009) and DPPH (2,2-diphenyl-1-picrylhydrazyl) (Antolovich et al. 2002; Amarowicz et al. 2004), which determine the scavenging activity, are used to estimate the antioxidant activity of fruits and vegetables. FRAP (ferric reducing antioxidant power) identifies the mechanism of reducing power (Antolovich et al. 2002). The TEAC assay

(Trolox equiv. antioxidant capacity) identifies the mechanism of antioxidant activity (Huang et al. 2005). TRAP (total radical-trapping antioxidant parameter) (Antolovich et al. 2002) and ORAC are also (the oxygen radical absorption capacity) (Prior et al. 2003) both used to determine the mechanism of antioxidant activity.

It is also important to identify the bioactive compounds in the studied material and in extracts used in these studies, which cause the beneficial health effects. Information regarding the chemical composition, antioxidant activity and bioactivity of plants is important for their potential use as food.

The objective of this study was to analyze the pulp of the Brazilian fruit *O. monacantha* and the leaf of *U. aurantiaca.* In this study, the nutritional potential, bioactive compound levels (carotenoids, flavonoids, tocopherol and ascorbic acid) and antioxidant activity are reported. The methods DPPH, ABTS and TRAP are used to determine these characteristics and to identify the compounds that contribute to the activity of these two extracts.

2. Materials and Methods

2.1. Plant materials

The fruit from (urumbeba) *O. monacantha* (*Om*) and the leaf from (cansanção) *U. aurantiaca* (*Ua*) were directly obtained in Águas Claras, Viamão, Rio Grande do Sul, Brazil (Latitude 30° 09' 26.67" S and Longitude 50° 52' 33.01" W) and Lami, Porto Alegre, Rio Grande do Sul, Brazil (Latitude 30° 13' 19" S and Longitude 51° 5' 5" O), respectively. The fruits were collected in June, and the leaves were collected in September 2012, and their vouchers were deposited in the Institute of Natural Sciences (ICN) Herbarium (UFRGS, Porto Alegre, Rio Grande do Sul, Brazil) under the numbers 191981 (*Om*) and 157756 (*Ua*).

One lot (300 g) of *Ua* leaves and one lot (2 Kg) of *Om* fruits were collected. The leaves were selected and cleaned. The damages leaves were discarded and the remainder were freeze-dried (- 40 °C for 48 h). The fruits were picked and carefully selected to obtain a uniform batch by degree of maturity as determined by the intensity of their color. After the fruit was divided in half, the pulp with seed was removed and freeze-dried (- 40 °C for 72 h). The dried samples were homogenized with a mortar and pestle, and the samples were stored in sealed plastic bags at - 40 °C before analyses.

2.2. Standards

The standards used for the construction of calibration curves were (±)-α-tocopherol (purity $\geq$ 96%) and (+)-γ-tocopherol (purity $\geq$ 96%) and were purchased from Sigma- Aldrich Corp. (St. Louis, MO, USA). The carotenoids β-carotene (purity > 93%), β-cryptoxanthin (purity > 97%), zeaxanthin (purity > 95%), and α-carotene (purity > 95%) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Lutein (purity > 95%) was purchased from Indofine Chemical Company Inc. (Hillsborough, NJ, USA). The ascorbic acid (99.0 – 100.5%) was purchased from Casa da Química (Diadema, São Paulo, Brazil). The flavonoid luteolin-7-O-glucosidase (purity > 90%) was purchased from Indofine Chemical Company Inc. (Hillsborough, NJ, USA). quercetin (purity $\geq$ 98%) was purchased from Sigma-Aldrich (Steinheim, Germany), and myricetin (purity $\geq$ 96%) and kaempferol (purity $\geq$ 97%), were purchased from Sigma-Aldrich (Lyon, France). Chromatographic analyses were performed using the HPLC grade solvents methanol, methyl-tert-butyl and acetonitrile (J.T.Baker, Xalostoc, Mexico). Formic acid and HCl were acquired from Labsynth Ltda (Diadema, São Paulo, Brazil), and the supra pure sulfuric acid (PM 98.08) was from Vetec Ltda (Duque de Caxias, Rio de Janeiro, Brazil).

2.3. Centesimal Composition and physico-chemical analysis

The moisture (method 920.151), ash (method 940.26), protein (N x 6.25) (968.20) and lipid content (962.20) were determined according methods (AOAC, 1997). The total dietary fiber (TDF) and insoluble dietary fiber (IDF) are expressed as $g.100^{-1}$ g dw. The soluble dietary fiber (SDF) was calculated by subtracting the IDF from the TDF (AOAC, 1997). The hydrogen potential (pH) (method 968.20) was determined using a digital pHmeter (Quimis Q400M, São Paulo, Brazil). The titratable acidity (TA) was calculated using method (942.15B). The amount of soluble solids (SS) (method 932.12) was determined with a digital PAL-3 refractometer (Atago Co., Taiwan, China) and expressed in °Brix (AOAC, 1997). The reducing and non-reducing sugars were determined at the Instituto Adolfo Lutz (IAL, 2005).

2.4. Determination of pigment contents and total phenolic compounds by spectrometry

The pigment content was determined based on the methodology proposed by Brahmi et al. (2012) with some modifications. The leaves and fruit samples (0.25 g) were homogenized in an (IKA T-25 Ultra-Turrax Digital, Staufen, Germany) with 80% acetone (v/v), and the extract was then centrifuged in sealed tubes at $5,000 \times$ g for 15 min. The supernatant was filtered and collected. The absorbance was read at 663 and 647 nm for chlorophyll a and chlorophyll b, respectively, and at 470 nm for the carotenoid content using a Shimadzu model UV-1800 (Kyoto, Japan). The calculation was performed according to the method of (Lichtenthaler & Buschmann, 2001).

The phenolic compound analysis was performed according to the method of (Swain & Hillis, 1959). A standard curve was constructed using 30 to 300 $mg.g^{-1}$gallic acid to quantitate the phenolic compounds.

2.5. Antioxidant activity in vitro

2.5.1. DPPH (Radical scavenging assay) and ABTS^{+} (Radical cation scavenging)

Sequestering of the (2,2- diphenyl-1-picrylhydrazyl) (DPPH) radical was used to determine the antioxidant activity according to the method of Brand-Wiliams et al. (1995) and the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method of Kuskoski et al. (2005) was also used. For both methods, the extracts were obtained from a 2.5 g sample diluted in 50:50 v/v methanol:water and 70:30 v/v acetone:water sequentially, according to the method of Larrauri, Rupérez and Saura-Calixto (1997) using three different dilutions extract:water (2:0.5, 2:2 and 2:10 v/v).

2.5.2. TRAP (Total radical-trapping antioxidant)

TRAP was measured and calculated as previously described by (Dresch et al. 2009). The ethanol extract concentrate was diluted with water. The system was calibrated using Trolox. After 2 h, 180 µL is placed in the system in a 96-well plate and then 20 µL sample (final concentration 5 µg/ mL), 20 µL Trolox (final concentration 1 µM) or 20 µL vehicle (99.25% water/0.75% DMSO) is added, which represents the maximum radical generation. The results were transformed into a percentile rank, and the area under the curve (AUC) was calculated using the GraphPad software (San Diego, CA, USA). The smaller the AUC compared to the system, the higher the total reactive antioxidant potential of the sample. The TAR index was determined by measuring the initial decrease of luminol luminescence calculated as the ratio I_0/I, where (I_0) is the initial emission of CL before the addition of the antioxidant and (I) is the instantaneous CL intensity after the addition of an aliquot of the sample or the reference compound (Trolox).

2.6. Determination bioactive compounds

The determination of bioactive compounds was performed on the samples in two different extracts (50:50 v/v methanol:water and 70:30 v/v acetone:water for the antioxidant assays DPPH (2,2- diphenyl-1-picrylhydrazyl) and ABTS 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) and in an ethanolic extract (ethyl alcohol 95% P.A) for TRAP (total radical-trapping antioxidant).

2.6.1. HPLC analysis of α- tocopherol and γ-tocopherol

The determination of tocopherol was based on the methodology proposed by Freitas et al. (2008) with some modifications for the extract. The freeze-dried (1 g) *Om* and *Ua* samples were macerated with absolute methanol until the pigments were completely removed and then filtered using a vacuum system. The extract was concentrated under reduced pressure in a rotary evaporator at ambient temperature (Fisatom, Model 801, St. Paul), diluted in 5 mL methanol, and placed in amber vials. The solvent was removed under nitrogen flow, and the extracts were stored at − 10 °C the tocopherol was quantified. The analyses were performed on an HPLC system (Agilent 1100) equipped with an online degasser, a quaternary pump, an automatic injector, and spectra (UV-Visible Agilent 8453) coupled to a C^{18} polymeric column from Vydac (218TP54) (250 mm × 4.6 mm i.d., 5 μm.) adjusted to 30 °C. Data acquisition and processing were performed using the ChemStation® software (Waldbronn, Germany). For quantification, a calibration curve was constructed with individual solutions of α-tocopherol and γ-tocopherol diluted with methanol (0.002 − 1 mg.g^{-1}) and (0.001 − 0.10 mg.g^{-1}), and the limits of detection (LOD) and quantification (LOQ) were (0.004 - 0.012 mg.g^{-1}) and (0.002 − 0.006 mg.g^{-1}), respectively.

2.6.2. HPLC analysis of carotenoid and Vitamin A

The carotenoid extract was prepared according to (Mercadante & Rodriguez Amaya, 1998). The analyses were performed using the same HPLC system described above. The carotenoids were separated on a polymeric reverse phase column YMC C^{30} (250 mm x 4.6 mm id., 3 µm) adjusted to 33 °C, with a mobile phase gradient elution beginning with water/methanol/MTBE at 5:90:5 and reaching 0:95:5 after 12 minutes, 0:89:11 after 25 minutes, 0:75:25 after 40 minutes and 00:50:50 after 60 minutes with a flow rate of 1 mL/min and injection volume of 5 µL (Zanatta and Mercadante, 2007). Data acquisition and processing were performed using the ChemStation® software (Waldbronn, Germany). Quantification was performed by constructing calibration curves for the carotenoids in the following concentration ranges: 3-700 µg.g^{-1} for β-carotene, 0.05-280 µg.g^{-1} for α-carotene, 0.2-850 µg.g^{-1} for lutein, 2-100 µg.g^{-1} for cryptoxanthin and 1-40 µg.g^{-1} for zeaxanthin. The following LOD and LOQ scores were obtained, respectively: 0.063 and 0.192 µg/g for β-carotene; 0.069 and 0.210 µg/ g for lutein; 0.021 and 0.065 µg/ g for β-cryptoxanthin; 0.015 and 0.045 µg/ g for zeaxanthin; and 0.032 and 0.100 µg/ g for α-carotene.

The vitamin A activity was calculated assuming a bioconversion factor based on the (WHO/FAO, 1967). The factor equivalence corresponds to 6 µg β-carotene or 12 µg α-carotene and β-cryptoxanthin.

2.6.3. HPLC-DAD analysis of ascorbic acid

The determination of ascorbic acid was based on the methodology proposed by Rosa et al. (2007) with some modifications. The extraction was performed with 5 g fresh *Ua* and 2 g freeze-dried *Om*, which were homogenized in an (IKA T-25 Ultra-Turrax Digital, Staufen, Germany) with 20 mL 0.05 M suprapure 96% sulfuric acid

(Vetec Ltda, Rio de Janeiro) for 1 minute, centrifuged at 22,000 x g for 15 min and then filtered through a 0.45 μm PTFE filter. The analyses were performed with a high performance liquid chromatography unit (Shimadzu, model CBM 20A, Kyoto, Japan), equipped with an online degasser, a ternary pump, an automatic injector (SIL-20AHT), and a photodiode array detector (SPD-M20A) and coupled to a C^{18} polymeric column Vydac (218TP54) (250 mm × 4.6 mm i.d., 5 μm.) adjusted at 28 °C. The data acquisition and processing were performed using the LC Operation® software (Chiyoda-ku, Tokyo, Japan). The ascorbic acid was eluted isocratically using 0.05 M supra pure sulfuric acid as the mobile phase with a flow rate of 1 mL/min and an injection volume of 10 μL. For the quantification of ascorbic acid, a calibration curve in ranging from 0.01 to 1 mg.g^{-1} was used. The limit of detection (LOD) and quantitation (LOQ) were 0.087 mg.g^{-1} and 0.264 mg.g^{-1}, respectively, for ascorbic acid.

2.6.4. HPLC-DAD analysis of flavonols and flavones

The determination of the extract and mobile phase of the flavonoids was according to the methodology of (Huber et al. 2009). The analyses were performed using the same HPLC system described above. The concentrations for the calibration curves were 10 – 500 μg.g^{-1} for luteolin, 10 – 2500 μg.g^{-1} for quercetin, 1 – 80 μg.g^{-1} for kaempferol and 1 – 50 μg.g^{-1} for myricetin. The limit of detection (LOD) and quantitation (LOQ), respectively, were as follows: luteolin (0.058 – 0.177 μg.g^{-1}), quercetin (0.055 – 0.167 μg.g^{-1}), kaempferol (0.0027 – 0.0082 μg.g^{-1}) and myricetin (0.0022 – 0.0069 μg.g^{-1}).

2.7. Statistical analysis

The results were evaluated by a one-way analysis of variance (ANOVA), and the mean values were analyzed by Tukey's test using the STATISTICA® 12.0 software (Statsoft Inc, Tolson, USA).

3. Results and Discussion

3.1. Chemical composition

Before freeze-drying, the *Urera aurantiaca* (*Ua*) and *Opuntia monacantha* (*Om*) samples have moisture contents of 82.51 ± 0.64 g/ 100g and 79.90 ± 0.11 g/ 100g, respectively (Table 1).

Table 1. Proximate composition and physico-chemical analysis of (mean ± SD) the pulp *O. monacantha* and of the leaf *U. aurantiaca* freeze-dried (g.100^{-1} g dw)

Parameters	Pulp *O. monacantha*	Leaf *U. aurantiaca*
Moisture$^\diamond$	16.97 ± 0.49[a]	6.56 ± 0.10[b]
Dry matter$^\diamond$	83.03 ± 0.64[b]	93.44 ± 0.11[a]
Ash$^\diamond$	3.50 ± 0.16[b]	16.65 ± 0.80[a]
Protein$^\diamond$	3.54 ± 0.10[b]	20.98 ± 0.71[a]
Lipid$^\diamond$	1.77 ± 0.08[a]	1.69 ± 0.04[a]
Carbohydrate	74.22	54.12
Total dietary fiber (TDF)$^\diamond$	61.74 ± 0.24[a]	39.62 ± 0.67[b]
Insoluble dietary fiber (IDF)$^\diamond$	29.45 ± 0.74[a]	36.53 ± 0.83[a]
Soluble dietary fiber (SDF)$^\diamond$	3.35 ± 0.02[a]	3.09 ± 0.70[a]
pH$^\diamond$	5.44 ± 0.03[b]	8.16 ± 0.01[a]

Titratable acidity (TA)$^{\diamond}$	0.40 ± 0.27^a	0.24 ± 0.30^b
Soluble solids (^{0}Brix - SS)$^{\diamond}$	8.06 ± 0.03^a	0.95 ± 0.03^b
Total sugars$^{\diamond}$	22.91 ± 0.26^a	nd*
Reducing sugars$^{\diamond}$	10.57 ± 0.22^b	nd*

$^{\diamond}$Mean of three replicates ($n = 3$). Different letters in the same line represent significant difference $p < 0.05$ by Tukey's test.
*Not determined.

Freeze-drying (- 40 ºC for 48 h for *Ua* and 72 h to *Om*) effectively removed the free water, and helps preserve the chemical and phytochemical properties during storage. A high amount of total dietary fiber (TDF) is desirable characteristic for fruits and vegetables. *Ua* has a TDF of 39.62 ± 0.67 g/ 100g dw and *Om* has a TDF of 61.74 ± 0.24 g/ 100g dw and are important sources of total fiber; therefore, they are great alternatives to vegetables and conventional fruits such as spinach 2.10 g/ 100g fw and kiwi 2.70 g/ 100g fw, respectively (NEPA, 2011).

The proximate composition analysis showed higher protein and ash contents in the *Om* sample compared to other fruits (fresh weight) of same family Cactaceae, (*Pilosocereus pachycladus, Pilosocereus gounellei and Cereus jamacaru*) with protein values of 2.10 g/ 100g, 2.65 g/ 100g and 2.35 g/ 100g and ash values of 0.63 g/ 100g, 0.91 g/ 100g and 0.43g/ 100g, respectively (Nascimento, et al. 2010). Only the protein value 20.70 g/ 100g dw was previously reported by Kinupp & Barros (2008) for *Ua*. Compared to the value for lyophilized taioba leaf (*Xanthosoma sagittifolium)*, the protein 29.4 g/ 100g dw and lipid 10.6 g/ 100g dw were higher than *Ua*, but the value ash was lower 8.24 g/ 100g dw (Jackix et al. 2013).

The TDF was higher compared to the raw cladodes, consumed in Mexican diet, of two cultivars (*Opuntia ficus indica*), cvs. Milpa Alta and Atlixco 47.48 g/ 100g dw and 51.14 g/ 100g dw, respectively and lower compared to the (insoluble dietary fiber)

IDF and (soluble dietary fiber) SDF 41.80 g/ 100g dw, 44.07 g/ 100g dw and 5.68 g/ 100g dw and 7.07 g/ 100g dw, respectively (Ramírez-Moreno et al. (2013). Furthermore, the *Ua* leaf had TDF and IDF values higher than the taioba leaf 35.2 g/ 100g dw and 28.4 g/ 100 g dw, respectively and a lower value for the SDF 6.82 g/ 100 g dw (Jackix et al. 2013). The beneficial effects of fiber in human health are widely known, and dietary fiber together with other functional foods may contribute to the prevention of chronic diseases (Roehring, 1988).

Physicochemical characteristics represent important attributes for the commercialization and use of fruits and vegetables. Similar values for *Om* were found by Oliveira et al. (1992), who determine the physicochemical parameters of *Om* fruit. The values were as follows: pH 5.3, TA 0.20%, SS 12.0 (°Brix) and reducing sugars 7.9 g/ 100 g fw. *Om* presents a higher soluble solids content, almost 9 times higher than the values found for *Ua*, an important fruit quality.

3.2. Content of pigments and total phenolics

Fruits and vegetables are a primary food source providing essential nutrients for sustaining life. They also contain a variety of phytochemicals, such as phenolics, which provide important health benefits (Liu et al. 2002). The association of diets rich in phytochemicals with the prevention of cancer has intensified interest in chlorophyll as a class of plant pigments with potential chemopreventative effects (Ferruzzi & Blakeslee, 2006). The concentrations of pigments and totals phenolics of the analyzed plants are shown in Table 2.

Table 2. Pigment contents and total phenolic compounds analysis of (mean ± SD) the pulp *O. monacantha* and of the leaf *U. aurantiaca* freeze-dried (mg/ g^{-1}dw.).

Parameters	Pulp *O. monacantha*	Leaf *U. aurantiaca*
Chlorophyll a (Chl a)$^\diamond$	0.63 ± 0.02^b	8.85 ± 0.83^a
Chlorophyll b (Chl b)$^\diamond$	0.34 ± 0.01^b	3.53 ± 0.41^a
Total Chlorophyll (TChl)$^\diamond$	0.98 ± 0.15^b	12.39 ± 0.95^a
Total carotenoids (TC)$^\diamond$	0.16 ± 0.01^b	1.88 ± 0.10^a
Total phenolic content (TPC)$^\diamond$	6.61 ± 0.01^b	11.26 ± 0.18^a

$^\diamond$Mean of three replicates. ($n = 3$). Different letters in the same line represent significant difference $p < 0.05$ by Tukey's test.

The phenolic content (TPC) for *Ua* was higher than for *Om*. Phenolic compounds may be a major determinant of the antioxidant potential of foods Parr & Bolwell, (2000) and represent a natural source of antioxidants present mainly in fruits and vegetables.

Chlorophylls are located in chloroplasts and are associated with carotenoids (Gross, 1991). Chlorophyll a for *Om* was higher than chlorophyll b in both samples. According to the Gross (1991), chlorophyll a the most abundant and most important member of this family, corresponding to approximately 75% of the green pigments found in plants. Chlorophyll b and carotenoids are called "accessory pigments". They absorb these pigments and transfer them to the centers of reactions (Streit et al. 2005).

For TPC, *Om* was higher than the fresh color orange of *Opuntia megacantha* and fresh color dark purple *Opuntia ficus indica* with values of 1.0 ± 0.01 mg GAE.g^{-1} fw and 1.1 ± 0.01 mg GAE.g^{-1} fw, respectively (Cayupán et al. 2011). The *Ua* leaves also presented higher values compared to (leaf + stem + flower) (*Urtica dioica*) containing 9.25 ± 0.32 mg/g GAE.g^{-1} dw (Kirca & Arslan, 2008).

However, for Chl a, chlorophyll b (Chl b) and total chlorophyll (TChl), the *Om* pulp showed higher values, with the relative the pulp of yellow and orange (*O.*

megacantha) (0.017 ± 0.30 mg/ g fw, 0.016 ± 0.20 mg/ g fw, 0.033 ± 0.60 mg/ g fw and

0.016 ± 0.10 mg/ g fw, 0.039 ± 0.20 mg/ g fw, 0.056 ± 0.20 mg/ g fw, respectively) with

zero weeks of ripening, when the chlorophyll content is higher (Cayupán et al. 2011).

The largest amount of pigments was detected in the *Ua* leaves, with the highest

concentration of chlorophyll a, the most prominent pigment in green vegetables. Leafy

vegetables also contain several types of photosynthetic pigments, which are

chlorophylls and carotenoids (Kimura & Rodriguez-Amaya, 2002). Kukrić et al. (2012)

found 0.882 ± 0.00 mg/ g for Chl a fw, 0.285 ± 0.01 mg/ g for Chl b fw, 1.17 ± 0.00 mg/

g for TChl fw and 0.323 ± 0.00 mg/ g total carotenoids (TC) fw for (*Urtica dioica* L.)

leaves.

3.3 Antioxidant activity in vitro (AA)

According to Apak et al. (2013), a number of protocols have been proposed to

determine the antioxidant activity. However, two methods, ABTS and DPPH, that use

the same mechanism (scavenging activity) were performed for repeatability. In addition

the TRAP method (antioxidant activity), they are variants of the ORAC assay, but use a

broader range of initiators, probes, and endpoint measurements, including those used in

the ORAC and TEAC assays (Apak et al. 2013).

The results of the DPPH and ABTS assays are expressed as g dw/ g dpph and

µM trolox/ g dw, respectively, and are shown in Table 3.

Table 3. Antioxidant activity analysis of (mean ± SD) the pulp *O. monacantha* and of

the leaf *U. aurantiaca* freeze-dried.

Parameters	Pulp *O. monacantha*	Leaf *U. aurantiaca*
DPPH(g d.w/ g dpph)$^{\lozenge}$	577.31 ± 0.56[a]	119.87 ± 0.84[b]

ABTS (µM trolox/ g d.w) $^{\diamond}$	149.03 ± 1.50^{b}	257.85 ± 0.60^{a}
AUC (%)$^{\diamond}$	$993510.00 \pm 5372.02^{a}$	$190894.00 \pm 20605.01^{b}$
TAR (I_0/I)$^{\diamond}$	1.77 ± 0.16^{b}	41.15 ± 1.23^{a}

$^{\diamond}$Mean of three replicates. ($n = 3$). Different letters in the same line represent significant difference $p < 0.05$ by Tukey's test.

The TRAP is based on the original studies of (Wayner et al. 1985). Through the analysis of TAR and TRAP, the *in vitro* uptake of free radicals can be assessed. According to Dresch et al. (2009) TRAP is an accurate method, and its main advantage is that it applies to antiradicals with different kinetic behaviors; therefore, TRAP can be used for antioxidants that exhibit distinct lag phases or do not exhibit phase lags, and may thus be used for any antioxidant compound or complex mixture independent of the kinetic emission profile of the compound. The TRAP results are expressed as the area under the curve (AUC) (Fig. 1) and (Fig. 1B) the total antioxidant reactivity (TAR I_0/ I).
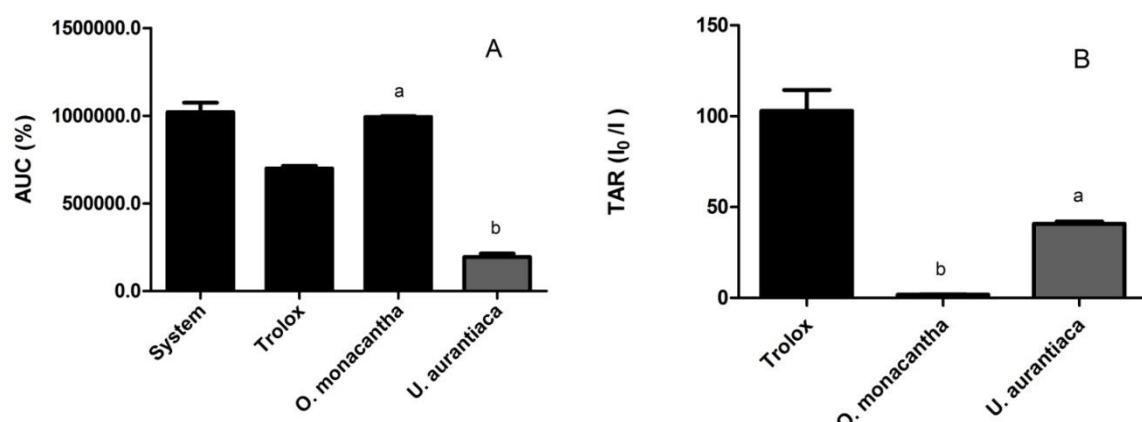
Figure 1A-B - Representative value obtained from analysis total radical-trapping antioxidant (TRAP - AUC and TAR) of pulp *Om* and leaf *Ua*. The values are expressed as the (mean $\pm$ SD) ($p < 0.05$).

According Dresch et al. (2009), the loss of chemiluminescence (Fig. 1 C) can be assessed by measuring the AUC. When the extent of the decay of the luminescence is large, the expected AUC value is low, such that the sudden loss of chemiluminescence

(TAR) and the AUC value are inversely proportional. The smaller the value of the AUC, the higher the antioxidant activity of the extracts.
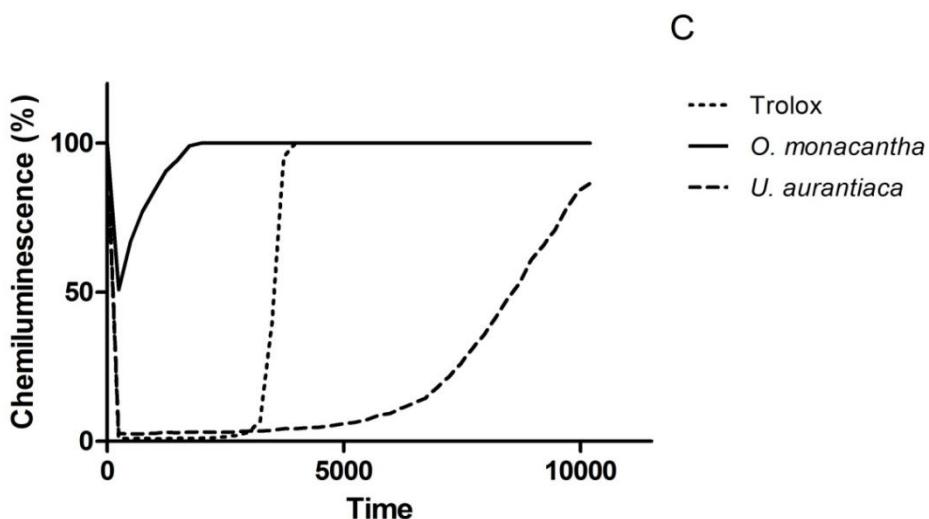
Figure 1C - Representative value obtained from analysis total radical-trapping antioxidant (Chemiluminescence) of pulp *Om* and leaf *Ua*. The values are expressed (%).

The results of the DPPH and ABTS analysis revealed that the natural antioxidants in *Ua* had better scavenging activity. For the TRAP – AUC (Fig. 1A), *Ua* also showed better antioxidant activity. According to Dresch et al. (2009), the AUC has the advantage of incorporating both the inhibition and duration of inhibition of the free radical generator. The results of the TAR (Fig. 1B) analysis also revealed the *Ua* was reactive, causing a sudden reduction in the chemiluminescence of the luminal (Fig. 1C), which was longer than for *Om*. Comparing the values of *Om* with fruit from the same Cactaceae family using the DPPH and ABTS methods shows that *Om* has more antioxidant activity than (*Cereus hildmannianus*) (3249.77 ± 158.57 g fw/g dpph and 19.61 ± 0.75 µM trolox/ g fw) (Pereira et al. 2013).

To identify the bioactive compounds that provide the antioxidant activity in the samples, the extracts were evaluated by DPPH, ABTS and TRAP using the same chromatographic conditions to identify the carotenoids, flavonoids, ascorbic acid and

tocopherol. The bioactive compounds that confer higher the antioxidant activity in the TRAP analysis for the *Ua* leaf and *Om* fruit may be related to tocopherols and carotenoids, the major components in these plants. (Figure 2 A-B).
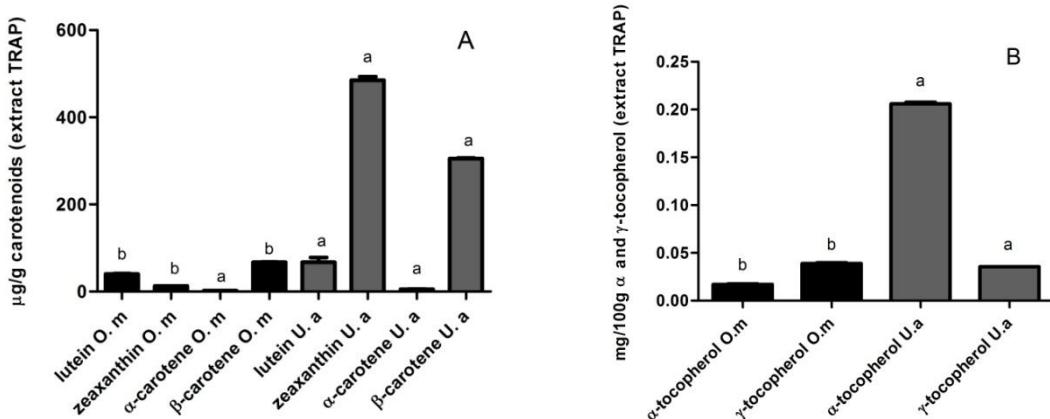
Figure 2A-B - Representative value obtained from alcoholic extract (TRAP) injected into the HPLC conditions of carotenoids and tocopherol. Values are expressed as (mean ± SD) (p < 0.05). Abbreviation: O.m (*O. monacanha*) and U.a (*U. aurantiaca*)

Ua had more antioxidant activity by all the antioxidant methods. It has a high concentration of phenolic compounds, antioxidant activity, carotenoids and tocopherol and flavonoids, except luteolin, and ascorbic acid. The carotenoids for *Om* and *Ua* were 40.42 ± 0.91 µg/ g and 67.32 ± 1.96 µg/ g for lutein, 12.96 ± 0.02 µg/ g and 485.09 ± 8.09 µg/ g for zeaxanthin, 2.09 ± 0.02 µg/ g and 5.14 ± 0.78 µg/ g for α-carotene and 67.44 ± 0.17 µg/ g and 305.03 ± 1.53 µg/ g for β-carotene, respectively. However, zeaxanthin was increased nearly 20 times in the extract that was not saponified. According Kimura, Rodriguez-Amaya, Godoy, (1990) & Rodriguez- Amaya (1989), some carotenoids, such as α-carotene, β-carotene, γ-carotene, and β-cryptoxanthin can resist saponification. Moreover, lutein, violaxanthin, and other dihydroxy-, trihydroxy-, and epoxycarotenoids are considerably reduced during saponification and the subsequent washing step (Khachik, Beecher, Whittaker, 1986, Kimura, Rodriguez-Amaya, Godoy, 1990, Riso & Porrini 1997).

The α- and γ-tocopherol values of *Om* and *Ua* were 0.016 ± 0.00 mg/100 g, 0.03 ± 0.00 mg/100 g and 0.20 ± 0.01 mg/100 g, 0.03 mg/100 g, respectively. For α- and γ-tocopherol, an increase was only observed in the *Om* fruit. With ethanol extraction, the value increased 0.0034 ± 0.00 mg/100 g to 0.016 ± 0.00 mg/100 g mg/100 g for α-tocopherol and 0.0012 ± 0.00 mg/100 g to 0.03 ± 0.00 mg/100 g compared to the methanol extraction used to quantify tocopherol. This is because ethanol is bipolar and easily dissolves nonpolar organic compounds.

The DPPH and ABTS only detected lutein in *Om* and *Ua*, with concentrations of 8.33 ± 0.21 µg/ g and 15.52 ± 0.14 µg/ g lutein, respectively. Water extracts polar compounds, which may have interacted in the extraction of carotenoids for the DPPH and ABTS analyses. Moreover, the extract was not macerated unlike the extract for the TRAP analysis, which influences the release of carotenoids.

For the other analyses (ascorbic acid and flavonoids), antioxidant activity, was not detected in the extract by TRAP and DPPH and ABTS. The polyphenol and ascorbic acid contents were highest in the water extracts, indicating that the majority of polyphenols in *Om* and *Ua* are polar. Ascorbic acid is a highly polar compound Kong et al. (2012), which was not extracted in the extracts for DPPH and ABTS and TRAP.

3.4. Content of tocopherol, carotenoid and Vitamin A

Lipophilic antioxidants include carotenoids and tocopherols, among others (Carvalho et al. 2013). Specific carotenoids are important due to their provitamin A activity (e.g., β-carotene) or a protective effect towards age related macular degeneration (e.g., lutein) Bowen, et al. (2002); Krinsky & Johnson, (2005), major compounds found in the *Ua* and *Om*. Tocopherols, as effective antioxidants, have been proposed to protect against carcinogenesis (Yang et al. 2013).

Table 4 shows the values detected for tocopherol and carotenoids. Five carotenoids were identified, lutein, zeaxanthin, β-cryptoxanthin, β-carotene, and α-carotene, through chromatographic separation; however, the β-cryptoxanthin levels found below the curve (< 1.0 µg/g) for the two samples. Two tocopherols were identified, α-tocopherol and γ-tocopherol, through chromatographic separation.

Table 4. Carotenoids, α- tocopherol and γ-tocopherol analysis of (mean ± SD) the pulp *O. monacantha* and of the leaf *U. aurantiaca* freeze-dried.

Parameters	Pulp *O. monacantha*	Leaf *U. aurantiaca*
Lutein (µg/ g d.w) [◊]	133.04 ± 0.84^b	808.09 ± 5.95^a
Zeaxanthin (µg/ g d.w) [◊]	1.18 ± 0.01^b	24.89 ± 0.18^a
β-cryptoxanthin (µg/ g d.w) [◊]	nq*	nq*
α-carotene (µg/ g d.w) [◊]	7.09 ± 0.57^b	282.29 ± 1.89^a
β-carotene (µg/ g d.w) [◊]	102.42 ± 0.94^b	613.25 ± 4.74^a
Total carotenoids (µg/ g d.w) [◊]	243.73	1728.52
Vitamin A (µg/ g d.w) [◊]	17.66	125.73
α- tocopherol (mg/ 100g d.w) [◊]	0.0034 ± 0.00^b	0.22 ± 0.01^a
γ-tocopherol (mg/ 100g d.w) [◊]	0.0012 ± 0.00^b	0.04 ± 0.00^a

[◊]Mean of three replicates ($n = 3$). Different letters in the same line represent significant difference $p < 0.05$ by Tukey's test.
*Below detection limit.

Lutein and β-carotene are the major compounds in both samples, although in *Ua*, the concentrations are significantly higher. According to Lakshminarayana et al. (2005) & Calvo (2005), lutein and β-carotene are two of the major carotenoids in vegetables. For α-carotene and zeaxanthin, the values found for *Ua* were significantly higher compared to the values found in studies of common vegetables.

The total carotenoid content of *Om* was lower compared to individual carotenoids. According Rodriguez-Amaya et al. (2008) brief exposure of the extract containing chlorophyll during the analysis, can cause rapid photodegradation and isomerization.

Rodriguez-Amaya et al. (2008) found values for organic kale of 54 µg/g fw for α-carotene and 3 µg/g fw for zeaxanthin. For lutein, the value was 63 µg/g fw for rocket and for 104 µg/ g fw for taioba (*Xanthosoma sp*). Based on the amount of carotenoids, *Ua* is a great alternative, with high potential for cultivation and propagation. According to Wisniewska & Subczynski (2006), the presence of zeaxanthin and/or lutein in the diet may be beneficial for reducing the incidence of the two common eye diseases of ageing, age-related macular degeneration and cataract formation.

The *Om* fruit also showed higher values of carotenoids compared to fruit of the same family Cactaceae, *Cereus hildmannianus* and common fruits, such as guava (*Psidium guajava*), camu-camu (*Myrciaria dubia*) and acerola (*Malpighia glabra*). According Pereira et al. (2013) values for (*Cereus hildmannianus*) were 0.55 ± 0.15 µg/ g fw for lutein, 0.019 ± 0.00 µg/ g fw for zeaxanthin, 0.18 ± 0.00 µg/ g fw for α-carotene and 0.086 ± 0.00 µg/g fw for α-carotene. Common fruits, such as guava showed values for β-carotene 12 µg/ g fw and zeaxanthin 0.3 µg/ g fw, camu-camu for α-carotene and lutein (1.1 µg/ g fw and 1.3 µg/g fw, respectively) and for acerola (Rodriguez-Amaya et al. 2008). This is a great alternative for consumption because of its nutritional quality and ease of cultivation and propagation.

Vegetables and fruits contain significant amounts of β-carotene and other provitamin A carotenoids, such as α-carotene and β-cryptoxanthin that can be absorbed and converted to vitamin A in the human body (Rodriguez-Amaya, 2001). The *Ua* leaves also had the highest vitamin A, with 7 times the values found in the *Om* pulp.

The tomato (10 μg/ g fw) and malabar spinach (24 μg/ g fw) have vitamin A contents lower than the *Om* pulp and *Ua* leaves (Kidmose et al. 2006).

Tocopherols are a group of four (α, β, γ e δ) lipophilic antioxidants synthesized by photosynthetic organisms, occurring mainly in leaves and seeds (Munné-Bosch & Alegre, 2002). The *Ua* leaves had the highest α- and γ-tocopherol content, with more than 60 and 30 times the values found in the *Om* pulp, respectively.

Chun et al. (2006) compiled data from the literature for the tocopherol content of fruits and vegetables, and showed values for α-tocopherol of 0.71 ± 0.23 mg/100 g and γ-tocopherol of 0.02 ± 0.02 mg/100 g for fresh peaches. Significantly higher results with respect to pulp *Om* freeze-dried. For raw spinach, the values were 1.96 ± 0.43 mg/100 g for α-tocopherol and 0.21 ± 0.06 mg/100 g for γ-tocopherol. The results were also significantly higher than the freeze-dried *Ua* leaves.

According with Bauernfeind (1980), the amount of tocopherols in fruits and vegetables is affected by the species, variety, maturity, growing conditions (weather, growing season, intensity of sunlight, and soil state), uneven distribution of groups of tocopherols, and time and manner of harvesting.

3.5 Content of flavones and flavonols and ascorbic acid

Vitamin C and the flavonoids are both strong antioxidant agents and their biological activities are partially synergistic (Isler et al. 1988).

According Huber et al. (2009) of the six principal classes of flavonoids, flavonols (myricetin, quercetin, and kaempferol) and flavones (luteolin) are the most widely distributed in plant foods, where they are found as glycosides. In flavonoid analysis, hydrolysis is usually performed and the resulting aglycone identified and quantified.

Table 5 shows the values found for the flavones and flavonols and ascorbic acid. Three flavonoids were identified, luteolin, quercetin and kaempferol, through chromatographic separation; however, myricetin was not detected in the two samples, and the quercetin in *Om* were below the curve (< 10 μg/ g).

Table 5. Flavones and flavonols and ascorbic acid analysis of (mean ± SD) the pulp *O. monacantha* and of the leaf *U. aurantiaca* freeze-dried.

Parameters	Pulp *O. monacantha*	Leaf *U. aurantiaca*	N
Luteolin (μg/g d.w)[◊]	440 ± 3.87[a]	130 ± 0.96[b]	5
Myricetin (μg/g d.w)[◊]	nd*	nd*	5
Quercetin (μg/g d.w)[◊]	nq**	1970 ± 6.98[a]	5
Kaempferol (μg/g d.w)[◊]	20 ± 0.45[b]	80 ± 0.62[a]	5
Ascorbic acid (mg/100g d.w)[◊]	13 ± 0.02[a]	2.5 ± 0.01[b]	2

[◊]Mean of three replicates ($n = 3$). Different letters in the same line represent significant difference $p < 0.05$ by Tukey's test.
* Not determined
** Below detection limit

Using a previously optimized and validated method proposed by Huber et al. (2009), the findings above indicate that the optimum conditions vary with the sample. Before initiating flavonoid analyses, the conditions for extraction/hydrolysis were optimized for each type of food, compared to fruits of the genus *Opuntia* and conventional vegetables, such as kale (*Brassica oleraceae*) and arugula (*Eruca sativa*). The extraction/hydrolysis was repeated five times for both samples. The sample weight, lyophilized or fresh sample, the HCl molarity, the concentration of methanol (50 or 62.5%), the reflux time (90 °C), use of antioxidant (ascorbic acid or BHT), and volume were verified. Before HPLC analysis, unfiltered samples and samples filtered with

filters (0.45 μm PTFE and SPE) were used. The use of 50% methanol and PTFE filters gave the best results. The antioxidant ascorbic acid was used, but there was no difference compared to BHT.

For *Ua*, the extraction/hydrolysis was more effective with 1 g freeze-dried sample homogenized with 50% methanol and 1.6 M HCl for 5 h/90°C. The volume was brought up to 40 mL. Huber et al. (2008) used extraction/hydrolysis for kale with 50% methanol and 1.2 M HCl for 6 h/90 °C. The values were 327 ± 94 μg/g fw for quercetin, 336 ± 77 μg/g fw for kaempferol and not determined for luteolin and myricetin. The quercetin in *Ua* was significantly higher and the kaempferol was lower compared to the values reported by the author.

For *Om*, the extraction/hydrolysis was more effective with 5 g freeze-dried sample homogenized with 50% methanol and 1.0 M HCl for 0.5 h/90°C. The volume was brought up to 25 mL. Kuti (2004) used extraction/hydrolysis for (*O. ficus indica*) with 50% methanol and 1.0 N HCl for 0.5 h/90 °C. The values were 43.2 ± 2.50 μg/g fw for quercetin and 2.2 ± 0.30 μg/g fw for kaempferol. Luteolin and myricetin were not evaluated in this study. The quercetin in *Om* was lower, and the kaempferol was higher compared to the values reported by the author.

Ascorbic acid identification was possible through chromatographic separation and quantification of the compounds, whereas for *Ua*, it was only possible to separate the compounds with a fresh sample. According Stevens et al. (2006) during lyophilization, vitamin C can be lost or degraded.

According Franke et al. (2004) the value for broccoli was 52.5 mg/ 100 g for ascorbic acid, which are significantly higher than for *Ua*. For fruit, Pereira et al. (2013) found values for (*Cereus hildmannianus*) of 25 mg/ 100 g for ascorbic acid, with values almost 2 times higher than *Om*. The food levels of vitamin C and flavonoids vary

greatly depending on the species and variety, growing location, harvesting time, storage, processing, and other conditions, and also because of methodological differences (Franke et al. 2004).

4. Conclusions

The study identified a potential use for pulp fruit and leaves not commonly used in Brazil. The leaves of *Urera aurantiaca* have high nutritional potential compared to conventional vegetables. The pulp of *Opuntia monacantha*, compared to other plants in the genus *Opuntia*, have potential nutritional quality for introduction into the diet of the population. However, the immediate impact of this study also may be to improve the use of species, such as *O. monacantha* and *U. aurantiaca*, which have high nutritional potential and could be used in programs to improve the quality of food among the population. In addressing the lack of research on food plants, this study highlights the potential of species as an important source of nutritional compounds and antioxidants available in the native Brazilian flora. Furthermore, a better understanding of the nutritional components of these species will contribute to conservation efforts.

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CHARACTERIZATION, BIOACTIVE COMPOUNDS AND ANTIOXIDANT

PROPERTIES OF TUBER, LEAF AND FLOWER OF *Tropaeolum pentaphyllum*

Lam.

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CHARACTERIZATION, BIOACTIVE COMPOUNDS AND ANTIOXIDANT PROPERTIES OF TUBER, LEAF AND FLOWER OF *Tropaeolum pentaphyllum* Lam.

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ABSTRACT

The aim of this study was to evaluate the physicochemical content, the bioactive compound content, and antioxidant properties of the tuber, leaves and flowers of the species *Tropaeolum pentaphyllum* Lam. (*Tp*) The tuber, leaves and flowers displayed significant differences ($p < 0.05$) in all parameters with high lutein contents (642.50 ± 4.56 µg/ g dry weight (dw) and 243.23 ± 0.89 µg/ g dw) in the leaves and flowers, respectively. High luteolin (4450 ± 12.50 µg/ g dw) and quercetin (3750 ± 8.09 µg/ g dw) contents were observed in the leaves. In addition, the antioxidant qualities of leaf and flower extracts were revealed using the TAR method. The study revealed the potential of the species *pentaphyllum*, particularly in comparison with the leaves and flowers of *Tropaeolum majus*, which have been investigated in other studies.

Keywords: Tropaeolum pentaphyllum Lam., nutritional composition, bioactive compounds, antioxidant properties.

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1. Introduction

The specie *Tropaeolum pentaphyllum* Lam. is native to South Brazil and belongs to the family Tropaeolaceae. The aerial portions of the plant are known because of their ornamental properties and because the leaves and flowers are edible and are used in salads, where these parts impart a peppery flavor similar to the flavor of the tuber.

Antioxidant and bioactive properties have been reported in leaves and flowers of *Tropaeolum majus* (Nasturtium), which is a plant of the same genus. In a study of the petals of orange Nasturtium flowers, amounts of phenolics and ascorbic acid were reported and free radical scavenging activity was exhibited in vitro (Garzón & Wrolstad, 2009). Additionally, a study performed by Rodriguez-Amaya, Kimura & Amaya-Farfan (2008) reported the amount of lutein in flowers and the amounts of lutein and β-carotene in leaves of *T. majus*.

A diet rich in fruits and vegetables is widely acknowledged to have a protective effect on human health, contributing to the prevention of degenerative diseases. This effect has been attributed to several compounds found in edible plants, such as ascorbic acid, carotenoids, tocopherols, phenolic acids and flavonoids (Huber, Hoffmann-Ribani & Rodriguez-Amaya, 2009).

The antioxidant capacity of vegetables is directly related to the presence of bioactive compounds. However, of paramount importance is the estimation of bioactive compounds analyzed to provide antioxidant activity. The chemical diversity of natural antioxidants makes it difficult to separate, detect and quantify individual antioxidants from a complex food matrix. Currently, there is no single antioxidant assay for food labeling because of the lack of standard quantification methods (Apak et al. 2013).

In recent years, a wide range of assays been adopted to measure the antioxidant capacity of foods, including ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl), with mechanisms of scavenging activity; FRAP (ferric reducing antioxidant power), with mechanisms of reducing power and TRAP (total radical-trapping antioxidant parameter), with mechanisms of antioxidant activity (Antolovich et al. 2002; Amarowicz et al. 2004).

The determination of the type of bioactive compounds is of great importance for the characterization of the species. However, the extracts that were used in the determination of antioxidant activity by DPPH and ABTS radical and TRAP methods also indicated the bioactivity of this action, which determines the beneficial health effects.

The aim of this study was to analyze the tuber, leaves and flowers of the species *Tropaeolum pentaphyllum* by nutritional characterization and by the determination of bioactive compounds and of antioxidant activity using different methods in addition to the compounds that contribute to its activity in different extracts.

2. Materials and Methods

2.1. Plant materials

The three parts (tubers, leaves and flowers) of the plant *Tropaeolum pentaphyllum* Lam. (*Tp*), which is known as "crem", were obtained in the region of Lami, Porto Alegre, Rio Grande do Sul, Brazil (Latitude 30° 13' 19" S and Longitude 51° 5' 5" O). The tubers were acquired in June, and the leaves and flowers were obtained in September 2012.

Their vouchers were deposited in the Institute of Natural Sciences (ICN) Herbarium (UFRGS, Porto Alegre, Rio Grande do Sul, Brazil) under the accession

number 157404. One lot (1 kg, 200 g, 50 g) of the *Tp* tubers, leaves and flowers were collected, respectively. The tubers were selected, peeled, chopped and then washed, and the leaves and flowers were selected, cleaned, with injured parts discarded, and freeze-dried (-40 °C for 48 h). The dried samples were homogenized and mashed using a mortar and pestle, and the samples were stored in sealed plastic bags at -40 °C until analyses.

2.2. Reagents and standards

The standards used for the construction of calibration curves were (±)-α-tocopherol (purity ≥ 96%) and (+)-γ-tocopherol (purity ≥ 96%) and were purchased from Sigma- Aldrich Corp. (St. Louis, MO, USA). The carotenoids β-carotene (purity > 93%), β-cryptoxanthin (purity > 97%), zeaxanthin (purity > 95%), and α-carotene (purity > 95%) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Lutein (purity > 95%) was purchased from Indofine Chemical Company Inc. (Hillsborough, NJ, USA). The ascorbic acid (99.0 – 100.5%) was purchased from Casa da Química (Diadema, São Paulo, Brazil). The flavonoid luteolin-7-O-glucosidase (purity > 90%) was purchased from Indofine Chemical Company Inc. (Hillsborough, NJ, USA). quercetin (purity ≥ 98%) was purchased from Sigma-Aldrich (Steinheim, Germany), and myricetin (purity ≥ 96%) and kaempferol (purity ≥ 97%), were purchased from Sigma-Aldrich (Lyon, France). Chromatographic analyses were performed using the HPLC grade solvents methanol, methyl-tert-butyl and acetonitrile (J.T.Baker, Xalostoc, Mexico). Formic acid and HCl were acquired from Labsynth Ltda (Diadema, São Paulo, Brazil), and the supra pure sulfuric acid (PM 98.08) was from Vetec Ltda (Duque de Caxias, Rio de Janeiro, Brazil).

2.3. Chromatography

HPLC analysis was performed on an Agilent 1100 Series HPLC (Agilent series 1100, Santa Clara, CA, USA) system equipped with a quaternary solvent pumping system (G1311A – DE14917573 Agilent 1100 Series, Waldbronn, Germany), spectra a UV/Vis detector (G1314B - DE71358944 Agilent 1100 Series, Waldbronn, Germany), automatic injector (G1312 – 67305 Agilent 1100 Series, Santa Clara, CA, USA) and ChemStation® software (Waldbronn, Germany). HPLC-DAD analysis was performed in a Shimadzu Prominence UFLC – MODEL CBM-20A (serial L202350 – 19270CD, Kyoto, Japan) system equipped with a ternary solvent pumping system LC 20AD (serial L201051 – 29053, Kyoto, Japan), spectra a diode array detector SPD-M20A (serial L201550 – 11010, Kyoto, Japan), automatic injector (SIL-20AHT, Kyoto, Japan) and LC Operation® software (Chiyoda-ku, Tokyo, Japan).

2.4. Centesimal composition and physicochemical analysis

The moisture (method 920.151), ash (method 940.26), protein (N x 6.25) (968.20) and lipid content (962.20) were determined. The total dietary fiber (TDF) and insoluble dietary fiber (IDF) are expressed as g.100 g^{-1}dw. The soluble dietary fiber (SDF) was calculated by subtracting the IDF from the TDF (AOAC, 1997).

The reducing and non-reducing sugars were determined at the Instituto Adolfo Lutz (IAL, 2005). The titratable acidity (TA) was calculated using (method 942.15B). The hydrogen potential (pH) (method 968.20) was determined using a digital pHmeter (Quimis Q400M, São Paulo, Brazil). The amount of soluble solids (SS) (method 932.12) was determined with a digital PAL-3 refractometer (Atago Co., Taiwan, China) and expressed in °Brix (AOAC, 1997).

2.5 Instrumentation for the mineral analysis

For the macro, micro and trace elements analysis two different methods were performed. Although, the same instrument was used, a Model contra 700 high-resolution continuum source atomic absorption spectrometer (Analytik Jena, Jena, Germany), with a graphite furnace and a flame atomizer in two separate compartments. The spectrometer is equipped with a xenon short-arc lamp operating in a hot-spot mode, a prism pre-monochromator, an echelle grating monochromator for high resolution, and a charge-coupled device (CCD) array detector.

For cadmium (228.802 nm) and chromium (357.869 nm) determination the graphite furnace atomizer compartment was used and the method was optimized according Duarte et al (2013); however, no chemical modifier was necessary. For macro and micro elements the flame compartment was used. An air-acetylene flame was used for the determination of Cu (324.754 nm), Fe (248.327 nm), K (404.720 nm), Mg (202.582 nm), Na (589.592 nm), Rb (780.027 nm) and Zn (231.857 nm), and a nitrous oxide-acetylene flame for Ca (239.856 nm). The instrumental parameters were optimized and the method was adapted according (Boschetti et al. 2013). Although, it was necessary to submit the *T. pentaphyllum* tuber sample to a microwave digestion; this pre-treatment procedure is described in the appendix A.

2.6. Determination of total carotenoids, chlorophyll and phenolic compounds

The methodology for the determination of chlorophyll a, chlorophyll b and total carotenoid concentrations was proposed by Brahmi et al. (2012) with some modifications to the extract; described in the appendix A. The methodology for the determination of phenolic compounds was proposed by Swain & Hillis (1959). A

standard curve was constructed to quantify gallic acid in the concentration range from 30 to 300 mg g^{-1}.

2.7. Antioxidant activity in vitro

2.7.1. DPPH and ABTS^{+} radical

The extracts for both methods are described in the appendix A. The methodology based on sequestering the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical Brand-Williams et al. (1995) and the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method Kuskoski et al. (2005) were used to determine the antioxidant activity.

2.7.2. TRAP (Total radical-trapping antioxidant)

The extracts and procedure of TRAP are described in the appendix A. The analysis was calculated and measured as previously described by (Dresch et al. 2009). The results were transformed into a percentile rank, and the area under the curve (AUC) was calculated utilizing the GraphPad software (San Diego, CA, USA). The smaller the AUC is (in comparison to the system), the higher the total reactive antioxidant potential of the sample is. The TAR index was determined by measuring the initial decrease in luminol luminescence, which is calculated as the ratio I_0/I, where (I_0) is the initial emission of CL (before the addition of the antioxidant) and (I) is the instantaneous CL intensity after the addition of an aliquot of the sample or of the reference compound (Trolox).

2.8. Determination bioactive compounds for HPLC analysis

The determination of bioactive compounds was performed on the samples in two different extracts (50:50 v/v methanol:water and 70:30 v/v acetone:water for the antioxidant assays DPPH (2,2- diphenyl-1-picrylhydrazyl) and ABTS 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) and in an ethanolic extract (ethyl alcohol 95% P.A) for TRAP (total radical-trapping antioxidant).

2.8.1. HPLC analysis of α- and γ-tocopherol

The determination of tocopherol was based on the methodology proposed by Freitas et al. (2008), with some modifications to the extract; described in the appendix A. The analyses were performed using an HPLC system that was equipped with an online degasser, a quaternary pump, an automatic injector, and spectra, which was coupled to a Vydac C18 polymeric column (250 mm × 4.6 mm i.d., 5 μm) adjusted at 30°C. The data acquisition and processing were performed using the ChemStation® software. For the chromatographic analysis, the concentrated extract was diluted in methanol, placed in an ultrasound bath (Unique, Model USC 1400) for 15 minutes and filtered (0.45 μm PTFE filter) for subsequent injection into the chromatograph. For quantification, a calibration curve was constructed using individual solutions of α-tocopherol and γ-tocopherol diluted with methanol (0.002 – 2.0 μg g-1 and 0.007 – 0.18 μg g-1, respectively), and the limits of detection (LOD) and quantification (LOQ) were 0.00012 - 0.0004 μg g^{-1} and 0.0010 – 0.0030 μg g^{-1}, respectively.

2.8.2. HPLC analysis of carotenoids

The analyses were performed using the same HPLC system described above. The carotenoids were separated on a polymeric reverse phase column YMC C^{30} (250 mm x 4.6 mm id., 3 μm) adjusted to 33 °C, with a mobile phase gradient elution beginning with water/methanol/MTBE at 5:90:5 and reaching 0:95:5 after 12 minutes, 0:89:11 after 25 minutes, 0:75:25 after 40 minutes and 00:50:50 after 60 minutes with a flow rate of 1 mL/min and injection volume of 5 μL (Zanatta and Mercadante, 2007). Data acquisition and processing were performed using the ChemStation® software. Quantification was performed by constructing calibration curves for the carotenoids in the following concentration ranges: 3-700 $μg.g^{-1}$ for β-carotene, 0.05-280 $μg.g^{-1}$ for α-carotene, 0.2-850 $μg.g^{-1}$ for lutein, 2-100 $μg.g^{-1}$ for cryptoxanthin and 1-40 $μg.g^{-1}$ for zeaxanthin. The following LOD and LOQ scores were obtained, respectively: 0.063 and 0.192 μg/g for β-carotene; 0.069 and 0.210 μg/ g for lutein; 0.021 and 0.065 μg/ g for β-cryptoxanthin; 0.015 and 0.045 μg/ g for zeaxanthin; and 0.032 and 0.100 μg/ g for α-carotene. The carotenoid extract was prepared according to (Mercadante & Rodriguez Amaya 1998).

2.8.3. HPLC-DAD analysis of ascorbic acid

The determination of ascorbic acid was based on the methodology proposed by Rosa et al. (2007), with some modifications; described in the appendix A. The analyses were performed in a high performance liquid chromatography unit that was equipped with an online degasser, a ternary pump, an automatic injector, and a photodiode array detector, which was coupled to a Vydac C^{18} polymeric column (250 mm × 4.6 mm i.d., 5 μm.) adjusted at 28°C. The data acquisition and processing were performed using the LC Operation® software. The ascorbic acid was isocratically eluted using 0.05 M

Suprapur sulfuric acid as the mobile phase, with a flow rate of 1 mL/ min and with an injection volume of 10 μL. The calibration curve was determined using individual solutions of diluted with 0.05 M supra pure sulfuric acid in a concentration range from 10 to 1000 μg.g^{-1}. The limits of detection (LOD) and of quantitation (LOQ) were 87 μg.g^{-1} and 264 μg.g^{-1}, respectively, for ascorbic acid.

2.8.4. HPLC-DAD analysis flavonols and flavones

The analyses were performed using the same HPLC system as described above. The determination of the extract and mobile phase flavonoids were according the methodology proposed by (Huber et al. 2009). The identification of the flavonols and flavones was performed by the combined use of retention times with standards and with the absorption spectra obtained using the diode array detector. The chromatograms were processed at a fixed wavelength of 370 nm for flavonoids. The concentration ranges of the calibrated curve were 10 – 5000 μg g^{-1} for luteolin; 10 – 4000 μg g^{-1} for quercetin; 1 – 80 μg g^{-1} for kaempferol and 1 – 50 μg g^{-1} for myricetin. The limits of detection (LOD) and of quantitation (LOQ) were as follows: luteolin (58.49 – 177.25 μg g^{-1}); quercetin (55.38 – 167.83 μg g^{-1}); kaempferol (2.72 – 8.26 μg g^{-1}) and myricetin (2.29 – 6.94 μg g^{-1}), respectively.

2.8.5. Statistical analysis

The results were evaluated by a one-way analysis of variance (ANOVA), and the mean values were analyzed by Tukey's test using the STATISTICA® 12.0 software (Statsoft Inc, Tolson, USA).

3. Results and Discussion

3.1. Proximate composition and physicochemical property

The centesimal composition and physicochemical analysis of the tubers, leaves and flowers of *Tropaeolum pentaphyllum* (*Tp*) is presented in Table 1.

Table 1. Proximate composition and physicochemical analysis of (mean ± SD) the tuber, leaf and flower of the *T. pentaphyllum* freeze-dried (g. 100^{-1} g)

Parameters	*T. pentaphyllum* tuber	*T. pentaphyllum* Leaf	*T. pentaphyllum* flower
Moisture$^{\lozenge}$	5.74 ± 0.08^{c}	7.87 ± 0.23^{b}	8.13 ± 0.39^{a}
Dry matter$^{\lozenge}$	94.26 ± 0.42^{a}	92.13 ± 0.32^{b}	91.87 ± 0.32^{c}
Ash$^{\lozenge}$	3.53 ± 0.22^{b}	14.55 ± 0.86^{a}	1.67 ± 0.01^{c}
Protein$^{\lozenge}$	6.56 ± 0.02^{b}	16.28 ± 0.02^{a}	3.63 ± 0.26^{c}
Lipid$^{\lozenge}$	0.17 ± 0.00^{c}	0.90 ± 0.02^{b}	1.39 ± 0.02^{a}
Carbohydrate$^{\lozenge}$	84.00	61.46	85.44
Total dietary fibre (TDF) $^{\lozenge}$	8.94 ± 0.10^{b}	27.78 ± 0.15^{a}	5.22 ± 0.76^{c}
Insoluble dietary fibre (IDF) $^{\lozenge}$	8.37 ± 0.03^{b}	25.76 ± 0.60^{a}	3.34 ± 0.10^{c}
Soluble dietary fibre (SDF) $^{\lozenge}$	0.57 ± 0.05^{b}	2.03 ± 0.01^{a}	1.88 ± 0.06^{a}
pH$^{\lozenge}$	5.12 ± 0.03^{c}	5.76 ± 0.01^{b}	6.11 ± 0.01^{a}
Acidity$^{\lozenge}$	1.28 ± 0.27^{b}	1.80 ± 0.63^{a}	0.60 ± 0.00^{c}
Soluble solids ^{0}Brix$^{\lozenge}$	5.60 ± 0.38^{a}	0.88 ± 0.00^{b}	0.14 ± 0.05^{c}

$^{\lozenge}$Mean of three replicates ($n = 3$). Different letters in the same line represent significant difference p < 0.05 by Tukey's test.

Before the tuber, leaf and flower samples had been freeze-dried; their moisture contents were $69.5 \pm 0.42\%$; $81.0 \pm 0.56\%$ and $85.0 \pm 0.32\%$, respectively. The 48 hour drying process was effective at removing the free water present. The leaves showed higher values with respect to ash, protein and fiber contents ($p < 0.05$) compared with flowers and tubers. However, the flowers showed higher values in lipids and carbohydrates ($p < 0.05$) compared with leaves and tubers. The analysis of ash, which is the first step for the subsequent analysis to characterization these minerals, provides advanced information concerning the nutritional value of food, primarily its mineral content.

Comparing the parameters found in the literature for other species, the results are consistent with Ishida et al. (2000), who evaluated the tubers and leaves of two cultivars (Koganesengan (KS) and Beniazuma (BA)) of sweet potatoes (*Ipomoea batatas poir*). For the cv. tubers, BA had similar values for moisture, at 69.9 g/100 g, compared with the *Tp* tubers. For the leaves (KS and BA), the values of 84.9 g/ 100g and 87.1 g/100 g fw were greater compared with those values for *Tp* leaves. Concerning the protein and ash contents, the values found for *Tp* (tubers and leaves) were significantly higher compared with the two cv. KS and BA (2.13 g/ 100g, 1.28 g/ 100g and 1.43 g/ 100g, 1.08 g/100 g fw) and leaves (3.80 g/ 100g, 3.68 g/ 100g and 1.88 g/ 100g, 1.53 g/100 g fw), respectively, which was studied by (Ishida et al. 2000).

The lipid contents of *Tp* tubers and leaves were similar to the values (0.20 g/ 100g, 0.33 g/100 g fw) for KS and BA tubers, and the leaf had similar values for BA, at 1.03 g/100 g fw, and higher compared with KS, at 0.33 g/100 g fw, in a study performed by (Ishida et al. 2000). In addition, the (insoluble dietary fiber) IDF content of *Tp* tubers was similar to KS tubers, at 9.0 g/100 g dw, and cv. BA was lower, at 6.13 g/100 g dw. For the (soluble dietary fiber) SDF and (total dietary fiber) TDF (with

exception of TDF cv. BA), the values of the KS and BA tubers presented higher contents compared with *Tp*, at 2.73 g/ 100g, 1.43 g/ 100g and 11.7 g/ 100 dw, respectively, and of TDF cv. BA, with a value of 7.56 g/ 100 g dw. The most commonly used form of the tuber is previously peeled and then pickled. However, the samples were lyophilized without bark for a better comparison, which may influence the lower concentration of SDF compared with sweet potato.

In contrast, the values for TDF, SDF and IDF of *Tp* leaves were lower compared with the leaves of cv. KS and BA sweet potatoes, with presented values of 45.9 g/ 100g, 45.7 g/ 100g, 6.83g/ 100g, 5.77 g/ 100g and 39.1 g/ 100g, 39.9 g/100 g dw, respectively (Ishida et al. 2000). No study has been reported with *Tp*; however, this difference can to be due to the species, genus, soil, climate and condition of cultivation.

The *Tp* flowers were also compared with the edible flowers of Roselle (*Hibiscus sabdariffa* L.), which were analyzed by (Jung et al. 2013). The moisture, ash and protein contents of this study were lower than the values reported for Roselle flowers. The values found were 10.38 g/ 100g, 7.57 g/ 100g and 7.17 g/100 g dw, respectively, with the exception of the lipid content, which was 0.77 g/100 g dw. The other parameters were not found in literature for flowers.

Significant differences (p < 0.05) were observed between the mean concentrations or values of all the analyzed parameters for the *Tp* tuber, leaf and flower samples. The flower was slightly acidic compared with the tuber and leaf; furthermore, the acidity of the tuber and flower showed a highly significant and inverse correlation (r = - 0.83 and - 1.00), respectively, with pH. In addition, the acidity of the leaf showed a highly significant and positive correlation (r = 1.00) with pH.

According to Galdón et al. (2012) the pH value of 5.7 ± 0.2 of the potato cultivar Palmera Lagarteada was similar to tuber *Tp*. However, the acidity was almost 5 times

lower, with a value of 0.28 ± 0.02 in comparison with the *Tp* tuber. According Jung et al. (2013), the acidity and °Brix values for the *Tp* flowers were lower compared with the flowers of (*H. sabdariffa*) with values of 2.62 ± 0.01 and 1.80 ± 0.01, respectively; however, the pH of the *Tp* flowers was almost 3 times higher. For the leaves, according to Vasconcelos et al. (2011), the pH value of the *Tp* leaves was similar compared with the pH value of a conventional rocket of 5.68 and presented a higher acidity value of 0.37 and a lower value in relation to °Brix of 4.92.

3.2. Mineral composition

The mineral content present in food can be considered beneficial or toxic to human health depending on its concentration. The investigation of the mineral composition realized in this work (table 2) showed that the *T. pentaphyllum* tuber sample is poor in Fe and Cu content, with concentration under the limit of quantification (LOQ): 46 µg g^{-1} and 5.2 µg g^{-1}. Based on the results obtained for K, Mg and Ca, the majority elements present in the sample, and for Zn, Na and Rb this root can be used as nutritional complement in the human diet. The potentially toxic elements Cd and Cr presented low concentrations values (1.200 ng/ day men; 1.680 ng/ day female for Cr and 26 ng/ day men; 30 ng/ day female for Cd, Luis et al. (2014), giving to *T. pentaphyllum* tuber an edible status. According to Luis et al. (2014), toxic metal for sweet potatoes (white-fleshed) were 0.001 ng g^{-1} for Cd and 0.034 ng g^{-1} for Cr, with both values lower when compared with the *Tp* tuber. The macromineral K, Mg, Ca showed values lower, being 0.451%, 0.07062%, 0.05463%, respectively and higher Na 564.4 µg g^{-1} for sweet potatoes. The micromineral Cu and Fe both showed values higher, being 1.264 µg g^{-1} and 7.195 µg g^{-1} and Zn 2.499 µg g^{-1} value lower when compared with *Tp* tuber.

Table 2. Mineral content results obtained for a *T. pentaphyllum tuber* sample by HR-CS GF AAS (N = 5 measurments ± standard deviation) and HR-CS FAAS (N = 3 measurments ± standard deviation).

Element	Concentration
*Cd (ng g^{-1})	24 ± 2
*Cr (ng g^{-1})	554 ± 27
**Zn (µg g^{-1})	23±1
**Na (µg g^{-1})	74±3
**Rb (µg g^{-1})	8.8±0.5
**Cu (µg g^{-1})	< LOQ
**Fe (µg g^{-1})	< LOQ
**K (%)	1.14±0.05
**Mg (%)	0.092±0.006
***Ca (%)	0.158±0.009

* Analyzed by HR-CS GF AAS, ** Analyzed by HR-CS FAAS with air-acetylene flame, *** Analyzed by HR-CS FAAS with nitrous oxide-acetylene flame. Fe_{LOQ} = 46 µg g^{-1}; Cu_{LOQ} = 5.2 µg g^{-1}.

3.3. Contents of phenolic compounds, total chlorophyll and carotenoids

The concentrations of phenolic compounds total (TPC), total chlorophyll and carotenoids of the *Tp* tubers, leaves and flowers are shown in Table 3.

Significant differences (p < 0.05) were observed between the mean concentrations or values of all the analyzed parameters for the *Tp* tubers, leaves and flowers. In the study by Isabelle et al. (2010), the TPCs of 66 vegetables, including the sweet potato and the leaf of the sweet potato (*Ipomoea batatas* L.), were evaluated. The found values were significantly smaller, at 0.59 mg gallic acid equivalent (GAE)/ g and 3.97 mg GAE/ g, respectively, compared with the *Tp* tuber and leaves. For the *Tp* flowers, the found value were also significantly higher compared with the concentration

of phenolic compounds in an ethanol extract (7.27 ± 0.20 mg GAE/g) found for the Roselle (*H. Sabdariffa*) flower by (Jung et al. 2013).

Table 3. Total carotenoids, chlorophyll and phenolic compounds analysis of (mean ± SD) the tuber, leaf and flower of the *T. pentaphyllum* freeze-dried (mg/ g^{-1}dw.).

Parameters	*T. pentaphyllum* tuber	*T. pentaphyllum* leaf	*T. pentaphyllum* flower
Chlorophyll a (Chl a) $^\Diamond$	na*	4.25 ± 0.44^a	0.51 ± 0.02^b
Chlorophyll b (Chl b) $^\Diamond$	na*	1.62 ± 0.20^a	0.44 ± 0.01^b
Total Chlorophyll (TChl) $^\Diamond$	na*	5.87 ± 0.61^a	0.95 ± 0.02^b
Total carotenoids (TC) $^\Diamond$	na*	0.88 ± 0.10^a	0.02 ± 0.01^b
Total phenolic content (TPC) $^\Diamond$	2.79 ± 0.02^c	6.83 ± 0.09^b	21.30 ± 0.01^a

$^\Diamond$Mean of three replicates ($n = 3$). Different letters in the same line represent significant difference $p < 0.05$ by Tukey's test.
*Not applied

According to Hue et al. (2011), the chlorophyll a (Chl a) values found for sweet potatoes leaves (*I. batatas*) of six varieties at the 1 to 18 days development stage ranged between 0.55 ± 0.01 mg Chl a/ g fw and 1.61 ± 0.03 mg Chl a/ g fw. The chlorophyll b (Chl b) values ranged between 0.246 ± 0.01 mg (Chl b)/ g fw and 0.780 ± 0.03 mg (Chl b)/ g fw, with both values lower when compared with the *Tp* leaves.

For the *Tp* flowers, the (Chl a) value presented was lower compared with the (Chl a) value for two leaves and a bud of the (*Camellia sinensis* L.) cultivar Anji Baicha, which originated from Anji, with a value of 0.69 ± 0.01 mg (Chl a)/ g fw. However, for (Chl b), the value presented for the *Tp* flower was higher when compared

with *C. sinensis* for the same cultivar and location, with a value of 0.05 ± 0.00 mg (Chl b)/ g fw (Wei, et al. 2012).

The total chlorophyll content of the *Tp* leaves and flowers showed a highly significant and positive correlation (r = 0.84 and 0.87), respectively, with the total carotenoid content. According to Hue et al. (2011), the total carotenoid (TC) values found for sweet potato leaves ranged between 0.13 ± 0.01 mg (TC)/ g and 0.36 ± 0.10 mg (TC)/ g fw, presenting values lower when compared with the *Tp* leaves. According to Seroczyńska et al. (2006), the (TC) values found for flowers (*Cucurbita maxima* Duch.) ranged from 0.01 mg (TC)/ g to 0.18 mg (TC)/ g fw, with the minimum found value lower compared with the *Tp* flower.

3.4. Antioxidant properties in vitro

In recent years, several protocols with different mechanisms have been used to determine antioxidant activity to obtain reliable results. In this study, two methods (ABTS and DPPH) with the same mechanism (scavenging activity) were used to obtain repeatability. In addition to the TRAP method (antioxidant activity), these methods are variant ORAC assays in principle; however, these methods use a broader range of initiators, probes, and endpoint measurements (including those measurements used in ORAC and TEAC assays) (Apak et al. 2013).

The results of DPPH and ABTS, which are expressed as g d.w/ g DPPH and μM Trolox/ g d.w, respectively, are shown in (Table 4).

Table 4. Antioxidant properties analysis of (mean ± SD) the tuber, leaf and flower of the *T. pentaphyllum* freeze-dried.

Parameters	*T. pentaphyllum* tuber	*T. pentaphyllum* Leaf	*T. pentaphyllum* flower

DPPH (g /g dpph) $^\Diamond$	585.05 ± 1.03^c	126.00 ± 0.86^a	249.15 ± 0.17^b
ABTS (µM trolox/g) $^\Diamond$	162.80 ± 0.04^c	266.12 ± 0.21^a	273.83 ± 0.87^a
AUC (%) $^\Diamond$	$270851.00 \pm$ 10530.59^c	$436796.00 \pm$ 9214.93^a	$348006.00 \pm$ 12898.33^b
TAR (I_0/I) $^\Diamond$	21.88 ± 1.28^c	43.08 ± 2.02^b	49.24 ± 4.65^a

$^\Diamond$Mean of three replicates ($n = 3$). Different letters in the same line represent significant difference $p < 0.05$ by Tukey's test.

Through the analysis of TAR and TRAP, the *in vitro* uptake of free radicals can be assessed. According to Dresch et al. (2009), the TRAP method is accurate, and its main advantage is that this method applies to antiradicals with different kinetic behaviors. Therefore, TRAP applies equally well for antioxidants that exhibit distinct lag phases and/or do not exhibit phase lags and, thus, may be used for any antioxidant compound (or complex mixture), independent of the compound's kinetic emission profile. The results of the TRAP method are expressed by the area under the curve (AUC) (Fig. 1A) and (Fig. 1B) by total antioxidant reactivity (TAR – I_0/ I).
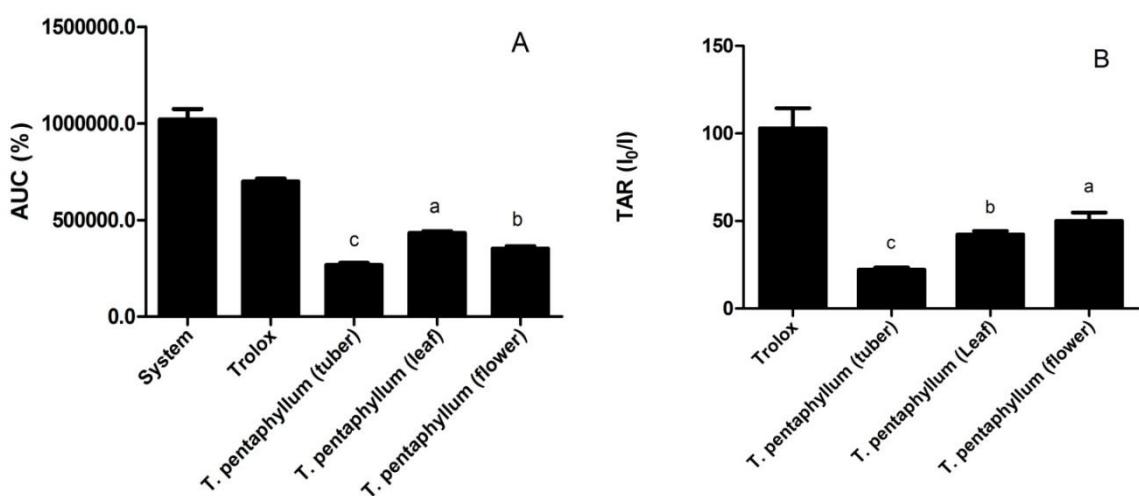
Figure 1A-B - Representative value obtained from analysis total radical-trapping antioxidant) (TRAP - AUC and TAR) of tuber, leaf and flower *Tp*. The values are expressed as the (mean ± SD) ($p < 0.05$).

According Dresch et al. (2009), loss in chemiluminescence (Fig. 1 C) can be assessed by measuring the AUC. The total radical-trapping antioxidant potential (TRAP) is one of the most used assays for testing antioxidant activity using chemiluminescence, and the parameter measured for indicate the quantity of antioxidants present in the plant extract is the area under the curve (AUC).
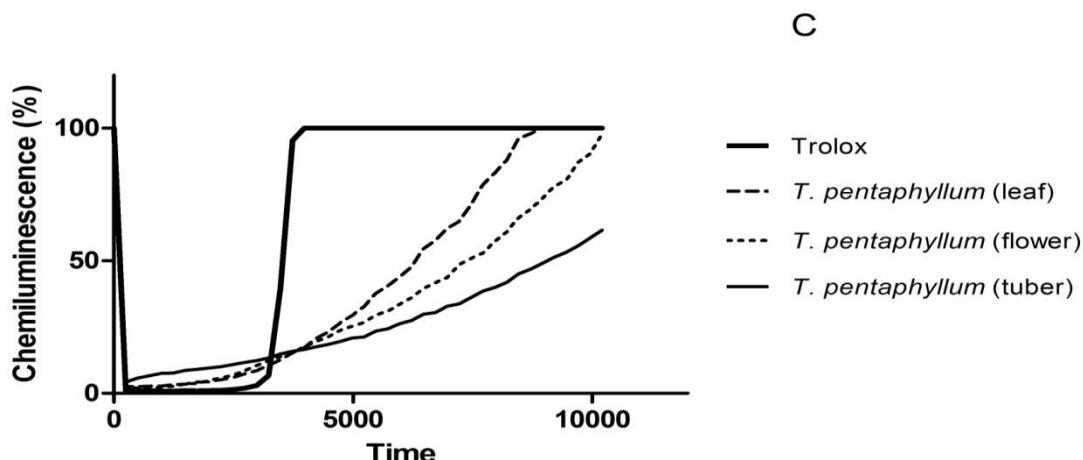
Figure 1C - Representative value obtained from analysis total radical-trapping antioxidant (Chemiluminescence) of tuber, leaf and flower *Tp*. The values are expressed (%).

For the DPPH analysis, the *Tp* tuber, leaf and flower values were significantly different ($p < 0.05$) and varied between 585.05 ± 1.03; 126.00 ± 0.86 and 249.15 ± 0.17 g dw/ g DPPH, respectively. For the ABTS analysis, the *Tp* tuber value was significantly different ($p < 0.05$) compared with the *Tp* leaf and flower values, varying between 162.80 ± 0.04; 266.12 ± 0.21 and 273.83 ± 0.87 µM Trolox/ g dw, respectively. The results of the DPPH analysis revealed that the natural antioxidant content for the *Tp* leaf was greater compared with that for the *Tp* tuber and flower. The results of the ABTS analysis showed that natural antioxidant content for the *Tp* flower was greater compared with that for the *Tp* tuber and leaf and showed better scavenging activity. However, the antioxidant capacity is directly related to the presence of bioactive compounds. To estimate which of the bioactive compounds analyzed provides

antioxidant activity, the DPPH, ABTS and TRAP extract samples were evaluated under the same chromatographic conditions studied in this work (carotenoids, flavonoids, ascorbic acid and tocopherol). According to the results for the DPPH and ABTS extracts, no antioxidant activity was detected in all samples analyzed.

Regarding to (TRAP - AUC) (Fig. 1A) the tuber showed higher amount of antioxidants and was statistically different ($p < 0.05$) of the leaf and flower. However, the flower revealed higher quality of its antioxidants by TAR method, followed of the leaf. According to Rossato et al. (2009) TRAP and TAR are not proportional because these indexes depend of the presence of efficient and inefficient antioxidants in the extract, and sometimes there are antioxidants of relatively low reactivityThe results of the TAR (Fig. 1B) analysis revealed that the flower obtained the highest TAR (highreactivity of the extract), also being statistically different ($p < 0.05$) of the tuber and the leaf. The flower presented higher amounts of phenolics compounds totals, α-tocopherol and γ-tocopherol, compared with the leaf. However, the flower indicated the presence of more efficient antioxidants.

The tuber presented lower amount of bioactive compounds and smaller scavenging free radical by methods DPPH and ABTS in relation to leaf and flower, revealing consistency between the antioxidant assays. Only in the alcoholic extract of the tuber was observed the presence of flavonoids, such as luteolin 194.10 ± 0.30 µg/ g, myricetin 29.41 ± 0.08 µg/ g and kaempferol 30.24 ± 0.20 µg/ g (Figure 2) what may have influenced in the higher amount of antioxidants as revealed by TRAP-AUC method.
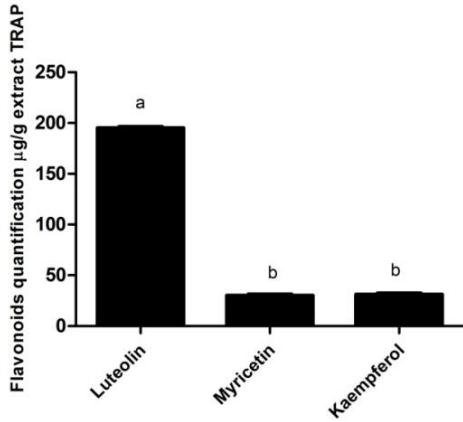
Figure 2. Representative value obtained from alcoholic extract (TRAP) of tuber, injected into the HPLC conditions of flavonoids. Values are expressed as (mean ± SD) ($p < 0.05$).

In contrast, antioxidant activities were detected for the carotenoids and tocopherol in the TRAP extracts for the leaf and flower samples and only for ascorbic acid in the flower sample. Based on the results, we can say that the bioactive compounds that confer higher antioxidant activity in the TRAP analysis for the leaf and flower are carotenoids and tocopherols because these compounds were present in the greatest quantities (Figure 2.1 A-B). In addition, the analysis of ascorbic acid detected antioxidant activity only for the flower sample, with a value of 9.6 ± 0.02 µg/ g.
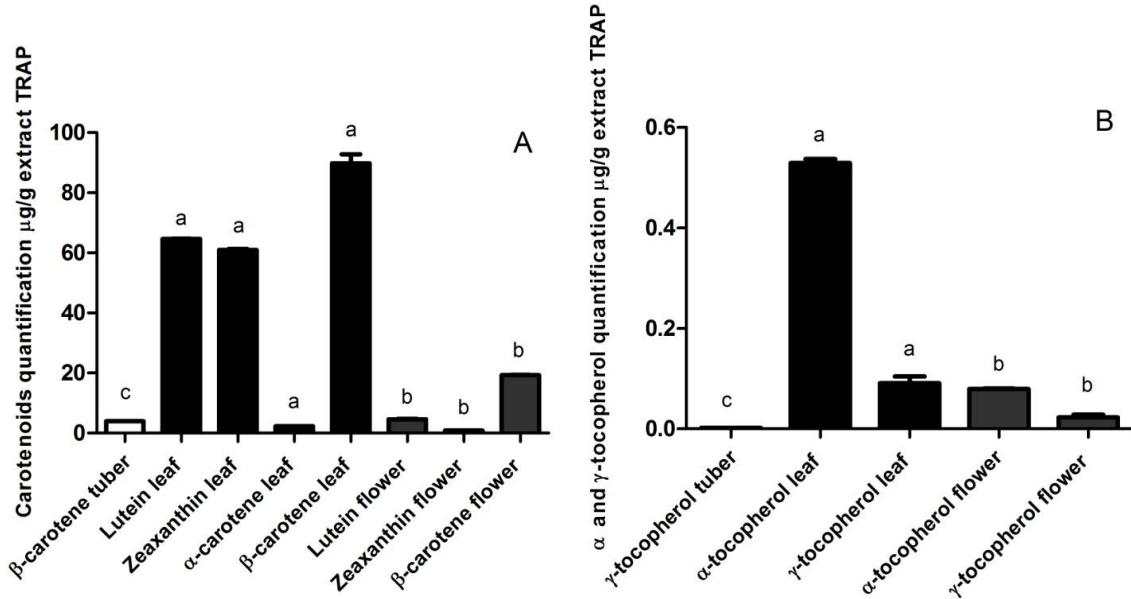
Figure 2.1 A-B - Representative value obtained from alcoholic extract (TRAP) of tuber, leaf and flower injected into the HPLC conditions of carotenoids and tocopherol. Values are expressed as (mean ± SD) ($p < 0.05$).

The leaf and flower antioxidant activities were analyzed using the high concentrations of carotenoids and flavonoids, and ascorbic acid and tocopherol activities were only found in the flower sample. For carotenoid quantification, the leaf and flower values found were 64.61 ± 0.10 µg/ g and 4.59 ± 0.24 µg/ g for lutein; 60.94 ± 0.35 µg/ g and 0.84 ± 0.00 µg/ g for zeaxanthin; 2.31 ± 0.00 µg/ g for α-carotene and 89.79 ± 2.99 µg/ g and 10.27 ± 0.14 µg/ g dw for β-carotene, respectively. However, zeaxanthin in the leaf sample, which increased to nearly 3 times the concentration the extract, did not saponify. According to Kimura, Rodriguez-Amaya, Godoy, (1990) & Rodriguez-Amaya (1989), some carotenoids (α-carotene, β-carotene, γ-carotene, and β-cryptoxanthin) can resist saponification. Moreover, lutein, violaxanthin, and other dihydroxy-, trihydroxy-, and epoxycarotenoids are reduced considerably during saponification and during the subsequent washing step (Riso & Porrini 1997, Kimura, Rodriguez-Amaya, Godoy, 1990; Khachik, Beecher, Whittaker, 1986).

In total carotenoids (expressed in µg/ g for a comparison with the quantification of carotenoids by HPLC), the *Tp* leaf sample presented a value of 884.38 µg/ g and the *Tp* flower sample presented a value of 21.01 µg/ g; these values are significantly lower when compared with the results of the quantification of carotenoids by HPLC (Table 6 – results expressed in µg/ g). This result may have occurred by the brief exposure of the extract containing chlorophyll during the analysis. According to Rodriguez-Amaya et al. (2008), photodegradation and isomerization can occur rapidly under such conditions.

For α- and γ-tocopherol quantification of leaf and flower samples, the values found ranged from 0.09 ± 0.18 µg/ g − 0.03 ± 0.04 µg/ g dw and 0.28 ± 0.00 µg/ g − 0.10±0.00 µg/ g dw, respectively. With respect to α- and γ-tocopherol, an increase was observed only in the leaf sample. With the ethanol extract, the value increased by 0.09 ± 0.18, with a value of 0.53 ± 0.00 µg/ g for α-tocopherol, and by 0.03 ± 0.04 µg/ g, with

a value of 0.09 ± 0.01 µg/ g, compared with the methanol extract used in the quantification of tocopherol because ethanol is often considered bipolar and easily dissolved by nonpolar organic compounds.

In the DPPH and ABTS extracts, only lutein was detected in the leaf and flower samples, with concentrations of 9.34 ± 1.07 and 5.48 ± 0.12 µg/ g lutein, respectively. Water can be used to extract polar compounds, which, in fact, may have interacted in the carotenoid extraction analysis of DPPH and ABTS. Moreover, in contrast to the extract for the TRAP analysis, this extract was not macerated, which is a factor that influences the release of carotenoids.

3.5. Lipophilic antioxidant contents of (*Tp*) tuber, leaf and flower samples

Carotenoids and tocopherols are included among the lipophilic antioxidants. Table 5 shows the values found for carotenoids and for tocopherols. Five carotenoids were identified (lutein, zeaxanthin, β-cryptoxanthin, β-carotene, and α-carotene) using chromatographic separation; however, the zeaxanthin and β-cryptoxanthin quantification levels that were found were below the curve (< 1.0 µg/ g) for the tuber sample. Two tocopherols were identified (α- tocopherol and γ-tocopherol) in *Tp* leaf and flower samples using chromatographic separation but were not determined in the *Tp* tuber sample.

Table 5. Carotenoids and α- and γ-tocopherol analysis of (mean ± SD) the tuber, leaf and flower of the *T. pentaphyllum* (µg/ g dw).

Parameters	Rt (min)	*T. pentaphyllum* tuber	*T. pentaphyllum* leaf	*T. pentaphyllum* flower

Lutein$^{\lozenge}$	17.3-18.3	0.22 ± 0.02^{c}	642.50 ± 4.56^{a}	243.23 ± 0.89^{b}
Zeaxanthin$^{\lozenge}$	21.0-21.9	nq*	23.20 ± 0.82^{a}	14.23 ± 0.04^{b}
β-cryptoxanthin$^{\lozenge}$	32.9-33.2	nq*	21.71 ± 0.09^{a}	2.58 ± 0.03^{b}
α-carotene$^{\lozenge}$	37.1-38.1	0.063 ± 0.00^{c}	8.14 ± 0.02^{a}	3.61 ± 0.02^{b}
β-carotene$^{\lozenge}$	42.8-44.5	4.20 ± 0.02^{c}	467.74 ± 2.39^{a}	132.06 ± 0.76^{b}
Total carotenoids$^{\lozenge}$	-	4.48	1163.29	395.71
α- tocopherol$^{\lozenge}$	6.1	nd**	0.09 ± 0.05^{b}	0.28 ± 0.07^{a}
γ-tocopherol$^{\lozenge}$	7.1	nd**	0.03 ± 0.02^{b}	0.10 ± 0.04^{a}

$^{\lozenge}$Mean of three replicates ($n = 3$). Different letters in the same line represent significant difference $p < 0.05$ by Tukey's test.
*Below detection limit.
**Not determined

The three parts of the *Tp* were significantly different ($p < 0.05$), with lutein and β-carotene identified as the major compounds for all samples (with the exception of lutein for the tuber sample), although their concentrations in the leaves are significantly higher compared with the flowers. Isabelle et al. (2010) evaluated the carotenoid contents of 66 vegetables, and lutein was not detected in sweet potato. However, for the *Tp* tuber *Tp*, 0.22 µg/ g of lutein was found. For the sweet potato leaves, 61.84 µg/ g of lutein was found, which is almost 11 times lower than that found for the *Tp* leaves. The β-carotene contents of the *Tp* tuber and leaf samples were also higher when compared with 0.30 µg/ g for sweet potato and with 48.97 µg/ g for sweet potato leaves. Rodriguez-Amaya, et al. (2008) evaluated the lutein and β-carotene contents in the leaves of *Tropaeolum majus* and found values of 69 µg/ g and 136 µg/ g, respectively, which were also lower in comparison with the *Tp* leaf values.

The study by Tlili et al. (2009) evaluated lutein and β-carotene in the flowers of *Capparis spinosa*; the found values for samples GM, KH and ST were lower, with

118.6 ± 4.92, 86.7 ± 1.9 and 195.9 ± 2.13 µg/ g for lutein and with 46.9 ± 0.84, 53.8 ±

1.07 and 80.7 ± 0.9 µg/ g for β-carotene, respectively, when compared with *Tp* flower

values. Rodriguez-Amaya et al. (2008) evaluated lutein in yellow and orange flowers of

Tropaeolum majus and found 450 µg/g and 350 µg/ g of lutein, respectively, which are

lower values with respect to *Tp* leaf values. Specific carotenoids, major compounds

found in the *Tp* leaf and flower, are important because of their protective effect against

age-related macular degeneration (e.g., lutein) (Krinsky & Johnson, 2005).

Concerning α-carotene and zeaxanthin, the values found for the leaf and flower

samples are significantly higher when compared with the values found in studies with

common vegetables. In the study of Isabelle et al. (2010), the amount of zeaxanthin in

the *Tp* flower was only lower when compared with the Chinese kale sample, at 15.16

µg/g, and for the *Tp* leaf when compared with seaweed, at 33.67 µg/ g. For *Tp* leaf and

flower samples, the α-carotene content was only lower when compared with carrot, at

14.49 µg/ g. However, the *Tp* leaf sample had zeaxanthin and α-carotene concentrations

that were significantly higher when compared with sweet potato leaf, at 3.33 µg/ g and

0.30 µg/ g, respectively.

However, the *Tp* flower showed values higher for β-cryptoxanthin in comparison

with acerola, at 2.1 µg/ g, and the leaf showed values higher in comparison with candied

papaya, at 20 µg/ g (Rodriguez-Amaya, et al. 2008).

Tocopherols act as antioxidants because of their capacity to scavenge lipid

peroxyl radicals from unsaturated lipid molecules, preventing the propagation of lipid

peroxidation (Traber, 2007).

The values found for α and for γ-tocopherol were significantly different between

the *Tp* leaf and flower samples. The values of α-tocopherol in the leaf and flower

samples were higher when compared with the γ-tocopherol content values. According to

Sanchéz-Machado et al. (2006), the flowers of (*Moringa oleifera*) had higher α-tocopherol and γ-tocopherol contents, at 305.7 ± 23.9 µg/ g and 9.1 ± 0.90 µg/ g dw, respectively, when compared with *Tp* flower contents. In addition, the α-tocopherol and γ-tocopherol contents of the *Tp* leaf were significantly lower when compared with the sweet potato leaves, at 7.23 µg/ g and 0.56 µg/ g fw, respectively (Isabelle, et al. 2010).

3.6. Flavone, flavonol and ascorbic acid contents

Vitamin C and the flavonoids are both extremely strong antioxidant agents, and their biological activities are partially synergistic (Isler et al. 1988).

According Huber et al. (2009), of the six principal classes of flavonoids, flavonols (e.g., myricetin, quercetin, kaempferol) and flavones (e.g., luteolin) are the most widely distributed in plant foods, where these compounds are found in their glycoside forms. During flavonoid analysis, hydrolysis is usually performed, and the resulting aglycone is identified and quantified.

Table 6 shows the measured values for flavones, flavonols and ascorbic acid. Using chromatographic separation, four flavonoids were identified (luteolin, myricetin, quercetin and kaempferol) in *Tp* leaves; three flavonoids were identified in *Tp* flowers, with the exception of lutein; and in *Tp* tubers, it was only possible to identify quercetin.

Table 6. Flavonoids and ascorbic acid analysis of (mean ± SD) the tuber, leaf and flower of the *T. pentaphyllum* (µg/ g dw).

Parameters	Rt (min)	Tuber *T.pentaphyllum*	Leaf *T. pentaphyllum*	Flower *T. pentaphyllum*
Luteolin[◊]	8.7	nd*	4450 ± 12.50^a	nd*
Myricetin[◊]	9.7	nd*	40 ± 0.13^a	5.00 ± 0.03^b
Quercetin[◊]	12.8	30 ± 0.10^c	3750 ± 8.09^a	840 ± 4.02^b

| Kaempferol$^{\Diamond}$ | 19.8 | nd* | 30 ± 0.80^{a} | 20 ± 0.09^{b} |
| Ascorbic acid$^{\Diamond}$ | 3.5 | 26 ± 0.01^{c} | 160 ± 0.30^{a} | 82 ± 0.10^{b} |

$^{\Diamond}$Mean of three replicates ($n = 3$). Different letters in the same line represent significant difference $p < 0.05$ by Tukey's test.
*Not determined

Using a previously optimized and validated method proposed by Huber et al. (2009), the findings presented above indicate that the optimum conditions vary with the different parts of the *Tp* plant (tuber, leaf and flower). Before initiating flavonoid analyses, the conditions for the extraction/ hydrolysis were optimized for each part of the plant compared with conventional vegetables. The extraction/ hydrolysis procedures were repeated six times ($n = 6$) for tuber samples, four times ($n = 4$) for leaf samples and two times ($n = 2$) for flower samples. The following items were verified: the weight of the sample; lyophilized or fresh sample; the molarities of HCl; the concentration of methanol (50 or 62.5%); the reflux time (90 °C); the use of an antioxidant (ascorbic acid or BHT); and the volume of methanol used. Before injection into HPLC, unfiltered and filtered samples with filters (0.45 µm PTFE and SPE) were also observed. From the second repetition because these methods presented the best results the use of methanol 50% and PTFE filters were established. The antioxidant (ascorbic acid) was also established; however, there was no difference compared with BHT.

For tuber and leaf samples, the extraction/ hydrolysis procedures were more effective with 20 g freeze-dried samples of tuber and 1 g freeze-dried samples of leaves, which were both homogenized with methanol 50% and HCl 1.2 M for 2 h at 90 °C. The volumes used were 110 mL for tuber samples and 20 mL for leaf samples. Chu et al. (2000) used extraction/hydrolysis procedures for potato and sweet potato leaves (green) with methanol 60% and 6 M HCl for 2 h at 90 ºC. The values found were 0.01 ± 0.00 µg/ g fw for myricetin; 0.05 ± 0.00 µg/ g fw for quercetin; 0.50 ± 0.00 µg/ g fw for

kaempferol and not determined for luteolin. The content of quercetin in *Tp* tuber was significantly lower in comparison with those values reported by the author. In contrast, the values found in this study for *Tp* leaves were significantly higher when compared with the quercetin value in sweet potato leaves (green), at 143.78 ± 5.06 µg/ g fw, and similar with the content of myricetin, at 38.88 ± 1.79 µg/ g fw. In addition, this study also identified high amounts of luteolin and kaempferol.

For flowers, the extraction/hydrolysis procedure was more effective with 1 g of fresh sample, which was homogenized with methanol 50% and HCl 1.2 M for 2 h at 90 °C. The volume used was 32.5 mL for flowers. Kaisson et al. (2011) used extraction/hydrolysis for twelve edible flowers from Thailand with methanol/ HCl (100: 1, v/ v), which contained 2% tert-butylhydroquinone, for 12 h at 35 ºC. The values found for a (*Tagetes erecta*) sample were 4.0 ± 0.60 µg/ g dw for myricetin; 145.6 ± 2.4 µg/ g dw for quercetin and 23.7 ± 2.3 µg/ g dw kaempferol. The contents of myricetin and quercetin in *Tp* flower samples were highest, and the kaempferol content was lower in comparison with those values reported by the author.

The ascorbic acid was identified in freeze-dried *Tp* tuber and leaf samples, whereas for *Tp* flowers, it was only possible to perform separation using a fresh sample. According to Isabelle et al. (2010), ascorbic acid values found for sweet potato and sweet potato leaves were 1.0 µg/ g fw for both samples, which are significantly lower than the results found for *Tp* tuber and leaf samples. However, according to Garzón & Wrolstad (2009), for Nasturtium (*Tropaeolum majus*) flowers, 715 µg/ g ascorbic acid was found, with values higher than that for *Tp* flowers.

4. Conclusions

This study presented the characterization of physicochemical characteristics, bioactive compound content and antioxidant properties of the tuber, leaf and flower samples of the species *Tropaeolum pentaphyllum* Lam. The tuber and leaf samples of *T. pentaphyllum* demonstrated antioxidant potential and nutritional quality in comparison with sweet potato and sweet potato leaves. Furthermore, the leaves of *T. pentaphyllum* exhibited high amounts of flavonoids and carotenoids. The flowers displayed high quality antioxidants, as determined by TAR methods. Additionally, a high lutein content was found compared with the lutein content of the flowers of *Tropaeolum majus*, which was reported by other studies. However, the tuber revealed high reactivity, as determined by TRAP – AUC methods. In addition, the leaf, flower, and tuber extracts presented antioxidant activities, as determined by the TRAP method. This study highlights the potential of this species as an important source of nutritional compounds that are available in the flora of South Brazil.

Acknowledgments

Appendix A. Supplementary data

Reagents and standards mineral composition

For the mineral composition the standard solutions were prepared daily by appropriate dilution of the stock standard solutions of 1000 mg L^{-1} (Specsol, Brazil) for Cd, Cu, Cr, Fe, Rb and Zn and of 10,000 mgL^{-1} for Ca, K, Mg and Na, which were prepared from $CaCl_2$, KCl and NaCl salts (Merck, Darmstadt, Germany). Nitric acid, purified in a quartz sub-boiling still (Kürner Analysentechnik, Rosenheim, Germany) were used to prepare the aqueous calibration solution and 10 g L^{-1} Cs (Sigma-Aldrich, USA) were added to all standards and blank solutions to stabilize the solutions, during flame atomization. All containers and glassware used for mineral determination were soaked in 1.4 mol L^{-1} nitric acid for at least 24 h and rinsed three times with deionized water before use.

Sample preparation mineral composition

To realize the mineral analysis the root samples were submitted to two different pre-treatment procedures. For the direct sampling analysis, used into the trace elements determinations, the samples were ground in a micro-mill A-11 Basic (IKA-Werke, Germany) and after that sieved through a 200 µm polyester sieve and kept in sealed plastic vials until further processing.

For the macro and micro elements analysis the samples were digested by a microwave oven MARS-6 (CEM, Matthews, EUA), with the manufacturer recommended equipment conditions for plant material. The microwave was equipped with four closed teflon vessels. The digestion procedure included the addition of 0.5 mg

of the sample and 10 mL of purified nitric acid followed by the microwave program: step 1 – 20 min ramp with 900 W of power; step 2 – 10 min hold time with 900 W of power; and step 3 – 15 min cooling time with 0 W of power. After digesting and cooling, the resulting digests were transferred to 25 mL flasks and Cs, with final concentration of 1% (m/v), was added and the volume completed with ultrapure water. All samples were prepared in triplicate.

Preparation extract of total carotenoids, chlorophyll and phenolic compounds

The three parts of the *Tp* sample (0.25 g) were homogenized in an (IKA T-25 Ultra-Turrax Digital, Staufen, Germany) with 80% acetone (v/v), and the extract was centrifuged in sealed tubes at 5,000 × g for 15 min. The supernatant was filtered and collected, and the absorbance was read for chlorophyll a, chlorophyll b and total carotenoid using a Shimadzu model UV-1800 spectrophotometer (Kyoto, Japan).

Preparation extract of DPPH and ABTS^{+} radical

The extracts for both methods were obtained from 2.5 g of freeze-dried sample diluted in 50:50 v/v methanol:water and 70:30 v/v acetone:water using three different dilutions extract:water (2:0.5, 2:2 and 2:10 v/v). The samples were weighed in centrifuge tubes and sequentially extracted with 40 mL of methanol/water (50:50, v/v) at room temperature for 1 h. Next, the tubes were centrifuged at 22,000 x g for 15 min at 4°C, and the supernatant was recovered. Then, 40 mL of acetone/ water (70:30, v/v) was added to the residue at room temperature, extracted for 60 min and centrifuged.

Methanol and acetone extracts were combined, made up to a total volume of 100 mL with distilled water and used to determine antioxidant activity.

Preparation extract of TRAP

The freeze-dried samples (5 g, 0.5 g and 0.5 g, in triplicate) of tubers, leaves and flowers, respectively, were macerated with ethyl alcohol 95% P.A until pigments were completely removed and filtered using a vacuum system. The extract was concentrated under reduced pressure in a rotary evaporator at ambient temperature (Fisatom, Model 801, St. Paul), diluted in 5 mL of ethyl ether, and placed in amber vials. The solvent was removed under nitrogen flow, and the extracts were stored at -10°C until the time of analysis antioxidant. After, the concentrated extract was diluted in water (final concentration 5 µg/mL).

The reaction mixture, which contained AAPH (10 mM) and luminol (4 mM) in glycine buffer (0.1 M, pH 8.6), was incubated at 21°C for 2 hours and measured. AAPH is a source of peroxyl radicals that react with luminol, yielding chemiluminescence (CL). The system was calibrated using Trolox. After the 2 h incubation, 180 µL of the reaction mixture was placed in a 96-well plate and then 20 µL of sample, 20 µL of Trolox (final concentration 1 µM) or 20 µL of vehicle (99.25% water/0.75% DMSO), which represents the maximum radical generation, was added.

Preparation extract of α- and γ-tocopherol

The freeze-dried samples (5 g, 1 g and 0.2 g, in triplicate) of tubers, leaves and flowers, respectively, were macerated with absolute methanol until pigments were

completely removed and filtered using a vacuum system. The extract was concentrated under reduced pressure in a rotary evaporator at ambient temperature (Fisatom, Model 801, St. Paul), diluted in 5 mL of methanol, and placed in amber vials. The solvent was removed under nitrogen flow, and the extracts were stored at -10°C until the quantification of tocopherol.

Preparation extract of ascorbic acid

The extraction was performed using 5 g fresh flowers and using 2 g and 1 g freeze-dried samples of tuber and leaves, respectively, which were ground in an (IKA T-25 Ultra-Turrax Digital, Staufen, Germany) with 20 mL 0.05 M Suprapur 96% sulfuric acid for 1 minute, centrifuged at 22,000 x g for 15 min and then filtered through a 0.45 μm PTFE filter.

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CAPÍTULO 3: DISCUSSÃO GERAL

O Brasil é um país de dimensão continental, reconhecido por sua grande biodiversidade. Contém também em seu território a maior riqueza de espécies da flora, além dos maiores remanescentes de ecossistemas tropicais. A região sul devido à sua privilegiada amplitude de clima e relevo, também possui destaque na biodiversidade vegetal, entretanto, por desconhecimento e falta de pesquisa, tais plantas são subutilizadas, e em sua maioria, são conhecidas como plantas de mato ou silvestres.

Entretanto, a domesticação e incentivo para o cultivo de plantas nativas utilizadas por populações locais, mas ainda sem introdução no mercado nacional é a grande oportunidade para utilizar esses recursos genéticos naturais na segurança alimentar e nutricional, além de explorar os efeitos dos antioxidantes naturais em pesquisas em prol da saúde.

Vários estudos epidemiológicos demonstraram que o consumo de frutas e vegetais está associado a uma menor incidência de doenças crônicas e degenerativas. Entretanto, poucos estudos reportam dados sobre a caracterização nutricional, avaliação de compostos bioativos e suas propriedades antioxidantes de frutas, vegetais e tubérculos nativos não convencionais do sul do Brasil.

Com base no exposto acima, o primeiro artigo intitulado "Bioactive compounds, nutritional and antioxidant activity of pulp of *Opuntia monacantha* (Willd.) Haw. and leaves of *Urera aurantiaca* Wedd." teve como objetivo caracterizar as duas espécies pela sua importância na alimentação de pequenas comunidades do Rio Grande do Sul e para incentivar a introdução na dieta da população, a fim de aumentar a diversidade de alimentos e a segurança alimentar e nutricional.

Cabe ressaltar, que este é o primeiro estudo que caracteriza a folha da espécie *U. aurantiaca* e também a polpa da fruta *O. monacantha*, com excessão da análise físico-química, relatada em um estudo anterior reportado por Oliveira, et al., (1992) para os frutos de *O. monacantha* proveniente do Cariri paraibano.

Segundo os resultados encontrados, o estudo revelou o potencial das plantas avaliadas, especialmente em comparação com outras espécies silvestres do mesmo gênero *Opuntia* que foram investigadas em outros estudos. Além disso, a espécie *U. aurantiaca* apresentou alta quantidade de compostos bioativos e antioxidantes naturais, comparada com vegetais comumente consumidos pela população brasileira, como por exemplo, espinafre e couve. Conforme estudos é interessante investigar o potencial para a agroindústria, pela sua elevada quantidade de carotenoides, quercetina, clorofila e de

proteínas em relação às plantas convencionais, que pode ser utilizada em programas de melhoramento da qualidade de alimentos para a população.

No segundo artigo intitulado "Characterization, bioactive compounds and antioxidant properties of tuber, leaf and flower of *Tropaeolum pentaphyllum* Lam." tem como principal objetivo caracterizar as três partes comestíveis da planta, tais como tubérculo, folhas e flores. Esta espécie tem grande importância especialmente na alimentação da população com ascendência italiana, nos estados do Rio Grande do Sul, Santa Catarina e Paraná. Entretanto, o uso principal e tradicional é do tubérculo ralado em conserva no vinagre tinto, acompanhando pratos de sopas e carnes. Além disso, o cultivo das folhas e flores vem sendo difundido em comunidades locais na cidade de Porto Alegre, incentivando a divulgação e o consumo das folhas e flores em saladas. Porém, a espécie ainda é desconhecida pela maioria da população brasileira em termos etnobotânicos e nutricionais.

Segundo resultados, o estudo revelou o potencial da espécie *pentaphyllum*, especialmente em comparação com outras espécies, como a batata-doce e as folhas de batata-doce já difundidas na alimentação. Foi observado alto conteúdo de quercetina e luteolina nas folhas do *T. pentaphyllum*, sendo alto teor de quercetina em comparação com as folhas de batata doce. Além disso, o conteúdo de luteína e β-caroteno foram superiores aos encontrados nas folhas do *Tropaeolum majus*. Entretanto, o tubérculo apresentou alta reatividade pelo método TRAP-AUC e a melhor qualidade antioxidante pelo método TAR foi observado na flor.

Além da caracterização, este estudo pode apoiar o cultivo em larga escala e à inclusão da espécie não somente nos estados citados anteriormente, mas em toda população brasileira, ajudando a divulgar a espécie e permitindo que novos estudos sejam realizados.

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