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# *Arrabidaea chica* (Humb. & Bonpl.) Verlot: IDENTIFICAÇÃO DE COMPOSTOS BIOATIVOS E AVALIAÇÃO DO POTENCIAL ANTIOXIDANTE POR MÉTODOS QUÍMICOS (*in vitro)*

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**Orientadora:** Profª. Drª. Alessandra Santos Lopes
**Coorientador:** Prof. Dr. Renan Campos Chisté

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*Dedico a Deus, autor da minha vida e o maior apoio nos momentos difíceis, a minha amada filha, Isis, meu amor maior; a minha mãe, Oliete e avós Laura e Ediberto (in memorian), que foram exemplos de caráter e honestidade.*

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## RESUMO

*Arrabidaea chica* é uma planta medicinal da família Bignoniaceae, que ocorre na América tropical e é amplamente distribuída na Amazônia brasileira. Esta planta pode ser vista como uma fonte promissora de compostos bioativos, como compostos fenólicos e carotenóides, que são metabólitos secundários da planta que podem ser usados para retardar o dano oxidativo em sistemas alimentares e biológicos. Neste estudo, a composição de carotenóides e compostos fenólicos das folhas de *A. chica* foi identificada e quantificada por HPLC-DAD-MS/MS, além do teor de ácido ascórbico. Os principais compostos fenólicos identificados foram a escutelarina e a escutelareína (aglicona); enquanto luteína, β-caroteno e α-caroteno foram os principais carotenóides. Em relação à capacidade antioxidante *in vitro*, o extrato hidrometanólico das folhas de *A. chica* foi caracterizado como um eficiente sequestrante de radicais ABTS, além de proteger o triptofano contra a oxidação por oxigênio singleto de forma concentração-dependente ($IC_{50}$ = 177 μg/mL). Esses resultados estimularam o próximo estudo a investigar ainda mais o potencial antioxidante de extratos de *A. chica*, obtidos por extração assistida por ultrassom, usando solventes "verdes", contra espécies reativas de oxigênio (ROS) e nitrogênio (RNS) de relevância fisiológica e alimentar. A capacidade antioxidante de três extratos de folhas de *A. chica* obtidos com solventes de diferentes polaridades (água, etanol e etanol/água (1:1, v/v) foram testados contra o radical ânion superóxido ($O_2^{\bullet-}$), o peróxido de hidrogênio ($H_2O_2$), o ácido hipocloroso (HOCl), o radical peroxinitrito ($ONOO^-$), o oxigênio singleto $^1O_2$ e radical peroxil (ROO•). O extrato de *A. chica* preparado com etanol/água apresentou os maiores teores fenólicos (11,80 mg/g de extrato), sendo a scutelareína (flavona) o composto majoritário (≈57%), enquanto o etanol e a água sozinhos foram menos eficientes, resultando em alterações no perfil individual de compostos fenólicos, destacado pela ausência de escutelareína e pelos altos teores de ácidos fenólicos. Todos os extratos foram capazes de eliminar os ROS/RNS testados de forma concentração-dependente com baixos valores de $IC_{50}$, sendo o extrato obtido com etanol/água o mais eficaz para todos os ROS/RNS ($IC_{50}$ de 0,34 a 35,66 μg/mL). Em relação à capacidade antioxidante contra ROO•, todos os extratos apresentaram o mesmo comportamento antioxidante, com eficiência de eliminação cerca de cinco vezes maior que o Trolox. Portanto, as folhas de *A. chica* provaram ser uma fonte promissora de compostos bioativos com altas propriedades antioxidantes para serem utilizadas como antioxidantes naturais para inibir danos oxidativos em sistemas alimentícios e fisiológicos.



**Palavras-chave:** Planta Amazônica. ROS. RNS. Solvente verde. LC-MS

## ABSTRACT

*Arrabidaea chica* is a medicinal plant from the Bignoniaceae family, which occurs in tropical America and it is widely distributed in the Brazilian Amazonia. This plant can be seen as a promising source of bioactive compounds, such as phenolic compounds and carotenoids, which are secondary plant metabolites that can be used to delay oxidative damage in both food and biological systems. In this study, the composition of carotenoids and phenolic compounds of *A. chica* leaves were identified and quantified by HPLC-DAD-MS/MS, in addition to the ascorbic acid content. The main phenolic compounds identified were scutellarin and scutellarein (aglycone); while lutein, β-carotene and α-carotene were the main carotenoids. Regarding the *in vitro* antioxidant capacity, the hydromethanolic extract of *A. chica* leaves was characterized as an efficient ABTS radical scavenger, in addition to protect tryptophan against oxidation by singlet oxygen in a concentration-dependent manner ($IC_{50}$ = 177 μg/mL). These results stimulated the next study to further investigate the antioxidant potential of extracts of *A. chica,* obtained through ultrasound-assisted extraction, using "green" solvents, against reactive oxygen (ROS) and nitrogen (RNS) species of physiological and food relevance. The antioxidant capacity of three extracts of *A. chica* leaves obtained with solvents of different polarities (water, ethanol and ethanol/water (1:1, v/v) were tested against superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl), the peroxynitrite radical (ONOO−), the oxigênio singleto $^1O_2$ and peroxyl radical ($ROO^{\bullet}$). The A. chica extract prepared with ethanol/water presented the highest phenolic contents (11.80 mg/g extract), being scutelarein (flavone) the major compound (≈57%), while ethanol and water alone were less efficient resulting in changes in the individual profile of phenolic compounds, highlighted by the absence of scutelarein and by the high levels of phenolic acids. All extracts were able to scavenge the tested ROS/RNS in a concentration-dependent manner with low $IC_{50}$ values, being the extract obtained with ethanol/water the most effective for all the ROS/RNS ($IC_{50}$ from 0.34 to 35.66 μg/mL). Regarding the antioxidant capacity against $ROO^{\bullet}$, all the extracts showed the same antioxidant behavior, with scavenging efficiency about five times higher than Trolox. Therefore, *A. chica* leaves proved to be a promising source of bioactive compounds with high antioxidant properties to be used as natural antioxidants to inhibit oxidative damage in both food and physiological systems.



**Keywords**: Amazonian plant. ROS. RNS. Green solvent. LC-MS

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## LISTA DE ABREVIATURAS E SIGLAS

| | |
|---|---|
| ABTS | Ácido 2,2-Anzino-Bis(3-Etilbenzotiazolina-6-Sulfônico) |
| APCI | Atmospheric Pressure Chemical Ionization |
| DHR | di-hidrorodamina |
| DPPH | 2,2-difenil-1-picril-hidrazil |
| ESI | Electrospray |
| EtOH | Etanol |
| EtOH/ $H_2O$ | Etanol/Água |
| FRAP | Testes de Redução do Ferro |
| $H_2O$ | Água |
| $H_2O_2$ | Peróxido de Hidrogênio |
| HAT | Hydrogen Atom Transfer |
| $HO^{\bullet}$ | Radical Hidroxila |
| HOCl | Ácido hipocloroso |
| HPLCDAD-MS | Cromatografia Líquida acoplada aos detectores de arranjo de diodos e espectrômetro de massas |
| LOD | Limites de Detecção |
| LOQ | Limites de Quantificação |
| MB | Methylene blue |
| MTBE | Éter metil *tert*-butílico |
| NADH | β-nicotinamida dinucleido de adenina |
| NADPH | Nicotinamida adenina dinucleotído fosfato |
| NBT | Cloreto de nitroazul de tetrazio |
| $NO_2$ | Dióxido de nitrogênio |
| $^{\bullet}NO$ | Óxido nítrico |
| $^1O_2$ | Oxigênio singleto |
| OH | Grupo Hidroxila |
| $ONOO^-$ | Ânion peroxinitrito |

## LISTA DE ABREVIATURAS E SIGLAS

| | |
|---|---|
| ORAC | Capacidade de absorção do radical de oxigênio |
| PMS | Metossulfato de fenazina |
| RAE | Retinol Activity Equivalent |
| RNS | Espécies Reativas de Nitrogênio |
| $ROO^{\bullet}$ | Radical Peroxila |
| ROOH | Hidroperóxido orgânico |
| ROS | Espécies Reativas de Oxigênio |
| SET | Single Electron Transfer |
| SOD | Superóxido Dismutase |
| $t_{1/2}$ | Meia-vida |
| TEAC | Capacidade Antioxidante Equivalente do Trolox |
| TRAP | Método do potencial antioxidante reativo total |
| TRP | Triptofano |

**SUMÁRIO**

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## INTRODUÇÃO GERAL

O aumento da ingestão de frutas e vegetais na dieta tem sido associado à baixa incidência de doenças crônico-degenerativas, como câncer, diabetes, doenças cardiovasculares e Alzheimer (KOK et al. 2010; RODRIGUEZ-CONCEPCION et al. 2018; SZAJDEK; BOROWSKA, 2008; WILLETT, 2002;) e este efeito pode estar atribuído à presença de compostos bioativos, dentre estes, tocoferóis (vitamina E), o ácido ascórbico (vitamina C), compostos fenólicos e carotenoides que minimizam os efeitos oxidativos *in vivo*, ocasionados pelas espécies reativas de oxigênio (ROS) e de nitrogênio (RNS) (HALLIWELL; GUTTERIDGE, 2007; SEIFRIED et al., 2007).

O metabolismo humano, em condições normais, mantém o equilíbrio entre compostos oxidantes e a atuação dos sistemas de defesa antioxidantes para manter a homeostase fisiológica adequada (HALLIWELL; GUTTERIDGE, 2007; WILLETT, 2002). No entanto, a superprodução de ROS e RNS podem ocasionar um desequilíbrio, levando ao estresse oxidativo e nitrosativo, e consequentemente, pode implicar no desenvolvimento dessas doenças crônico-degenerativas. Um dos mecanismos responsável pela redução do risco dessas doenças envolve a desativação do efeito oxidativo dessas espécies (BUNKOVA; MAROVA; NEMEC, 2005; CHISTÉ et al., 2011; CHISTÉ; MERCADANTE, 2012)

No sistema alimentício, devido à sua natureza biológica, a ocorrência das ROS é inevitável e são as principais responsáveis pelo início da reação de oxidação dos alimentos (CHOE; MIN, 2006). As ROS podem alterar as propriedades funcionais das proteínas, lipídios e carboidratos devido à formação de dímeros e trímeros oxidados, o que resulta na diminuição da qualidade nutricional (CHOE; MIN, 2006; LEE; MIN; LEE, 2003; SHAHIDI; ZHONG, 2010).

Devido a essas consequências prejudiciais, a inibição da ação de ROS em sistemas alimentares é altamente desejável e, portanto, o uso de antioxidantes é um meio eficiente para retardar ou inibir a oxidação.

O papel dos antioxidantes é inegável para retardar ou inibir os danos oxidativos em uma molécula alvo (HALLIWELL; GUTTERIDGE, 2007). Dada a sua importância, há um crescente interesse em antioxidantes de matrizes naturais, como as plantas medicinais e dietéticas, que podem ajudar a atenuar os danos oxidativos (RIBEIRO et al., 2015; RIBEIRO et al., 2018; SHARIFIFAR; DEHGHN-NUDEH; MIRTAJALDINI, 2009) .

Nesse contexto, sabe-se que a flora brasileira é uma das mais ricas fontes de espécies com substâncias bioativas, dentre essas espécies, particularmente no bioma amazônico, a *Arrabidaea chica* (Humb. & Bonpl.) B. Verlot, também conhecida como "crajiru", "pariri" ou "chica", possui elevado potencial para a prospecção de compostos bioativos. *A. chica* é uma planta arbórea pertencente à família Bignoniaceae que contém aproximadamente 120 gêneros e 800 espécies (RIBEIRO et al., 2012; TAKEMURA, et al., 1995).

As propriedades corantes deste vegetal são devidas a três antocianinas do tipo 3-desoxiantocianidinas já reportadas para a *A. chica*: 6,7,3′,4′- tetra-hidroxi-5-metoxi flavilium, 6,7,4′- trihidroxi-5-metoxi flavilium e a carajurina (6,7-dihidroxi-5,4′dimetoxi flavilium), que é a antocianina majoritária (ZORN et al., 2001).

Com relação às propriedades antioxidantes das folhas da *A. chica*, avaliadas pelos métodos DPPH (2,2-difenil-1-picrilhidrazil), β-caroteno/ácido linoleico e TRAP (método do potencial antioxidante reativo total), foi observado que entre o extrato bruto apresentou maior atividade antioxidante quando comparado com os compostos fenólicos isolados da folha: escutelareína e apigenina (SIRAICH et al. 2013). De acordo com os mesmos autores, esta atividade pode ser atribuída à presença da mistura de flavonoides no extrato vegetal, dentre eles a isoscutelareína, 6-hidroxiluteolina, hispidulina, escutelareína, luteolina e apigenina.

Outros estudos também demonstraram que *A. chica* apresenta ação antioxidante na desativação de ROS de relevância fisiológica e alimentícia, como por exemplo o radical peroxila ($ROO^{\bullet}$) (SILVA et al., 2007). Além disso, o pós-tratamento com *A. chica*, em fibroblastos L929, minimizou o dano oxidativo, pela inibição de ROS intracelulares e do radical ânion superóxido ($O_2^{\bullet-}$) mitocondrial, induzido por irradiação UV-A e UV-B, (RIBEIRO et al., 2018).

Embora reconhecida a capacidade antioxidante da *A. chica*, não há relatos na literatura sobre o seu efeito antioxidante contra outras ROS e RNS de importância fisiológica e alimentícia.

Por outro lado, o teor de compostos bioativos e sua capacidade antioxidante podem variar entre as diferentes espécies de frutas e vegetais, e dentro de uma mesma espécie (BALASUNDRAM; SUNDRAM; SAMMAM, 2006). Podendo existir a interferência dos fatores intrínsecos (gênero, espécie, cultivar) e extrínsecos (meio ambiente, estocagem, processamento e armazenamento) (KING; YOUNG, 1999; TOMÁS-BARBERAN; ESPÍN, 2001). Tais diferenças podem ainda, estar relacionadas à complexidade e diversidade de estruturas deste grupo de compostos e aos métodos de extração, polaridade dos solventes e métodos analíticos empregados (BRAVO, 1998).

Desse modo, o presente estudo tem como objetivo identificar e quantificar os perfis de compostos bioativos presentes nos extratos das folhas da *A. chica* e avaliar o potencial antioxidante contra algumas ROS e RNS, com alta relevância em sistemas biológicos e alimentares.

Essa tese está dividida em capítulos. O primeiro capítulo é uma revisão da literatura; o segundo capítulo aborda a identificação e quantificação dos compostos bioativos do extrato hidrofílico da *A. chica,* bem como sua capacidade antioxidante; e o terceiro capítulo representa o estudo da capacidade de desativação *in vitro* de extratos hidrofílicos de *Arrabidaea chica* frente as principais ROS e RNS de importância em sistemas alimentícios e fisiológicos.

## REFERÊNCIAS

BALASUNDRAM N, SUNDRAM K, SAMMAM S Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. **Food Chemistry**, v. 99, n. 1, p. 191-203, 2006.

BRAVO, L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. **Nutrition Reviews**, v. 56, n. 11, p. 317-333, 1998.

BUNKOVA, R.; MAROVA, I.; NEMEC, M. Antimutagenic properties of green tea. **Plant Foods for Human Nutrition**, v. 60, n. 1, p. 25-29, 2005.

CHISTÉ, R. C., & MERCADANTE, A. Z. (2012). Identification and quantification, by HPLC-DAD-MS/MS, of carotenoids and phenolic compounds from the Amazonian fruit Caryocar villosum. Journal of Agricultural and Food Chemistry, 60(23), 5884–5892.

CHISTÉ, R.C, MERCADANTE, A.Z, GOMES A, FERNANDES E, LIMA J.L.F.D, BRAGAGNOLO N. *In vitro* scavenging capacity of annatto seed extracts against reactive oxygen and nitrogen species. **Food Chemistry**, v. 127, n. 2, p. 419-426, 2011.

CHISTÉ, R. C.; FREITAS, M.; MERCADANTE, A. Z.; FERNANDES, E. The potential of extracts of *Caryocar villosum* pulp to scavenge reactive oxygen and nitrogen species. **Food Chemistry**, v. 135, n. 3, p. 1740-1749, 2012.

CHOE, E.; MIN, D. B. Chemistry and reactions of reactive oxygen species in foods. **Critical Reviews in Food Science and Nutrition**, v. 46, n. 1, p. 1-22, 2006.

HALLIWELL, B.; GUTTERIDGE, J. M. C. Free Radicals in Biology and Medicine, 4a ed.; OUP: Oxford, United States, pp 280−525, 2007.

KOK T. M. C. M.; DE WAARD, P.; WILMS, L. C.; VAN BREDA, S. G. J. Antioxidative and antigenotoxic properties of vegetables and dietary phytochemicals: the value of genomics biomarkers in molecular epidemiology. **Molecular Nutrition & Food Research**, v. 54, n. 2, p. 208-217, 2010.

KING, A.; YOUNG, G. Characteristics and occurrence of phenolic phytochemicals. **Journal of the American Dietetic Association**, v. 99, n. 2, p. 213-218, 1999.

LEE, J.; MIN, S.; LEE, E. C.; MIN, D. B. Formation of volatile compounds in soy flour by singlet oxygen oxidation during storage under light. **Journal of Food Science**, v. 68, n. 6, p. 1933-1937, 2003.

RIBEIRO, A. B., BERTO, A., CHISTÉ, R. C., FREITAS, M., VISENTAINER, J. V, & FERNANDES, E. (2015). Bioactive compounds and scavenging capacity of extracts from different parts of *Vismia cauliflora* against reactive oxygen and nitrogen species. **Pharmaceutical Biology** v. 53, n. 9, p. 1267-1276, 2015.

RIBEIRO, A. F. C.; TELLES. T. C.; FERRAZ, V. P.; SOUZA-FAGUNDES E. M.; CASSALI, G.D.; CARVALHO, A. T.; MELO, M. M. Effect of *Arrabidaea chica* extracts on the Ehrlich solid tumor development. **Brazilian Journal of Pharmacognosy**, v. 22, n. 2, p. 364-373, 2012.

RIBEIRO, F. M., VOLPATO, H., LAZARIN-BIDÓIA, D., DESOTI, V. C., DE SOUZA, R. O., FONSECA, M. J. V., … SILVA, S. DE O. The extended production of UV-induced reactive oxygen species in L929 fibroblasts is attenuated by posttreatment with *Arrabidaea chica* through scavenging mechanisms. **Journal of Photochemistry and Photobiology B: Biology**, v. 178, p. 175-181, 2018.

RODRIGUEZ-CONCEPCION, M.; AVALOS, J.; BONET, M.L.; BORONAT, A.; GOMEZ-GOMEZ, L.; HORNERO-MENDEZ, D.; LIMON, M.C.; MELÉNDEZ-MARTÍNEZ, A.J.; OLMEDILLA-ALONSO, B.; PALOU, A.; et al. A global perspective oncarotenoids: Metabolism, biotechnology, and benefits for nutrition and health. **Progress in lipid research** s. v.70, p. 62–93, 2018.

SHAHIDI F, ZHONG Y. Lipid oxidation and improving the oxidative stability. **Chemical Society Reviews**, v. 39, n. 11, p. 4067-4079, 2010.

SHARIFIFAR F.; DEHGHN-NUDEH G.; MIRTAJALDINI, M. Major flavonoids with antioxidant activity from *Teucrium polium* L. **Food Chemistry**, v. 112, n. 4, p. 885-888, 2009.

SEIFRIED, H. E.; ANDERSON, D. E.; FISCHER, E. I.; MILNER, J.A review of the interaction among dietary antioxidants and reactive oxygen species. **The Journal of nutritional Biochemistry,** v. 18, n. 9, p. 567-579, 2007.

SILVA, E. M., SOUZA, J. N. S., ROGEZ, H., REES, J. F., & LARONDELLE, Y. Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. **Food Chemistry**, v. 101, n. 3, p. 1012-1018, 2007.

SIRAICHI, J. T. G.; FELIPE, D. F.; BRAMBILLA, L. Z. S.; GATTO, M. J.; TERRA, V. A.; CECCHINI, A. L.; CORTEZ, L. E. R.; RODRIGUES-FILHO, E.C.; DIÓGENES, Antioxidant capacity of the leaf extract obtained from *Arrabidaea chica* cultivated in Southern Brazil. **PLoS One**, v. 8, n. 8, p. e72733, 2013.

SZAJDEK, A.; BOROWSKA, E. J. Bioactive compounds and health-promoting properties of berry fruits: a review. **Plant Foods for Human Nutrition**, v. 63, n. 4, p. 147-156, 2008.

TAKEMURA, O. S.; IINUMA, M.; TOSA, H.; MIGUEL, O. G.; MOREIRA, E.; NOZAWA, Y. A flavone from leaves of *Arrabidaea chica* f. cuprea. **Phytochemistry**, v. 38, n. 5, p. 1299-1300, 1995.

TOMÁS-BARBERAN, F.; ESPÍN, J. C. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. **Journal of Science and Food Agriculture**, 81, 853-876, 2001.

WILLETT, W. C. Balancing life-style and genomics research for disease prevention. **Science**, v. 296, n. 5568, p. 695-698, 2002.

ZORN, B.; GARCI´A-PINERES, A. J.; CASTRO, V.; MURILLO, R.; MORA, G. MERFORT, I. ZORN, Bettina et al. 3-Desoxyanthocyanidins from *Arrabidaea chica*. **Phytochemistry**, v. 56, n. 8, p. 831-835, 2001.

# OBJETIVOS

## OBJETIVO GERAL

Determinar o perfil de compostos bioativos presentes nas folhas da *A. chica* da Amazônia e o potencial antioxidante *in vitro* de extratos da folha frente as principais ROS e RNS de importância fisiológica e alimentícia.

## OBJETIVOS ESPECÍFICOS

- Determinar as características físico-químicas das folhas da *A. chica;*
- Identificar e quantificar o perfil de compostos fenólicos, carotenoides e ácido ascórbico das folhas de *A. chica*;
- Avaliar a capacidade de diferentes extratos de *A. chica* na desativação *in vitro* das ROS e RNS de relevância fisiológica e alimentícia.

# CAPÍTULO I

## REVISÃO DE LITERATURA

# REVISÃO DE LITERATURA

## 1 *ARRABIDAEA CHICA* (HUMB. & BONPL.) VERLOT

A espécie *A. chica* (Humb. & Bonpl.) Verlot é uma planta do tipo arbórea (Figura 1), membro da família Bignoniaceae, que abrange 120 gêneros e 800 espécies, de ocorrência na América Tropical, desde o sul do México até o Brasil central (DEVIA et al., 2002). As plantas dessa família, no Brasil, são encontradas na extensão da Floresta Amazônica, Cerrado e Mata Atlântica. *A.chica* é conhecida, popularmente, como crajiru (no Amazonas), pariri (no Pará), além de carajiru, carajuru, coapiranga, puca-panga ou chica (VON-POSER et al., 2000).

**Figura 1** – *Arrabidaea chica* cultivada no campo experimental da EMBRAPA Amazônia Oriental (Belém, PA, Brasil).

Fonte: Autora, 2017

No Nordeste do Brasil, as folhas da *A. chica* produzem um pigmento vermelho escuro, quando submetidas à fermentação e são usadas pelos índios como tintura para pintar o corpo em rituais, para proteger a pele dos raios solares e repelir insetos (ZORN et al*,* 2001). Há ainda relatos da ação anti-inflamatória (OLIVEIRA et al*.,* 2009*),* e antibacteriana, antiparasitária, antifúngica e antitumoral (BARBOSA et al., 2008; DA ROCHA et al., 2011; HOFLING et al., 2010). Possuem ainda, propriedades terapêuticas para doenças da pele como psoríase e úlceras (TAKEMURA et al., 1995*).*

Outros estudos reportam, que o extrato hidroalcóolico de *A. chica* promoveu a inibição do crescimento de *Heliobacter pylori* e *Enterococcus faecalis (*MAFIOLETI et al., 2013). Além disso, o extrato bruto das folhas de *A. chica* estimula a síntese de colágeno *in vivo* e *in vitro e* induz a proliferação de fibroblastos *in vitro*, além de exibir capacidade antioxidante e ação antiulcerogênica, reduzindo a área lesionada (JORGE et al., 2008*).*

## 2. COMPOSTOS BIOATIVOS

Compostos bioativos são substâncias químicas sintetizadas de forma natural pelos diferentes organismos vivos, os quais produzem efeito biológico sobre outros organismos vivos. Esta atividade biológica pode ter efeito terapêutico para combater doenças e/ou produzir efeitos tóxicos em humanos e animais, podem produzir também como mecanismos de defesa para se proteger contra o meio ambiente, os chamados metabólitos secundários (COLEGATE; MOLYNEAUX, 2007). Os compostos bioativos de origem vegetal estão envolvidos em processos metabólicos das plantas e também na interação delas com o meio ambiente, as quais incluem: atração de insetos polinizadores ou dispersadores de sementes, proteção contra insetos ou animais herbívoros, proteção contra ação microbiana e proteção contra a radiação ultravioleta do sol (DENNY; BUTTRISS, 2007).

Existe grande interesse na adição de compostos bioativos, provenientes de fontes naturais (frutas e vegetais), em formulações que permitam oferecer benefícios à saúde associada e a baixa incidência de desenvolvimento de diversas desordens relacionadas ao estresse oxidativo, tais como cânceres, inflamações, doenças cardiovasculares, catarata, degeneração macular e outras (EISENHAUER et al. 2017; SAK, 2014; TAUCHEN et al., 2016)

Entre os compostos bioativos destacam-se os carotenoides, o ácido ascórbico e os compostos fenólicos, amplamente distribuídos no reino vegetal.

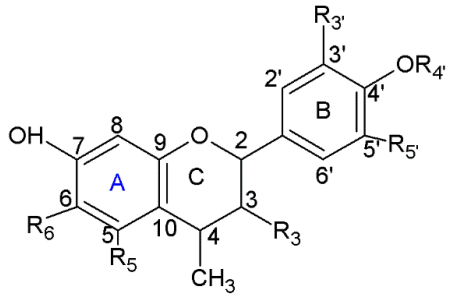
### 2.1 COMPOSTOS FENÓLICOS

Os compostos fenólicos são os antioxidantes mais expressivos oriundos da alimentação humana, e são quimicamente definidos como substâncias que possuem anel aromático com um ou mais grupos hidroxilas, incluindo seus grupos funcionais (IGNAT et al., 2011; SHAHIDI; NACZK, 2003). Estas substâncias podem também apresentar outros grupos substituintes em sua estrutura, como ésteres, metil-ésteres e glicosídios (HAN et al., 2007). Em relação a sua estrutura química, podem ser classificados como: não-flavonoides (estilbenos, ácidos fenólicos e álcoois fenólicos) e flavonoides (flavonóis, flavonas, flavanonas, catequinas, isoflavonoides

e antocianidinas) (BURNS et al, 2001; LEE et al, 2013). Dentre essas, a classe mais abundante é a dos flavonoides, que está presente em frutas, folhas, flores e sementes. Os flavonoides são responsáveis pelas cores amarelo, tons de azul e vermelho desses vegetais (ÂNGELO; JORGE, 2007; ROBARDS; ANTOLOVICH, 1997). Os flavonoides podem ser divididos em 6 subclasses em função do tipo de anel heterocíclico envolvido: flavonóis, flavonas, isoflavonas, flavanonas, antocianidinas e flavanóis (catequinas e proantocianidinas) (MANACH et al., 2004).

Os flavonoides possuem uma estrutura química caracterizada por dois anéis aromáticos (A e B), ligados por uma cadeia de três átomos de carbono, formando um heterociclo oxigenado (anel C) (Figura 2). O padrão de substituição e o grau de oxidação do anel C classificam os flavonoides e, dentro destas classes, cada composto é especificamente definido pelo padrão de substituição nos anéis A e B (MANACH et al., 2004). Estas substituições incluem oxigenação, alquilação, acilação, glicosilação e sulfatação (HOLLMAN; KATAN, 1999).

**Figura 2** – Estrutura geral dos flavonoides.

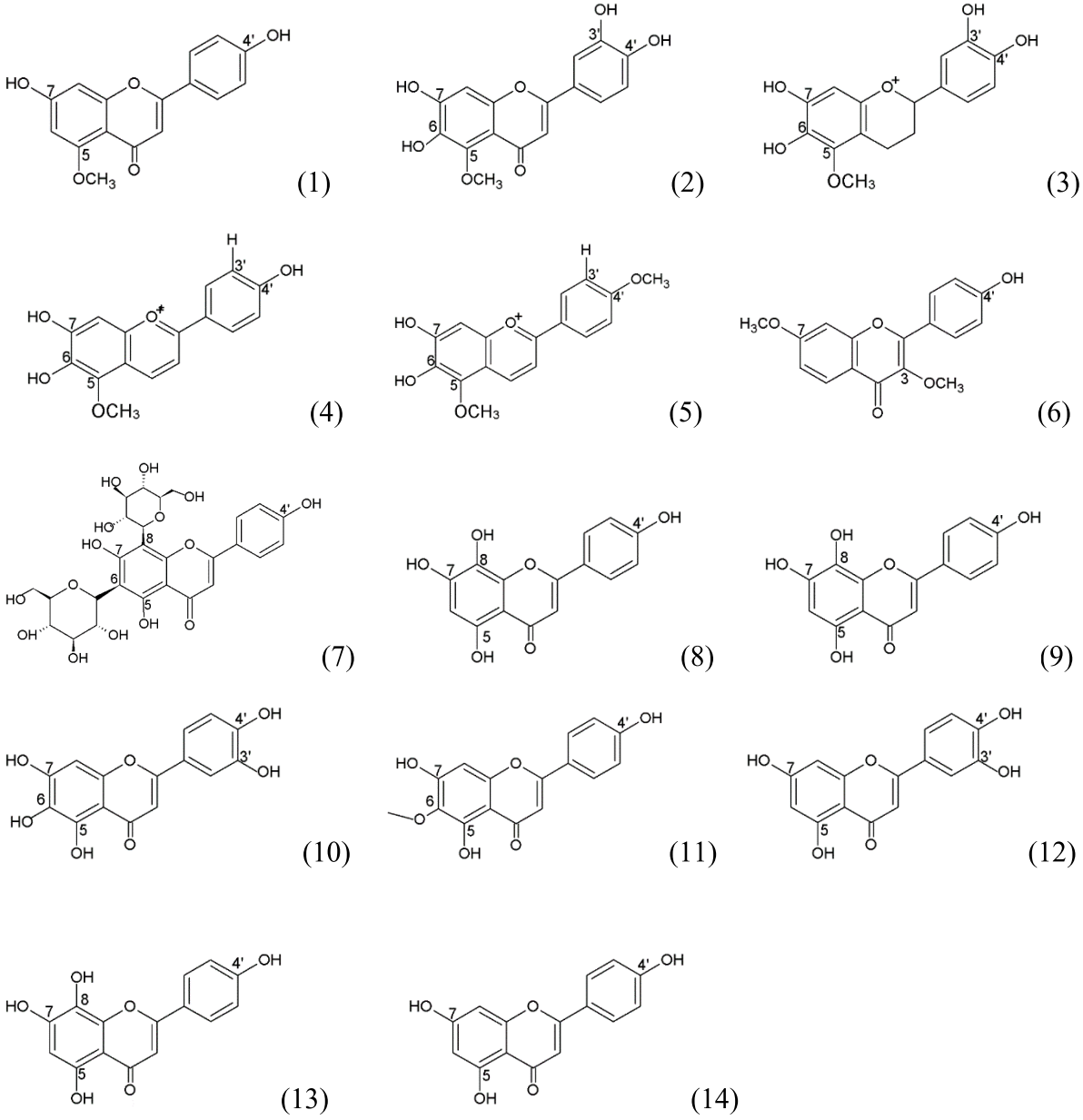



Fonte: MANACH et al., 2004

O primeiro estudo sobre compostos bioativos das folhas de *A. chica,* reporta o isolamento da 3-deoxiantocianidina, a carajurina (CHAPMAN et al., 1927), e foi proposto que a ocorrência deste raro pigmento em Bignoniaceae era provavelmente restrita a *A. chica* (SCOGIN 1980). Foi relatado ainda o isolamento de outras flavonas (Figura 3): 7,4'-di-hidroxi-5-metoxiflavona (1) e 6,7,3',4'-tetrahidroxi-5-metoxiflavona (carajuruflavona) (2) (TAKEMURA et al.,1995); e novas estruturas de agliconas foram estabelecidas principalmente com base nos dados de espectrometria de massa (MS) e Ressonância Magnética Nuclear (RMN), destacando-se as 3-desoxiantocianidinas: 6,7,3',4'-tetrahidoxi-5-metoxi-flavilium (3); 6,7,4'-trihidroxi-5-dimetoxiflavilium (carajurona) (4), juntamente com a já descrita 6,7-dihidroxi-5,4'-dimetoxiflavilium (carajurina) (5) (DEVIA et al*.,* 2002; ZORN et al., 2001). Adicionalmente, foi reportado o isolamento de três flavonoides 4'-hidroxi-3,7-dimetoxiflavona

(6); vicenina-2 (7) e kaempferol (8) (Barbosa et al., 2008), além da identificação da isoescutelareina (9), 6-hidroxiluteolina (10), hispidulina (11), luteolina (12), escutelareina (13) e apigenina (14), sendo os dois últimos os dois flavonóides majoritários (SIRAICH et al., 2013).

**Figura 3 -** Estruturas químicas dos flavonoides isolados das folhas de *A. chica*: 7,4'-di-hidroxi-5-metoxiflavona (1); 6,7,3',4'-tetrahidroxi-5-metoxiflavona (carajuruflavona) (2); 6,7,3',4'- tetrahidoxi-5-metoxi-flavilium (3); 6,7,4'-trihidroxi-5-dimetoxiflavilium (4); 6,7-di-hidroxi-5,4'-dimetoxiflavilium (carajurina) (5); 4'-hidroxi-3,7-dimetoxiflavona (6); vicenina-2 (7); kaempferol (8); isoescutelareina (9), 6-hidroxiluteolina (10), hispidulina (11), luteolina (12), escutelareina (13) e apigenina (14).




Com relação ao teor de compostos fenólicos, os extratos hidroalcoólicos (70:30) das folhas da *A. chica* apresentaram cerca de 41 mg /g extrato (PAULA et al., 2014).

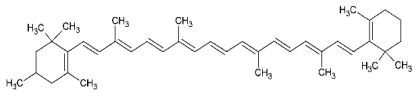
## 2.2 CAROTENOIDES

Os carotenoides são pigmentos naturais, lipossolúveis, amplamente distribuídos na natureza e sintetizados em plantas, algas, microorganismos (bactérias e fungos) e alguns artrópodes, como os insetos pertencentes a ordem Hemiptera (adelgídeos, pulgões e filoxerídeos) (RODRIGUEZ-CONCEPCION, 2018).

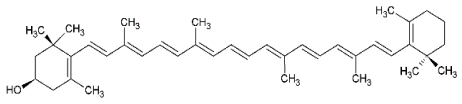
Os carotenoides pertencem à classe dos tetraterpenos ($C_{40}$), constituído por oito unidades isoprenoides. Podem ser acíclicos ou ciclizados em uma ou ambas as extremidades (Figura 4). Apresentam uma série de ligações duplas conjugadas, conferindo-lhes elevada reatividade e capacidade de absorver radiação eletromagnética na região do visível (360 -780 nm) (IGREJA, 2021; MERCADANTE, 2007). Em sua maioria, conferem a coloração amarela, laranja ou vermelho, no entanto, também existem carotenoides, como fitoeno e fitoflueno, que são incolores (IGREJA, 2021; RODRIGUEZ-CONCEPCION, 2018).

Mais de 750 carotenoides foram relatados, e são divididos em duas classes: carotenos, que possuem apenas átomos de carbono e hidrogênio na molécula, como por exemplo o β-caroteno, o α-caroteno e o licopeno; e as xantofilas, que são os derivados oxigenados dos carotenos, contendo pelo menos um átomo de oxigênio na estrutura, como por exemplo a β-criptoxantina, a luteína e a zeaxantina (IGREJA, 2021; TAPIERO et al., 2004).

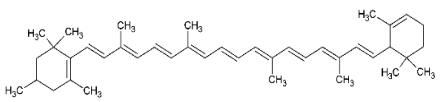
**Figura 4** – Estrutura de alguns carotenoides.



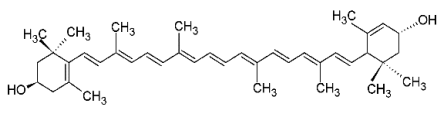
β-caroteno



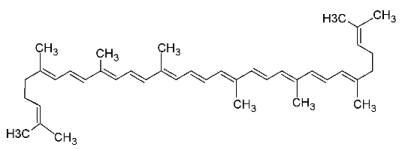
β-criptoxantina



α-caroteno



Luteina



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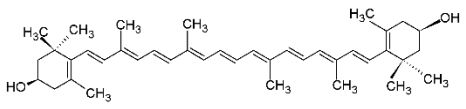

zeaxantina

Além do papel dos carotenoides como corantes naturais, têm sido reconhecido seu valor no contexto de alimentos funcionais, nutracêuticos e produtos afins, uma vez que alguns deles, incluindo β-caroteno e α-caroteno, apresentam atividade provitamina A, e há evidências de que podem contribuir no fortalecimento do sistema imunológico, e na diminuição do risco de desenvolver várias doenças degenerativas e cardiovasculares, catarata e envelhecimento (MELÉNDEZ-MARTÍNEZ; MAPELLI-BRAH; STINCO, 2018; RAO; RAO, 2007; RODRIGUEZ-AMAYA et al., 2008).

Além disso, esses compostos bioativos possuem propriedades antioxidantes, com reconhecida capacidade de desativação de espécies reativas de oxigênio (ROS) e de nitrogênio (RNS) (CHISTÉ et. al., 2011).

Estudos com vegetais folhosos demonstraram a identificação predominante de alguns carotenoides como a luteína, o β-caroteno, o α-caroteno e a zeaxantina (POP et al., 2014; RAJU, et al., 2007; ZEB; KHADIM; Ali, 2017). No entanto, em relação às folhas de *A. chica*, a composição de carotenoides não havia sido reportada até o desenvolvimento deste trabalho.

## 3. ESPÉCIES REATIVAS DE OXIGÊNIO (ROS) E DE NITROGÊNIO (RNS)

No organismo, as ROS e RNS desempenham um papel essencial na manutenção da condição fisiológica do corpo (BARREIROS et al., 2006; GOMES et al., 2006). Em contrapartida, a produção e o acúmulo excessiva de ROS e RNS, em decorrência de desordens fisiológicas, e a deficiência de antioxidantes endógenos e exógenos no corpo humano para inibir o efeito deletério dessas espécies induz ao estresse oxidativo, capaz de danificar componentes básicos para a função e sobrevivência das células (VIGNINI, 2011).

O distúrbio metabólico, causado pelo excesso de ROS, pode ser um importante mediador de danos potenciais às estruturas celulares, incluindo proteínas, lipídeos e DNA e os consequentes distúrbios biológicos e desenvolvimento de doenças crônicas ou degenerativas, como doenças cardiovasculares, oncológicas, endócrinas e diabetes *mellitus* (BUONOCORE; PERRONE; TATARANNO, 2010; BURTON; JAUNIAUX, 2011; HALLIWELL; GUTTERIDGE, 2015). Além disso, há a formação de macromoléculas nitradas, causadas pelas RNS, através da adição de um grupo nitro ($-NO_2$). Ocasionando modificação em alvos biológicos, como ácidos graxos poli-insaturados, resíduos de tirosina e triptofano em proteínas, bases do DNA e açúcares (MAZZULLI et al., 2006; VALKO et al., 2007).

Além da geração natural de ROS em sistemas fisiológicos, a formação de ROS também pode ocorrer em sistemas alimentícios, que por sua natureza biológica são altamente suscetíveis

à deterioração da qualidade, principalmente sob a condição de estresse oxidativo (BREWER, 2011). As ROS podem ser geradas durante o armazenamento e o processamento de alimentos, e são as principais responsáveis pela iniciação de reações de oxidação dos nutrientes, podendo reagir com lipídios, proteínas, açúcares e vitaminas, produzindo compostos voláteis indesejáveis com a degradação de ácidos graxos essenciais, aminoácidos e vitaminas, promovendo a geração de compostos potencialmente carcinogênicos comprometendo a segurança alimentar (CHOE; MIN, 2006). As ROS mais atuantes em sistemas alimentícios são os radicais hidroxila ($HO^{\bullet}$), peroxila ($ROO^{\bullet}$) e o oxigênio singleto ($^1O_2$) (CHOE; MIN, 2006).

O termo ROS, derivadas do oxigênio molecular com atividade redox e maior reatividade, inclui dois grupos, as radicalares: $O_2^{\bullet-}$, hidroperoxila ($HO_2^{\bullet}$), $ROO^{\bullet}$, $HO^{\bullet}$, e alcoxila ($RO^{\bullet}$); e algumas formas não-radicalares: $H_2O_2$, $^1O_2$ e o ácido hipocloroso (HOCl) (GOMES et al., 2005; HALLIWELL, 2012;). As espécies reativas de nitrogénio (RNS) são derivadas do óxido nitrico ($^{\bullet}NO$) e de seus metabólitos, incluindo não somente os radicais livres: $^{\bullet}NO$ e dióxido de nitrogênio ($^{\bullet}NO_2$), mas também espécies não-radicalares: ânion peroxinitrito ($ONOO^-$), ácido peroxinitroso (ONOOH) e o trióxido de dinitrogênio ($N_2O_3$) (GOMES et al., 2006).

O $O_2^{\bullet-}$ pode ser formado enzimaticamente e quimicamente a partir de oxigênio tripleto (oxigênio molecular, $^3O_2$). Essa espécie reativa é muito importante na redução do oxigênio para gerar outras ROS, como o $H_2O_2$, $HO^{\bullet}$ e o $^1O_2$. A dismutação do $O_2^{\bullet-}$, que pode ser catalisada pela enzima superóxido dismutase (SOD), produz uma molécula de $H_2O_2$, que é um oxidante menos reativo do que o $O_2^{\bullet-}$ (CHOE; MIN, 2006; PASTOR et al., 2004)

O $HO^{\bullet}$ é uma ROS altamente reativa capaz de oxidar qualquer molécula biológica vizinha ao seu sítio de formação. Pode ser formado por radiólise da água ou por decomposição de $H_2O_2$ por UV, catalases ou peroxidases (SYMONS; GUTTERIDGE, 1998). A reação do $O_2^{\bullet-}$ e $H_2O_2$ podem produzir $HO^{\bullet}$ por meio da reação de Haber-Weiss catalisada por ferro (Equação 1), na qual a Reação de Fenton também faz parte (CHOE; MIN, 2006; VALKO et al., 2007).

$$O_2^{\bullet-} + Fe^{3+} \longrightarrow O_2 + Fe^{2+} \qquad \text{(Conjunto de Equações 1)}$$

$$H_2O_2 + Fe^{2+} \xrightarrow{\text{reação de Fenton}} Fe^{3+} + OH^- + HO^{\bullet}$$

O $^1O_2$, outra espécie de oxigênio altamente reativa, que pode ser gerada tanto em sistemas fisiológicos quanto alimentares, é mais frequentemente produzida por reações de foto-sensibilização, onde fotossensibilizadores, como a clorofila e a riboflavina, absorvem a energia da luz e transfere-a para oxigênio tripleto para formar $^1O_2$. Duas moléculas de $ROO^{\bullet}$ reagem

entre si formando um intermediário tetraóxido que se decompõe formando $^1O_2$ pelo mecanismo de Russell (Equação 2) (HALLIWELL; GUTTERIDGE, 2007; MIYAMOTO et al., 2014).

$$2R\text{-}CHOO^{\bullet}\text{-}R' \xrightarrow{\text{Mecanismo Russell}} R\text{-}CHOH\text{-}R' + R\text{-}CO\text{-}R'^{-} + {}^1O_2 \qquad \text{(Equação 2)}$$

Em relação aos mecanismos de reação das ROS com componentes alimentares, o $^1O_2$ tem alta energia e é um composto eletrofílico, podendo ser gerado em resposta à mudança de temperatura, exposição à luz UV, redução da energia de ativação (presença de metais de transição) e danos físicos aos tecidos das matérias-primas que compõem os alimentos. O $^1O_2$ exibe uma alta reatividade com biomoléculas que possuem componentes insaturados, como os aminoácidos aromáticos e ácidos graxos poliinsaturados (CHOE; MIN, 2006; BREWER, 2011).

O $H_2O_2$ pode implicar indiretamente na oxidação dos lipídeos, pois ele é o precursor para a geração de $HO^{\bullet}$, que são fortes iniciadores da oxidação lipídica. O $HO^{\bullet}$ é uma molécula eletrofílica e possui elevado potencial de redução, podendo reagir com qualquer componente orgânico presente nos alimentos (CHOE; MIN, 2006).

O $HO^{\bullet}$ pode ser formado ainda por processos oxidativos catalisados por íons de metais de transição bivalentes, em particular ferro e cobre Matrizes alimentícias, como a carne vermelha, contendo quantidades significativas desses metais de transição podem estar suscetíveis a reações de oxidação (BREWER, 2011).

Nesse contexto, os esforços para inibir ou retardar as reações de oxidação em sistemas alimentícios e biológicos têm aumentado e, a presença de antioxidantes naturais é vista como uma estratégia promissora na inibição dessas reações, ao mesmo tempo que atendam a crescente exigência por parte dos consumidores pela incorporação de aditivos naturais em substituição aos aditivos alimentícios sintéticos.

## 4. ANTIOXIDANTES E MECANISMOS DE AÇÃO

Os antioxidantes são substâncias que, quando se encontram em baixas concentrações comparadas com as do substrato oxidável, previnem ou retardam, de maneira eficaz, um dano oxidativo desse substrato (HALLIWELL; GUTERIDGE, 1999) e podem atuar como inibidores de danos oxidativos em biomoléculas (HALLIWELL; GUTTERIDGE, 2007).

Em alimentos, os antioxidantes são aditivos adicionados para minimizar as alterações no sabor, aroma, cor ou valor nutritivo, quando resultantes de reações oxidativas. Os

antioxidantes sintéticos, como butil hidroxianisol (BHA), butil hidroxitolueno (BHT), galato de propila (PG) e terc-butil hidroquinona (TBHQ), são amplamente utilizados na indústria alimentícia, devido a sua elevada estabilidade, disponibilidade e ao baixo custo. No entanto, Estudos apontam que elevadas dosagens desses antioxidantes podem causar toxicidade em certos tecidos (HAM, et al. 2020 a; 2020 b) e danos ao DNA (BARAN et al., 2021; ESKANDANI et al., 2014; VANDGHANOONI, et al., 2013).

Com base nisso, as pesquisas têm valorizado o desenvolvimento e aplicação de substâncias antioxidantes provenientes de fontes naturais, como os compostos fenólicos, carotenoides, ácido ascórbico (vitamina C) e tocoferóis (vitamina E). Ao longo dos anos, a ação antioxidante destes compostos tem sido evidenciada por grande número de ensaios químicos *in vitro* (BENZIE, 2003; BERTO et al. 2015; LIU et al., 2018).

Os antioxidantes podem apresentar diferentes propriedades protetoras em diferentes estágios do processo de oxidação e atuar por diferentes mecanismos e são classificados em duas categorias principais: primários, que podem inibir ou retardar a oxidação por inativação de espécies reativas, interrompendo a cadeia de reações através de doação de elétrons ou átomos de hidrogênio, transformando-os em substâncias estáveis; e secundários, que apresentam uma grande variedade de mecanismos de ação, incluindo a quelação de íons metálicos (alteração de valência), sequestro de oxigênio nos estados tripleto e singleto, e a absorção de radiação ultravioleta (GORDON, 2001; MAISUTHISAKUL; SUTTAJIT; PONGSAWATMANIT, 2007; SILVA et al., 2010). No entanto, alguns antioxidantes apresentam mais de um mecanismo de ação e são descritos, na maioria das vezes, como antioxidantes de múltipla função (REISCHE; LILLARD; EITENMILLER, 2008).

Com relação aos mecanismos de ação dos antioxidantes para desativar os radicais livres, há dois principais: por transferência de um átomo de hidrogênio (*Hydrogen Atom Transfer*, HAT) (Equação 3) e por transferência de elétrons SET (*Single Electron Transfer*, SET) (Equação 4), para reduzir qualquer composto, incluindo metais, carbonilas e espécies radicalares. O conhecimento destas duas reações é muito importante para a compreensão e escolha dos métodos utilizados para avaliar a capacidade antioxidante de compostos bioativos (LEOPOLDINI, et al., 2004; LEOPOLDINI; RUSSO; TOSCANO, 2011; PRIOR et al., 2005).

$$R^{\bullet} + ArOH \rightarrow RH + ArO^{\bullet} \qquad \text{(Equação 3)}$$

onde o antioxidante ArOH reage como o radical livre $R^{\bullet}$, formando um novo radical mais estável que o inicial.

$R^{\bullet} + ArOH \rightarrow R^{-} + ArOH^{\bullet+}$ (Conjunto de equações 4)

$ArOH^{\bullet+} + H_2O \leftrightarrow ArO^{\bullet} + H_3O^{+}$

$R^{-} + H_3O^{+} \rightarrow RH + H_2O$

$M(III) + ArOH \rightarrow ArOH^{+} + M(II)$

Onde ArOH é o antioxidante e M(III) é um metal com estado de oxidação $3^{+}$. Na reação SET, ao contrário da reação HAT, o antioxidante ArOH doa um elétron ao radical li1vre $R^{\bullet}$, reduzindo o composto, incluindo metais, carbonilas e radicais. Também neste caso, o cátion radical resultante da transferência de elétron é mais estável.

A exemplo de alguns componentes presentes naturalmente nos alimentos, os carotenoides e os compostos fenólicos atuam como antioxidantes primários e podem reagir diretamente com os radicais livres dando lugar a um novo radical menos reativo que o radical livre inicial (GORINSTEIN et al., 2000; REISCHE, LILLARD; EITENMILLER, 2008), ou podem agir como antioxidantes secundários regenerando ou potencializando outros sistemas antioxidantes, como certas enzimas (GORINSTEIN et al., 2000).

O extenso sistema de ligações duplas conjugadas na molécula dos carotenoides confere elevada densidade eletrônica e é responsável pelo seu potencial antioxidante. Os carotenoides podem capturar o $ROO^{\bullet}$ tanto pelo mecanismo de doação de átomos de hidrogênio quanto pela transferência de elétrons, que leva à formação de uma variedade de radicais de carotenoides e, portanto, a múltiplos produtos finais (CHOE; MIN, 2006). Além disso, apresenta eficiência reconhecida na desativação do $^{1}O_2$, sendo que esta ação antioxidante aumenta com o aumento do número de ligações duplas conjugadas presente na estrutura dos carotenoides (RIOS et al., 2007; STAHL; SIES, 2005). Em sistemas biológicos, os carotenoides são capazes de desativar o $^{1}O_2$, que retorna ao estado fundamental ($^{3}O_2$). O carotenoide no estado excitado resultante, libera energia na forma de calor (*quenching* físico), retornando ao estado fundamental para efetuar a desativação de outras moléculas de $^{1}O_2$, ou forma produtos de oxidação de carotenoides (*quenching* químico), sendo o processo físico mais eficiente do que o químico (KIOKIAS; GORDON, 2004; STAHL; SIES, 2005). Além disso, os carotenoides podem ainda apresentar efeitos sinergísticos com outros antioxidantes como a vitamina E ou C (STAHL; SIES, 2005).

Quanto à atividade antioxidante dos compostos fenólicos, em particular aos flavonoides, está atribuída à capacidade de desativação dos radicais livres através da doação de átomos de hidrogênio ou elétrons, estabilização do radical flavanoil formado, capacidade de quelar íons metálicos, ou inibição de enzimas oxidativas; onde a estrutura química destes compostos é o

fator determinante para estas atividades (LEOPOLDINI, RUSSO, TOSCANO, 2011; MAQSOOD; BENJAKUL, 2010; PAIXÃO et al.,2007; PIETTA, 2000). Os grupos OH presente na molécula dos compostos fenólicos são capazes de doar hidrogênio ou elétrons aos $HO^{\bullet}$, $ROO^{\bullet}$ e ONOO-, estabilizando-os e transformando o flavonoide, por exemplo, em uma molécula radical relativamente estável (HEIM et al., 2002).

O potencial de eliminação de radicais livres dos compostos fenólicos depende do número e das posições dos grupos OH e de outros substituintes. Por exemplo, nos flavonoides, os múltiplos grupos OH apresentam maior potencial antioxidante do que compostos contendo um único grupo (GELDOF; ENGESETH, 2002; KUMAR et al., 2015). Além disso, a presença de glicosilações na molécula tendem a diminuir a capacidade antioxidante de alguns compostos quando comparados com as suas formas agliconas correspondentes (CAI et al., 2006; SAMRA et al., 2011).

Alguns estudos na literatura comprovaram a capacidade antioxidante de compostos fenólicos em relação à desativação das ROS e RNS (BARIZÃO et al, 2016; GOMES et al., 2007). Com relação ao potencial antioxidante da *A. chica*, frente a ROS e RNS de importância fisiológica e alimentar foi determinado somente os valores de ORAC (*Oxygen Radical Absorbance Capacity*), relativos à desativação do $ROO^{\bullet}$. Estudo avaliando a capacidade antioxidante no extrato misto das folhas da *A. chica* (metanol, água, etanol, água destilada e ácido clorídrico) reportaram o valor de ORAC de 261,4 µmol equivalentes de trolox (ET)/g peso fresco (SILVA et al., 2007). Este valor foi inferior ao das folhas de outra planta utilizada medicina popular, *Byrsonima crassifolia* (muruci) (778.8 µmol ET/g peso fresco), reportada no mesmo estudo, e que segundo o autor, pode ser considerada fonte promissora de compostos antioxidantes.

Para o radical DPPH (2,2-difenil-1-picrilhidrazil), que é um radical estável e não biológico, Jorge et al. (2008) observaram que o extrato metanólico acidificado (0,3% de ácido cítrico) das folhas da *A chica* foi capaz de desativar os radicais livres de DPPH com valor de $IC_{50}$ de 15,98 mg/mL.

Em um outro estudo avaliando as propriedades antioxidantes das folhas da *A. chica*, pelos métodos DPPH, β-caroteno/ácido linoleico e potencial antioxidante reativo total, foi observado que entre o extrato bruto e compostos puros (escutelareína e apigenina), obtidos a partir do extrato de *A. chica*, o extrato bruto apresentou a maior atividade antioxidante; o valor $IC_{50}$ do extrato para o método de DPPH foi de 13,51 mg/mL e o teste de descoloração do β-caroteno mostrou que a adição do extrato em diferentes concentrações (200 e 500 µg/mL) impediu a perda de cor do β-caroteno em 51,2% e 94%, respectivamente (SIRAICH et al. 2013).

Por estes motivos, com o decorrer dos anos, tem sido dado destaque à pesquisa de novos antioxidantes de matrizes naturais (CASTRO-VARGAS; BAUMANN; PARADA-ALFONSO, 2016; SHAHIDI, ZHONG 2010; SOUZA et al., 2017). Nesse contexto, os antioxidantes da *A. chica* possuem elevado potencial, na aplicação contra os danos oxidativos em produtos provenientes da indústria alimentícia e farmacêutica.

## REFERÊNCIAS


ÂNGELO, P. M.; JORGE, N. Compostos fenólicos em alimentos-uma breve revisão. **Revista Instituto Adolfo Lutz (Impresso)**, v. 66, n. 1, p. 01-09, 2007.

BARAN, A.; YILDIRIM, S.; GHOSIGHAREHAGHAJI, A.; BOLAT, ˙I.; SULUKAN, E.; CEYHUN, S. An approach to evaluating the potential teratogenic and neurotoxic mechanism of BHA based on apoptosis induced by oxidative stress in zebrafish embryo (Danio rerio). **Hum. Exp. Toxicol**, 40, 425–438, 2021.

BARBOSA WLR, PINTO LN, QUIGNARD E, VIEIRA JMS, SILVA JUNIOR JOC, ALBUQUERQUE S. *Arrabidaea chica* (HBK) Verlot: phytochemical approach, antifungal and trypanocidal activities**. Revista Brasileira de Farmacognosia**, v. 18, n. 4, p. 544-548, 2008.

BARIZÃO, E. O; VISENTAINER, J. V.; ALMEIDA, V. DE C.; RIBEIRO, D.; CHISTÉ, R.C., FERNANDES, E. Citharexylum solanaceum fruit extracts: Profiles of phenolic compounds and carotenoids and their relation with ROS and RNS scavenging capacities. **Food Research International** v. 86, p. 24-33, 2016.

BARREIROS, A. L. B. S.; DAVID, J. M.; DAVID, J. P. Estresse oxidativo: relação entre geração de espécies reativas e defesa do organismo. **Química Nova**, v. 29, n. 1, p. 113, 2006.

BENZIE, I. F. F. Evolution of dietary antioxidants, Comparative Biochemistry and Physiology a – **Molecular & Integrative Physiology,** v. 136, n. 1, p. 113–126, 2003.

BERTO, A., RIBEIRO, A. B., DE SOUZA, N. E., FERNANDES, E., & CHISTÉ, R. C. Bioactive compounds and scavenging capacity of pulp, peel and seed extracts of the Amazonian fruit *Quararibea cordata* against ROS and RNS. **Food Research International**, v. 77, p. 236-243, 2015.

BREWER, M. S. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. **Comprehensive reviews in food science and food safety**, v. 10, n. 4, p. 221-247, 2011.

BUONOCORE, G.; PERRONE, S.; TATARANNO, M. L. Oxygen toxicity: Chemistry and biology of reactive oxygen species. **Seminars in Fetal and Neonatal Medicine**, v. 15, n. 4, p. 186–190, 2010.

BURNS, J.; GARDNER PT, MATTHEWS, D.; DUTHIE, G.G; LEAN, M.E; CROZIER; A. Extraction of phenolics and changes in antioxidant activity of red wines during vinification. **Journal of Agricultural and Food Chemistry**, v. 49, n. 12, p. 5797-5808, 2001.

BURTON, G. J.; JAUNIAUX, E. Oxidative stress. **Best Practice & Research Clinical obstetrics & Gynaecology**, v. 25, n. 3, p. 287-299, 2011.

CASTRO-VARGAS, H. I.; BAUMANN, W.; PARADA-ALFONSO, F. Valorization of agroindustrial wastes: Identification by LC-MS and NMR of benzylglucosinolate from papaya (*Carica papaya* L.) seeds, a protective agent against lipid oxidation in edible oils. **Electrophoresis**, v. 37, n. 13, p. 1930-1937, 2016.

CAI, Y. Z.; SUN, M.; XING, J.; LUO, Q.; CORKE, H. Struture-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. **Life Science**. v. 78, n. 25, p. 2872-2888, 2006.

CHAPMAN, L. M.; GREENBERG, D. M.; SCHMIDT, C. L.A. Studies on the nature of the combination between certain acid dyes and proteins. **Journal of Biological Chemistry**, v. 72, n. 2, p. 707-729, 1927.

CHISTÉ, R.C, MERCADANTE, A.Z, GOMES A, FERNANDES E, LIMA J.L.F.D, BRAGAGNOLO N. *In vitro* scavenging capacity of annatto seed extracts against reactive oxygen and nitrogen species. **Food Chemistry**, v. 127, n. 2, p. 419-426, 2011.

CHOE, E.; MIN, D. B. Chemistry and reactions of reactive oxygen species in foods. **Critical Reviews in Food Science and Nutrition**, v. 46, n. 1, p. 1-22, 2006.

COLEGATE, S. M.; MOLYNEUX, R. J. (Ed.). **Bioactive natural products: detection, isolation, and structural determination**. CRC press, 2007.

DA ROCHA, C. Q,; VILELA, F. C.; CAVALCANTE, G. P.; SANTA-CECÍLIA, F. V.; SANTOSE-SILVA, L.; dos SANTOS, M. H.; GIUSTI-PAIVA, A.; ROCHA, C. Q. Anti-inflammatory and antinociceptive effects of *Arrabidaea brachypoda* (DC.) Bureau roots. **Journal of Ethnopharmacology**, v. 133, n. 2, p. 396-401, 2011.

DELADINO, L.; ANBINDER, P.; NAVARRO, A.; MARTINO, M. Encapsulation of natural antioxidants extracted from Ilex paraguariensis. **Carbohydrate Polymers**, v. 71, n. 1, p. 126-134, 2008.

DENNY, A.; BUTTRISS, J. Plant foods and health: focus on plant bioactives. **Synthesis report**, v. 4, p.1-64, 2007.

DEVIA B, LLABRES G, WOUTERS J, DUPONT L, ESCRIBANO-BAILON, M. T.PASCUAL-TERESA, S. de; ANGENOT, L.; TITS, M. New 3-deoxyanthocyanidins from leaves of *Arrabidaea chica*. **Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques**, v. 13, n. 2, p. 114-120, 2002.

EISENHAUER, B.; NATOLI, S., LIEW, G.; FLOOD, V. Lutein and zeaxanthin—food sources, bioavailability and dietary variety in age-related macular degeneration protection. **Nutrients**, v. 9, n. 2, p. 120, 2017.

ESKANDANI, M.; HAMISHEHKAR, H.; EZZATI NAZHAD DOLATABADI, J. Cytotoxicity and DNA damage properties of tertbutylhydroquinone (TBHQ) food additive. **Food Chemistry**, 153, 315–320, 2014.

GELDOF N, ENGESETH NJ. Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. **Journal Agriculture and Food Chemistry** 50:3050–5 2002.

GOMES, A.; FERNANDES, E.; LIMA, J.L.F.C. Fluorescence probes used for detection of reactive oxygen species. **Journal of Biochemical and Biophysical Methods**, v. 65, n. 2-3, p. 45-80, 2005.

GOMES, A., FERNANDES, E., LIMA, J.L.F.C. Use of fluorescence probes for detection of reactive nitrogen species: A review. **Journal of Fluorescence**, v. 16, n.1, p. 119-139, 2006.

GOMES, A., FERNANDES, E., SILVA, A. M. S., SANTOS, C. M. M., PINTO, D. C. G. A., CAVALEIRO, J. A. S., et al. 2-*Styrylchromones*: Novel strong scavengers of reactive oxygen and nitrogen species. **Bioorganic and Medicinal Chemistry**, v. 15, n. 18, p. 6027-6036, 2007.

GORDON, M. H. The development of oxidative rancidity in foods. In: Antioxidants in food. **Woodhead Publishing**, p. 7-21.2001.

GORINSTEIN, S.; KULASEK, W.; BARTNIKOWSKA, E.; LEONTOWICZ, M.; ZEMSER, M. The effects of diets, supplemented with either whole persimmon or phenol-free persimmon, on rats fed cholesterol. **Food Chemistry**, v. 70, n. 3, p. 303-308, 2000.

HALLIWELL, B; GUTTERIDGE, J.M.C. Free radicals in biology and medicine. 5a ed. **Oxford university press**, USA, 2015.

HALLIWELL, Barry. Free radicals and antioxidants: updating a personal view. **Nutrition reviews**, v. 70, n. 5, p. 257-265, 2012.

HAM, J.; LIM, W.; YOU, S.; SONG, G. BUTYLATED hydroxyanisole induces testicular dysfunction in mouse testis cells by dysregulating calcium homeostasis and stimulating endoplasmic reticulum stress. **Science of The Total Environment**, 702, 134775, 2020.

HAM, J.; LIM,W.; WHANG, K.Y.; SONG, G. Butylated hydroxytoluene induces dysregulation of calcium homeostasis and endoplasmic reticulum stress resulting in mouse Leydig cell death. **Environmental Pollution**, 256, 113421, 2020.

HAN X.; SHEN T.; LOU H. Dietary polyphenols and their biological significance. **International Journal of Molecular Sciences**, v. 8, n. 9, p. 950-988, 2007.

HEIM, K. E.; TAGLIAFERRO, A. R.; BOBILYA, D. J. Flavonoid antioxidants: chemistry, metabolism and struture-activity relationships. **The Journal of Nutritional Biochemistry**, v. 13, n. 10, p. 572-584, 2002.

HÖFLING, J. F., ANIBAL, P. C., OBANDO-PEREDA, G. A., PEIXOTO, I. A. T., FURLETTI, V. F., FOGLIO, M. A., & GONÇALVES, R. B. Antimicrobial potential of some plant extracts against Candida species. Brazilian Journal of Biology, 70(4), 1065-1068, 2010.

HOLLMAN, P. C. H; KATAN, M. B. Dietary flavonoids: intake, health effects and bioavailabily. **Food Chemistry Toxicological**, v. 37, n. 9/10, p. 937-9342, 1999.

JORGE, M.P, MADJAROF, C, RUIZ A.L.G., FERNANDES, A.T, FERREIRA RODRIGUES RA, DE OLIVEIRA, S IM, et al. Evaluation of wound healing properties of *Arrabidaea chica* Verlot extract. **Journal of Ethnopharmacology**, v. 118, n. 3, p. 361-366, 2008.

IGNAT, I.; VOLF, I.; POPA, V. I. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. **Food chemistry**, v. 126, n. 4, p. 1821-1835, 2011.

IGREJA, W. S., MAIA, F. D. A., LOPES, A. S., & CHISTÉ, R. C. Biotechnological production of carotenoids using low cost-substrates is influenced by cultivation parameters: A review. **International Journal of Molecular Sciences**, 22(16), 8819, 2021.

KIOKIAS, S.; GORDON, M. H. Antioxidant Properties of Carotenoids *in vitro* and *in vivo*. **Food Reviews International**, v.20, n.2, p.99–121, 2004.

KUMAR, Y., YADAV, D. N., AHMAD, T., & NARSAIAH, K.. Recent Trends in the Use of Natural Antioxidants for Meat and Meat Products. Comprehensive Reviews in **Food Science and Food Safety**, v. 14, n 6, p 796-812. 2015. http://dx.doi.org/10.1111/1541-4337.12156.

LEE, J. H.; PARK, K. H.; LEE, M. H.; KIM, H. T.; SEO, W. D., KIM, J. Y.; ... & HA, T. J. Identification, characterisation, and quantification of phenolic compounds in the antioxidant activity-containing fraction from the seeds of Korean perilla (*Perilla frutescens*) cultivars. **Food chemistry**, v. 136, n. 2, p. 843-852, 2013.

LEOPOLDINI, M.; MARINO, T.; RUSSO,N.; TOSCANO, M.. Antioxidant properties of phenolic compounds: H-atom versus electron transfer mechanism. **The Journal of Physical Chemistry A**, v. 108, n. 22, p. 4916-4922, 2004.

LEOPOLDINI, M.; RUSSO,N.; TOSCANO, M.. The molecular basis of working mechanism of natural polyphenolic antioxidants. **Food Chemistry**, v. 125, n. 2, p. 288-306, 2011.

LIU, Q., LI, X.; OUYANG, X.; CHEN, D.. Dual effect of glucuronidation of a pyrogallol-type phytophenol antioxidant: a comparison between scutellarein and scutellarin. **Molecules**, v. 23, n. 12, p. 3225, 2018.

MAFIOLETI, L., DA SILVA JUNIOR, I. F.; COLODEL, E. M. , FLACH, A., MARTINS, D. T. DE O. Evaluation of the toxicity and antimicrobial activity of hydroethanolic extract of *Arrabidaea chica* (Humb. & Bonpl.) B. Verl. **Journal of ethnopharmacology**, v. 150, n. 2, p. 576-582, 2013.

MAISUTHISAKUL, P., SUTTAJIT, M., & PONGSAWATMANIT, R. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. **Food chemistry**, v. 100, n. 4, p. 1409-1418, 2007.

MANACH, C; SCALBERT, A; MORAND, C: RÉMÉSY, C: JIMÉNEZ, L. Polyphenols: food sources and bioavailability, **The American Journal of Clinical Nutrition**, v. 79, n. 5, p. 727-747, 2004.

MAQSOOD, S.; BENJAKUL, S. Comparative studies of four different phenolic compounds on *in vitro* antioxidative activity and the preventive effect on lipid oxidation of fish oil emulsion and fish mince. **Food Chemistry.** v. 119, n. 1, p. 123-132, 2010.

MARTÍNEZ, C A.; MOSQUERA, O M.; NIÑO, J. Apigenin glycoside: an antioxidant isolated from *Alchornea coelophylla* pax & k. *Hoffm.*(euphorbiaceae) leaf extract. **Universitas Scientiarum**, v. 21, n. 3, p. 247-257, 2016.

MAZZULLI, J. R., LIND, S., ISCHIROPOULOS, H. Reactive oxygen and nitrogen species: weapons of neuronal destruction. In: Luo, Y., Packer, L. **Oxidative stress and age-related neurodegeneration.** New York: Taylor & Francis Group, LLC, cap. 5, p. 107-119, 2006.

MELÉNDEZ-MARTÍNEZ, A. J., MAPELLI-BRAHM, P., & STINCO, C. M. The colourless carotenoids phytoene and phytofluene: From dietary sources to their usefulness for the functional foods and nutricosmetics industries. **Journal of Food Composition and Analysis**, 67, 91-103, 2018.

MERCADANTE, A. Z. Carotenoids in foods: sources and stability during processing and storage. **Food colorants: chemical and functional properties**, v. 213, 2007.

MIYAMOTO, S., MARTINEZ, G. R., MEDEIROS, M. H. G., & DI MASCIO, P. Singlet molecular oxygen generated by biological hydroperoxides. **Journal of Photochemistry and Photobiology B**: Biology, 139, 24–33, 2014.

OLIVEIRA, D.P.C, BORRÁS, M.R.L, FERREIRA, L.C.L, LÓPEZ-LOZANO, J.L. Anti-inflammatory activity of the aqueous extract of *Arrabidaea chica* (Humb. & Bonpl.) B. Verl. on the self-induced inflammatory process from venoms amazonians snakes. **Revista Brasileira de Farmacognosia**, v. 19, n. 2B, p. 643-649, 2009.

PAIXÃO, N.; PERESTRELO, R.; MARQUES, J.C.; CÂMARA, J.S. Relationship between antioxidante capacity and total phenolic contente of red, rose and White wines. **Food Chemistry**, v. 105, n. 1, p. 204-214, 2007.

PASTOR, I.; ESQUEMBRE, R.; MICOL, V.; MALLAVIA, R.; MATEO, C. R. A ready-to-use fluorimetric biosensor for superoxide radical using superoxide dismutase and peroxidase immobilized in sol–gel glasses. **Analytical biochemistry**, v. 334, n. 2, p. 335-343, 2004.

PAULA, J. T., PAVIANI, L. C., FOGLIO, M. A., SOUSA, I. M., DUARTE, G. H., JORGE, M. P., ... & CABRAL, F. A. Extraction of anthocyanins and luteolin from *Arrabidaea chica* by sequential extraction in fixed bed using supercritical $CO_2$, ethanol and water as solvents. The Journal of Supercritical Fluids, 86, 100-107, 2014.

PIETTA, P-G. Flavonoids as antioxidants. **Journal of Natural Products**, v. 63, n. 7, p. 1035-1042, 2000.

POP, M. R., WEESEPOEL, Y., SOCACIU, C., PINTEA, A., VINCKEN, J.; GRUPPEN, H. Carotenoid composition of berries and leaves from six Romanian sea buckthorn (*Hippophae rhamnoides L.*) varieties. **Food Chemistry**, v. 147, p. 1-9, 2014.

PRIOR, R.L.; WU, X.; SCHAICH, K. Standardized methods for the determination of antioxidant capacity and phenolic in foods and dietary supplements. **Journal of Agricultural and Food Chemistry**, v. 53, n. 10, p. 4290-4302, 2005.

SIRAICHI JTG, FELIPE DF, BRAMBILLA LZS, GATTO MJ, TERRA VA, CECCHINI AL, et al. Antioxidant capacity of the leaf extract obtained from *Arrabidaea chica* cultivated in Southern Brazil. **PLoS One**, v. 8, n. 8, p. e72733, 2013.

RAJU, M.; VARAKUMAR, S.; LAKSHMINARAYANA, R.; PARTHASARATHY, T.; BASKARAN, V. Carotenoid composition and vitamin A activity of medicinally important green leafy vegetables. **Food Chemistry**, v. 101, n. 4, p. 1598-1605, 2007.

RAO, A. V.; RAO, L. G. Carotenoids and human health. **Pharmacological Research**, v. 55, n. 3, p. 207-216, 2007.

REISCHER, D. W.; LILLARD, D. A.; EITENMILLER, R.R. Antioxidants. In: AKOH, C.C.; MIN, D.B. **Food lipids: chemistry, nutrition, and biotechnology**. 3ª Ed. Boca Raton, FL: CRC. P. 409-430, 2008.

RIOS, A. de O.; MERCADANTE, Adriana Z.; BORSARELLI, C. D. Triplet state energy of the carotenoid bixin determined by photoacoustic calorimetry. **Dyes and pigments**, v. 74, n. 3, p. 561-565, 2007.

ROBARDS, K.; ANTOLOVICH, M. Analytical Chemistry of Fruit Bioflavonoids A Review. **Analyst.** v. 122, n. 2, p. 11R-34R, 1997.

RODRIGUEZ-AMAYA, D. B. A. **A guide to carotenoid analysis in foods**. Washington: International Life Sciences Institute - ILSI press, 2001.

RODRIGUEZ-AMAYA, D. B.; KIMURA, M.; GODOY, H. T., AMAYA-FARFAN, J. Updated Brazilian database on food carotenoids: Factors affecting carotenoids composition, **Journal of Food Composition and Analysis**, v. 21, n. 6, p. 445-463, 2008.

RODRIGUEZ-CONCEPCION, M.; AVALOS, J.; BONET, M.L.; BORONAT, A.; GOMEZ-GOMEZ, L.; HORNERO-MENDEZ, D.; LIMON, M.C.; MELÉNDEZ-MARTÍNEZ, A.J.; OLMEDILLA-ALONSO, B.; PALOU, A.; et al. A global perspective on carotenoids: Metabolism, biotechnology, and benefits for nutrition and health. **Prog. Lipid** Res. 2018, 70, 62–93.

SAK, K. Cytotoxicity of dietary flavonoids on different human cancer types. **Pharmacognosy reviews**, v. 8, n. 16, p. 122, 2014.

SAMRA, M. A.; CHEDA, V. S.; ECONOMOU, A; CALOKERINOS, A.; KEFALAS, P.Antioxidant/prooxidant properties of model phenolic compounds: Part I. Studies on equimolar mixtures by chemiluminescence and cyclic voltammetry. **Food Chemistry**, v. 125, n. 2, p. 622-629, 2011.

SCOGIN, R. Anthocyanins of the Bignoniaceae. **Biochemical systematics and ecology**, v. 8, n. 3, p. 273-276, 1980.

SHAHIDI, F. Antioxidants in food and food antioxidants. **Food/nahrung**, v. 44, n. 3, p. 158-163, 2000.

SHAHIDI, F. E; NACZK, M. **Phenolics in Food and Nutraceuticals**. CRC Press, 2003.

SHAHIDI F, ZHONG Y. Lipid oxidation and improving the oxidative stability. **Chemical society reviews**, v. 39, n. 11, p. 4067-4079, 2010.

SILVA, E. M., SOUZA, J. N. S., ROGEZ, H., REES, J. F., & LARONDELLE, Y. Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. **Food Chemistry**, v. 101, n. 3, p. 1012-1018, 2007.

SILVA, M. L. C.; COSTA, R. S.; SANTANA, A. S.; KOBLITZ, M. G. B. Compostos fenólicos, carotenóides e actividade antioxidante em produtos vegetais. **Semina: Ciências Agrárias**, v. 31, n. 3, p. 669-681, 2010.

SIRAICHI, J. T. G.; FELIPE, D. F.; BRAMBILLA, L. Z. S.; GATTO, M. J.; TERRA, V. A.; CECCHINI, A. L.; CORTEZ, L. E. R.; RODRIGUES-FILHO, E.C.; DIÓGENES, Antioxidant capacity of the leaf extract obtained from *Arrabidaea chica* cultivated in Southern Brazil. **PLoS One**, v. 8, n. 8, p. e72733, 2013.

SOUZA, A. L., MARTÍNEZ, F. P., FERREIRA, S. B., & KAISER, C. R. A complete evaluation of thermal and oxidative stability of chia oil. **Journal of Thermal Analysis and Calorimetry**, v. 130, n. 3, p. 1307-1315, 2017.

STAHL, W.; SIES, H. Bioactivity and protective effects of natural carotenoids. **Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease**, v. 1740, n. 2, p. 101-107, 2005.

SYMONS, M.C.R., AND GUTTERIDGE, J.M.C. Free radicals and Iron: Chemistry**, biology, and medicine**. p. 102. Oxford University Press Inc., New York, 1998.

TAKEMURA, O. S.; IINUMA, M.; TOSA, H.; MIGUEL, O. G.; MOREIRA, E.; NOZAWA, Y. A flavone from leaves of *Arrabidaea chica* f. cuprea. **Phytochemistry**, v. 38, n. 5, p. 1299-1300, 1995.

TAPIERO, H.; TOWNSEND, D. M.; TEW, K. D. The role of carotenoids in the prevention of human pathologies. **Biomedicine & Pharmacotherapy**, v. 58, n. 2, p. 100-110, 2004.

TAUCHEN, J. T., J., BORTL, L., HUML, L., MIKSATKOVA, P., DOSKOCIL, I., MARSIK, P., ... & HAVLIK, J. Phenolic composition, antioxidant and anti-proliferative activities of edible and medicinal plants from the Peruvian Amazon. **Revista Brasileira de Farmacognosia**, v. 26, n. 6, p. 728-737, 2016.

VALKO M.; LEIBFRITZ, D.; MONCOL, J.; CRONIN, M. T. D.; MAZUR, M.; TELSER, J. Free radicals and antioxidants in normal physiological functions and human disease. **The International Journal of Biochemistry & Cell Biology.** v. 39, n. 1, p. 44-84, 2007.

VANDGHANOONI, S.; FOROUHARMEHR, A.; ESKANDANI, M.; BARZEGARI, A.; KAFIL, V.; KASHANIAN, S.; EZZATI NAZHAD DOLATABADI, J. Cytotoxicity and DNA fragmentation properties of butylated hydroxyanisole**. DNA Cell Biol,** 32, 98–103, 2013.

VIGNINI A Stroke and oxidative stress. In: **Oxidative Stress and Free Radical Damage in Neurology**. Humana Press, 2011. p. 137-152.

VON POSER, G. L.*;* SCHRIPSEMA, J.*;* HENRIQUES, A. T.; JENSEN,S. R. The distribution of iridoids in Bignoniaceae. **Biochemical Systematics and Ecology**, v. 28, n. 4, p. 351-366, 2000.

ZEB, A.; KHADIM, N.; ALI, W. Changes in the polyphenolic profile, carotenoids and antioxidant potential of apricot (*Prunus armeniaca* L.) leaves during maturation. **Agriculture**, v. 7, n. 2, p. 9, 2017.

ZORN, B.; GARCI´A-PINERES, A. J.; CASTRO, V.; MURILLO, R.; MORA, G. MERFORT, I. ZORN, Bettina et al. 3-Desoxyanthocyanidins from *Arrabidaea chica*. **Phytochemistry**, v. 56, n. 8, p. 831-835, 2001.

# CAPÍTULO II

**Profile of phenolic compounds and carotenoids of Arrabidaea chica leaves and the in vitro singlet oxygen quenching capacity of their hydrophilic extract**

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**Profile of phenolic compounds and carotenoids of *Arrabidaea chica* leaves and the *in vitro* singlet oxygen quenching capacity of their hydrophilic extract**

## ABSTRACT

*Arrabidaea chica* (Brazilian name = *pariri*) is a plant species that belongs to the Bignoniaceae family, occurring in tropical America and widespread in the Amazonian region of Brazil. In this study, the phenolic compound and carotenoid profiles of *A. chica* leaves were determined by HPLC-DAD-MS. Scutellarin was identified as the main phenolic compound (15,147.22 μg/g, dry basis, d.b.) and lutein (204.28 μg/g, d.b.), β-carotene (129.5 μg/g, d.b.) and α-carotene (79.86 μg/g, d.b.) as the major carotenoids. Moreover, *A. chica* leaves presented 152.7 μg/g of ascorbic acid (fresh weight). For the first time, the carotenoid profile and ascorbic acid contents were reported for *A. chica* leaves. Regarding the antioxidant capacity, *A. chica* extract was able to scavenge ABTS radical (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), and for the first time, the protective effect against singlet oxygen ($^1O_2$) was reported. The *A. chica* extract was efficient ABTS radical scavenger (86.81 μM Trolox/g of fresh leaves) and inhibited the degradation of tryptophan by $^1O_2$ in a concentration-dependent manner with an $IC_{50}$ of 160 μg/mL. Thus, *A. chica* leaves can be exploited as a promising source of bioactive compounds that may be useful to human health or food systems against oxidative damage.

**Keywords**: Pariri, Amazonian plants, antioxidant capacity, bioactive compounds, singlet oxygen, LC-MS.

# 1. INTRODUCTION

High levels of reactive species in living systems can oxidize biomolecules, leading to tissue damage, cell death or the onset of various chronic-degenerative diseases, such as cancer, diabetes, kidney problems, hypertension, cardiovascular diseases, and atherosclerosis (HALLIWELL, 2012; KATOCH, et al., 2013). In food systems, the occurrence of reactive species is also responsible for the onset of food oxidation reactions (CHOE; MIN, 2015). Studies have shown a growing interest in scientific researches on bioactive compounds, such as phenolic compounds, carotenoids and ascorbic acid, from medicinal and dietary plants that may help to minimize the effects of oxidative damages both in foods and *in vivo* (DZIADEK, KOPEĆ, & TABASZEWSKA, 2019; RIBEIRO et al., 2015; SHARIFIFAR; DEHGHN-NUDEH; MIRTAJALDINI, 2009; SILVA, et al., 2005).

Brazilian flora, especially the Amazonian biome, is one of the richest sources in the world of species with bioactive substances, and among these species, *Arrabidaea chica* (Humb. & Bonpl.) B. Verlot, also known as "crajiru", "pariri" or "chica", is a tree plant belonging to the Bignoniaceae family, widely used in folk medicine as tea to treat gastric ulcers, anemia, infections, inflammation, herpes, among others (MAFIOLETI, et al, 2013; ZORN et al., 2001). The hydroethanolic extract of *A. chica* leaves with high contents of phenolic compounds (16%) demonstrated low oral, acute and subchronic toxicities and lack of cytotoxicity in Chinese hamster ovary cells (CHO-K1) (MAFIOLEbTI et al, 2013). Furthermore, *A. chica* presents high potential for the prospection of bioactive compounds (ARO et al., 2013). For example, the presence of flavonoids, anthocyanins, tannins and phytosterols were already reported in *A. chica* leaf extracts cultivated in other localities, distinct from the Amazon region (DEVIA et al., 2002; SIRAICHI et al., 2013). However, no information was found in the literature regarding the carotenoid profile and ascorbic acid contents in this species. Furthermore, the leaves of *A. chica* were reported to possess anti-inflammatory, antibacterial, antifungal, antiparasitic, antitumor, antiulcerogenic and antioxidant activities (RIBEIRO et al., 2012; MAFIOLETI et al., 2013; SIRAICHI et al., 2013).

Therefore, the objective of this study was to identify and quantify the bioactive compounds, by high performance liquid chromatography coupled to diode array and mass spectrometer detectors (HPLC-DAD-MS), of *A. chica* leaves cultivated in the Amazonian region of Brazil. In addition, the *in vitro* antioxidant capacity of the *A. chica* hydrophilic extract

to scavenge ABTS radical (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and to quench singlet oxygen ($^1O_2$) was assessed.

## 2. MATERIALS AND METHODS

### 2.1. CHEMICALS

Scutellarin, apigenin, quercetin, all-*E*-β-carotene, all-*E*-lutein, ascorbic acid, trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid), potassium persulphate, methylene blue (MB), methanol (MeOH), methyl-*tert*-butyl ether (MTBE), acetonitrile, sulfuric acid, formic acid and all other analytical grade salts were purchased from Sigma-Aldrich (St. Louis, USA). L-Tryptophan was obtained from Fisher Scientific (Pittsburgh, PA, USA). Ultrapure water was obtained from the Milli-Q System system (Millipore Corp., Milford, MA, USA). All phenolic compounds and carotenoids standards showed at least 95% of purity degree, as determined by HPLC-DAD.

### 2.2. SAMPLES

The *A. chica* leaves were collected from five plants in May 2017 from the Active Germplasm Bank of EMBRAPA (Brazilian Agricultural Research Corporation) Eastern Amazon Research Center, located in Belém, Pará State, Brazil (01°26'14.7" S and 48°26'52.2" W). For each plant, 500 g of leaves from the lower, middle and upper parts were collected to obtain a representative sample. The collected leaves were divided into two portions: one was used for proximate composition analysis and the other was freeze-dried using a benchtop lyophilizer (Liotop, L101, São Paulo, Brazil) and used in the bioactive compounds determination and antioxidant capacity assays. The freeze-dried leaves were milled in a knife mill, vacuum packed in plastic bags and stored at -18 °C until analysis.

### 2.3. PROXIMATE COMPOSITION

The contents of ashes, total lipids, moisture and total proteins (conversion factor of 6.25 from total nitrogen to total protein) were determined according to AOAC (ASSOCIATION OF ANALYTICAL CHEMISTS, 2000) and expressed as g/100 g (%). Total carbohydrates were calculated by difference [Total carbohydrates = 100 – (% moisture + % ashes + % total proteins + % total lipids)]. The total energetic value (kcal/100 g) was obtained according to the Atwater conversion factor for vegetables, as follows: total energetic value (kcal/100 g) =

(protein × 2.44 kcal/g) + (lipid × 8.37 kcal/g) + (total carbohydrate × 3.57 kcal/g) (FAO/WHO, 2003). All the experiments were carried out in triplicate (n = 3).

## 2.4. DETERMINATION OF BIOACTIVE COMPOUNDS

### 2.4.1. Equipments

The identification of phenolic compounds and carotenoids in A. chica leaf extracts was carried out by a Shimadzu HPLC (Prominence UFLC model, Kyoto, Japan) consisting of a binary pump (LC-20AD), a degasser unit (DGU-20A3R), an automatic injector (SIL-20AHT), and an oven (CTO-20A), connected in series to a DAD detector (SPD-M20A) and a mass spectrometer with an ion-trap as the *m/z* analyzer from Bruker Daltonics (AmaZon speed ETD, Bremen, Germany), equipped with electrospray (ESI) and atmospheric pressure chemical ionization (APCI) as the ionization sources for phenolic compounds and carotenoids identification, respectively.

The quantification of phenolic compounds, carotenoids and ascorbic acid was carried out by an Agilent HPLC (Agilent 1260 Infinity model, Santa Clara, CA, USA) equipped with a quaternary pump (G1311C), an automatic injector (G7129), an oven (G1316A) and a DAD detector (G1328C). For all the chromatographic analysis, samples and solvents were filtered using membranes of 0.22 and 0.45 μm, respectively, both from Millipore (Billerica, MA, USA).

### 2.4.2 HPLC-DAD-ESI-$MS^n$ analysis of phenolic compounds

The phenolic compounds of *A. chica* leaves were exhaustively extracted from about 0.5 g of the freeze-dried leaves with MeOH/water (8:2, v/v) in a mortar with a pestle (5 min), followed by vacuum filtration and the residue was subjected to the same extraction procedure for 3 times. The filtered extract was transferred to a 50 mL-flask and filled up with the same extraction solution.

The phenolic compounds were separated on a $C_{18}$ Synergi Hydro column (Phenomenex, 4 μm, 250 × 4.6 mm), set at 29 °C. A linear gradient of water with 0.5% formic acid (solvent A) and acetonitrile with 0.5% formic acid (solvent B) was used from A:B 99:1 to 50:50 in 50 min followed to 1:99 in 5 min, keeping the last ratio for another additional 5 min at 0.9 mL/min (CHISTÉ & MERCADANTE, 2012). The UV-visible (UV-vis) spectra were obtained between 200 and 600 nm, and the chromatograms were monitored at 270, 320, 360 and 480 nm. The column eluate was split to allow only 0.15 mL/min to enter the ESI source. The mass spectra (MS) were acquired after ionization in negative ion mode, with a scanning range of *m/z* from 100 to 1000, and the MS parameters set as described by Chisté and Mercadante (2012). The

phenolic compounds were tentatively identified by combining the following information: elution order, retention time on the $C_{18}$ column, UV-vis and mass spectra compared to data available in the literature (CHANDRASEKARA; SHAHIDI, 2011; EL-HELA, AL-AMIER; IBRAHIM, 2010; SIRAICHI et al., 2013; SUROWIEC; SZOSTEK; TROJANOWICZ, 2007; RIBEIRO, et al., 2016) and authentic standards. The quantification was carried out by comparison with external analytical curves of six points (3.12 to 100 µg/mL, in duplicate) for apigenin [$R^2$ = 0.99, limit of detection (LOD) = 0.17 µg/mL and limit of quantification (LOQ) = 0.51 µg/mL], scutellarin [$R^2$ = 0.99, LOD = 0.14 µg/mL and LOQ = 0.41 µg/mL] and scutellarein [$R^2$ = 0.98, LOD = 0.23 µg/mL and LOQ = 0.69 µg/mL]. LOD and LOQ values were calculated using the parameters (standard deviation and slope) of the analytical curves (ICH, 2005). The phenolic compound contents were expressed as µg/g of freeze-dried leaves (dry basis, d.b.), considering three independent extraction procedures (n = 3).

### 2.4.3. HPLC-DAD-APCI-MS/MS analysis of carotenoids

The carotenoids of freeze-dried *A. chica* leaves (0.5 g) were exhaustively extracted with acetone, followed by liquid-liquid partition to petroleum ether/diethyl ether (1:1, v/v) by washing with distilled water, saponified overnight with 10% KOH in methanol (1:1, v/v), re-partitioned and evaporated under vacuum (T < 38 °C). The dried carotenoid extract was solubilized in MTBE and filtered prior to HPLC injection, according to the procedures described by Chisté and Mercadante (2012).

The carotenoids were separated on a $C_{30}$ YMC (5 µm, 250 mm × 4.6 mm) column set at 29 °C, using as a mobile phase a linear gradient of MeOH (solvent A) and MTBE (solvent B), from A:B 95:5 to 70:30 in 30 min, followed by 50:50 in 20 min at 0.9 mL/min. The UV-vis spectra were recorded between 200-600 nm and the chromatograms were processed at 450 nm. The column eluate was directed to the APCI interface, and the mass spectra were obtained after APCI in positive ion mode, with a scan interval at *m/z* from 100 to 800, with the parameters reported by Chisté and Mercadante (2012). The carotenoids were tentatively identified based on the following combined information: elution order on the $C_{30}$ column, UV-vis [$\lambda_{max}$, spectral fine structure (%III/II), *cis*-peak intensity (%$A_B/A_{II}$)] and mass spectra compared to data available in the literature (DE ROSSO; MERCADANTE, 2007; DE FARIA;, DE ROSSO; MERCADANTE, 2009; CHISTÉ; MERCADANTE, 2012; BERTO, et al., 2015;). The assignment of *Z*-isomer of carotenoids was based on the observation of %III/II decrease and %$A_B/A_{II}$ increase (≈ 7-11% = 9*Z*; ≈45% = 13*Z* and ≈ 56% = 15*Z* carotenoid) as reported by De Rosso & Mercadante (2007). The carotenoids were quantified by HPLC-DAD by external five-

point analytical curves (3.12 - 100 µg/mL, in duplicate) of (all-*E*)-β-Carotene ($R^2$ = 0.99, LOD = 0.98 µg/mL and LOQ = 2.96 µg/mL) and (all-*E*)-Lutein ($R^2$ = 0.98, LOD = 0.20 µg/mL and LOQ = 0.62 µg/mL). The NAS-IOM conversion factor was used to calculate the vitamin A value, considering 12 µg of (all-*E*)-β-carotene and 24 µg of (all-*E*)-α-carotene, (9*Z*)-β-carotene and (13*Z*)-β-carotene corresponding to 1 µg of retinol activity equivalent (RAE). The activity used was 100% for (all-*E*)-β-carotene and 50% for (all-*E*)-α-carotene, (9*Z*)-β-carotene and (13*Z*)-β-carotene (NAS- IOM, 2001). The carotenoid contents were expressed as µg/g of freeze-dried leaves (d.b.), considering three independent extraction procedures (n = 3).

### 2.4.4. HPLC-DAD analysis of ascorbic acid

The ascorbic acid was extracted from 1 g of the freeze-dried *A. chica* leaves with an aqueous solution of 1% oxalic acid (3 times), followed by centrifugation (3000 x *g* at 4 °C). The supernatant was transferred to a 50 mL flask; the volume was filled up with the same solution and then filtered prior injection into the Agilent HPLC-DAD system. The ascorbic acid was separated on a $C_{18}$ Synergi Hydro column (Phenomenex, 4 µm, 250 × 4.6 mm) set at 25 °C, using an aqueous solution of sulfuric acid (0.001 M, pH 2.5) as the mobile phase, in isocratic mode, at 0.7 mL/min. The chromatogram was processed at 244 nm (RIBEIRO et al., 2016). The identification of ascorbic acid was based on the retention time (7 min), co-elution and UV-vis characteristics compared to the authentic standard (data not shown) analyzed under the same conditions. The ascorbic acid was quantified using six-point external analytical curve (1.6 - 100 µg/mL, in duplicate, $R^2$ = 0.99, LOD = 0.05 µg/mL and LOQ = 0.16 µg/mL) and the results were expressed as µg/g of fresh weight leaves (FW), considering three independent extraction procedures (n = 3).

## 2.5. *IN VITRO* ANTIOXIDANT CAPACITY OF *A. CHICA* EXTRACT

The extract used for the antioxidant capacity assays was obtained according to the same procedure described to the analysis of phenolic compounds.

### 2.5.1. Scavenging capacity of ABTS radical

The ABTS radical scavenging assay was carried out according to the method described by Re et al. (1999), with modifications. The radical cation ($ABTS^{\bullet+}$) was generated by the chemical reaction of ABTS (7 mM) with potassium persulfate (145 mM) held in the dark during 12-16 h before use. After formation of the $ABTS^{\bullet+}$, 3 mL of this solution (diluted with methanol to ~ 0.70 absorbance at 734 nm) was mixed with an aliquot (30 µL) of the *A. chica* extract at

concentrations ranging from 0.5 to 10 mg/mL or Trolox standard and the absorbance was recorded after 6 min at 734 nm. The analytical curve of trolox (102 to 2048 μM) was constructed and the results (n = 3) were expressed in μM of Trolox equivalent (TE)/g of FW leaves.

### 2.5.2. Quenching of singlet oxygen ($^1O_2$)

The ability of *A. chica* extract to quench $^1O_2$ was determined according to the method described by Chisté, Benassi and Mercadante (2011), with adaptations. The experiment was conducted by taking an aliquot (100 μL) of the *A. chica* extract (50 to 375 μg/mL) or quercetin standard (positive control, 0.78 to 4.75 μg/mL), which was added to a solution containing 950 μL of L-tryptophan (TRP) (40 μM) and 950 μL of MB (10 μM) as the sensitizer, under atmospheric air and at room temperature (25 °C), both solutions prepared in phosphate-buffered saline (pH 7.2). The blank experiment was performed under the same conditions, replacing 100 μL of the antioxidant by 100 μL of MeOH/water (8:2, v/v). The $^1O_2$ was generated by direct sensitization of MB by a 75 W incandescent bulb, which was used as the excitation source, and two filters (red and orange colored filters) was placed between the excitation source and the cuvette containing the reactants to only excite MB. The reaction was monitored by spectrophotometric measurements in the range of 200-800 nm during 20 min and the absorbance of TRP was recorded at 219 nm. The kinetic data obtained from the decay of the absorbance of TRP was fitted to a first-order reaction (Eq. 1) using Origin Pro 8 software (OriginLab Corporation, Northampton, MA, USA) to calculate the rate constants (Eq. 2). The percentage of protection of *A. chica* extract or quercetin against the oxidative damage of $^1O_2$ was calculated through Eq. 3.

$$Y = Y_\infty + A.exp^{(-\frac{x}{t_1})} \quad (1)$$

$$k = \frac{\ln 2}{t_1} \quad (2)$$

$$Protection\ against\ ^1O_2\ (\%) = \frac{K_{obs}^{TRP} - K_{obs}^{TRP+antioxidant}}{K_{obs}^{TRP}} \text{ x } 100 \quad (3)$$

where Y is the intensity absorbance of TRP; $Y_\infty$ is the intensity absorbance of TRP at infinite time; A is pre-exponential factor; *k* is the pseudo-first order rate constant; x is the reaction time; $t_1$ is half-life time (min); $K_{obs}^{TRP}$ is the observed pseudo-first order rate constant fitted to the TRP decay curve (obtained in the blank experiment); and $K_{obs}^{TRP+antioxidant}$ is the observed pseudo-first order rate constant fitted to the TRP decay curve in the presence of antioxidant.

The *in vitro* $IC_{50}$ value (inhibitory concentration to decrease by 50% the amount of reactive species in the tested media) was calculated from the curves of percentage of inhibition *versus* antioxidant concentration using OriginPro 8 software.

## 3. RESULTS AND DISCUSSION

The *A. chica* leaves presented high moisture (69.95 ± 0.18%), carbohydrates (22.84 ± 0.25%) and ashes (4.66 ± 0.17%) contents, while low contents of total protein (1.82 ± 0.05%) and total lipids (0.73 ± 0.04%) with a total energetic value of 92.07 ± 0.80 kcal/100 g (FW). This is the first report concerning the proximate composition of *A. chica* leaves and these results were lower than those reported for the Nigerian medicinal plants *Chromolina oduratum, Ipomoea aserifolia* and *Emilia santifolia* (EKPA, 1996), and those found in 40 leaf cultivars of sweet potatoes (*Ipomoea batatas* L.) (SUN, et al. 2014).

Regarding the ascorbic acid content, its value (152.7 ± 13.6 μg/g FW), reported for the first time in the present study, is within the range found for spinach (*Spinacia oleracea* L.) (14.0 - 460 μg/g FW) at different stages of growth and post-harvest storage (BERGQUIST; GERTSSON; OLSSON, 2006), but lower than broccoli (*Brassica oleracea L., Italian var., Marathon cv.*) inflorescences (665-1,077 μg/g FW) during harvesting and storage (VALLEJO; TOMÁS-BARBERÁN; GARCIA-VIGUERA, 2003). Therefore, A. *chica* leaves could be added to food preparations to increase ascorbic acid ingestion in a healthy diet, since ascorbic acid is considered one of the main antioxidant compounds found in vegetables. Such compound is important for the regeneration of vitamin E, inhibition of nitrosamine formation and in the absorption of inorganic iron, which may promote an enhancement in the immune system and protection against diseases related to oxidative stress (RIBEIRO, et al., 2007; RIBEIRO et al., 2016).

### 3.1. PHENOLIC COMPOUNDS PROFILE OF *A. CHICA* LEAVES

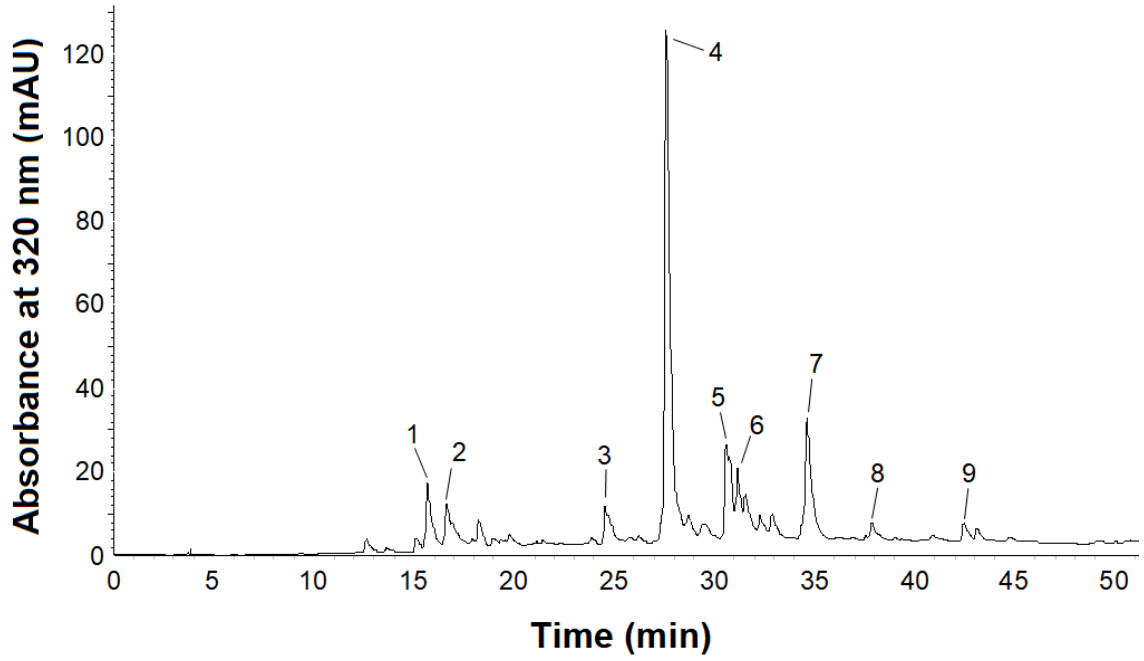
Regarding the profile of phenolic compounds of *A. chica*, as observed in Table 1, the HPLC-DAD-ESI-$MS^n$ allowed the assignment of nine separated phenolic compounds (Figure 1).

**Table 1**. Phenolic compounds profile of *Arrabidaea chica* leaves by HPLC-DAD-ESI-$MS^n$.

| Peak | Phenolic Compound* | Concentration (μg/g leaves)[a] | $t_R$ (min)[b] | $\lambda_{max}$ (nm)[c] | $[M-H]^-$ (*m/z*) | $MS^2$ *(m/z)*[d] | $MS^3$ *(m/z)*[d] |
|---|---|---|---|---|---|---|---|
| 1 | Feruloyl hexose (isomer 1)[e] | 1.088,07 ± 236.98 | 15.7 | 313 | 355 | 337, 212, **193**, 151 | [355→193]: **151** |
| 2 | Feruloyl hexose (isomer 2)[e] | 915.26 ± 217.24 | 16.7 | 309 | 355 | 337, 212, **193**, 151 | [355→ 193]: **161** |
| 3 | Feruloyl derivative[e] | 857.53 ± 72.65 | 24.6 | 273, 327 | 449 | 405, **273**, 257 | [449→273]: **255**, 231 |
| 4 | Scutellarin[e] | 15,147.22 ± 1660.84 | 27.7 | 282, 334 | 461 | **285**, 178 | [461→285]: 285**,** **267,** 243, 192,169 |
| 5 | Apigenin glucuronide[g] | 807.83 ± 221.15 | 30.7 | 267, 337 | 445 | **269**, 177 | [445→ 269]: **269,** 225 |
| 6 | Flavone-glucuronyl derivative[e] | 1,143.23 ± 209.03 | 31.0 | 275, 328 | 493 | 445, 431, **317**, 273 | [493→ 317]: 299, **273**, 259, 185 |
| 7 | Scutellarein[f] | 1,379.17 ± 91.57 | 34.6 | 282, 337 | 285 | **267** , 225, 200, 173 | [285→ 267]: 241, 227, 214, **198** |
| 8 | Methyl apigenin glucuronide[g] | 132.14 ± 12.95 | 37.6 | 266, 292, 335 | 459 | **283**, 178 | [459→283]: **268** |
| 9 | Apigenin[g] | 49.79 ± 40,80 | 42.5 | 267,293, 337 | 269 | 251, **225**, 201, 183 | [269→225]: nd |
| **Total phenolic (μg/g leaves)** | | 21,520.24 ± 2,763.21 | | | | | |

*Tentative identification based on Uv-visible and mass spectra as well as relative retention time on $C_{18}$ column and published data. [a]Mean ± standard deviation (n = 3, dry basis). [b]Retention time on the $C_{18}$ Synergi Hydro (4μm) column. [c]Solvent: gradient of 0.5% formic acid in water and acetonitrile with 0.5% formic acid. [d]In the $MS^2$ and $MS^3$, the most abundant ion is shown in boldface. The peaks were quantified as equivalent of scutellarin[e], scutellarein[f] and apigenin[g]. nd = not detected.

**Figure 1 -** HPLC-DAD chromatogram at 320 nm of phenolic compounds of *Arrabidaea chica* leaf extract. The peak characterization is given in Table 1.




**Peaks 1** and **2** were tentatively identified as feruloyl hexose isomers since both compounds presented deprotonated molecule ($[M-H]^-$) at *m/z* 355 with fragments at *m/z* 337 and an intense fragment at *m/z* 193 (ferulic acid) in the $MS^2$ spectra, corresponding to a neutral loss of a water molecule (18 u) and a hexose moiety (162 u), respectively. Such characteristics were already reported in the literature for this type of compounds (CHANDRASEKARA; SHAHIDI, 2011; RIBEIRO et al., 2016). **Peak 3** was assigned as feruloyl derivative due to the UV-Vis absorption at 327 nm (hydroxycinnamic acids) and the presence of $[M-H]^-$ at *m/z* 449, followed by a neutral loss of a feruloyl moiety (176 u) as the most intense fragment at *m/z* 273 in the $MS^2$ spectra. The ion at *m/z* 273 was further fragmented into the *m/z* 255, showing the presence of a hydroxyl group in the $MS^3$ spectrum. The same fragmentation pattern was previously reported for the feruloyl derivative compounds found in *Solanum diploconos* fruits (RIBEIRO et al., 2016).

**Peaks 4-9** are phenolic compounds, which belong to the flavone class, as can be observed by their characteristic UV-vis absorption spectra at the wavelength range of 334-337 nm. **Peak 4** was the major phenolic compound in *A. chica* leaves and identified as scutellarin, with $[M-H]^-$ at *m/z* 461 and a neutral loss of a glucuronyl moiety at *m/z* 285 ($[M-H-176]^-$ in the $MS^2$ spectrum, followed by an intense peak at *m/z* 267 in the $MS^3$ spectrum, which corresponds

to the elimination of a hydroxyl group. Scutellarin, identified in this leaf for the first time in the literature, was also confirmed based on co-elution and comparison with the same MS fragmentation pattern exhibited by the authentic standard. Considering that **peak 5** showed $[M-H]^-$ at *m/z* 445 and an intense fragment at *m/z* 269 ($MS^2$ spectra), corresponding to the loss of a glucuronyl unit (176 u), this compound was tentatively assigned as apigenin glucuronide, according to previous report (SUROWIEC et al., 2007). **Peak 6** was tentatively identified as flavone-glucuronyl derivative as showed a $[M-H]^-$ ion at *m/z* 493 with an intense $MS^2$ fragment at *m/z* 317, corresponding to the loss of a glucuronyl unit, followed by a neutral loss of 18 u at *m/z* 299 ($MS^3$ spectrum). **Peak 7** was identified as scutellarein due to the presence of a $[M-H]^-$ at *m/z* 285 and a $MS^2$ fragment at *m/z* 267 , which corresponds to a neutral loss of a hydroxyl group. The identification of scutellarein was confirmed by co-elution and the same MS fragmentation characteristics exhibited by the authentic standard and previous data (SIRAICHI et al., 2013). **Peak 8** exhibited $[M-H]^-$ at *m/z* 459, with consecutive losses of 176 u (glucuronide moiety) at *m/z* 283 ($MS^2$ spectrum), and 15 u (methyl group) at *m/z* 268 ($MS^3$ spectrum), being identified as methyl apigenin glucuronide (Abubakar et al., 2016; Shi et al., 2013). **Peak 9** was identified as apigenin, with $[M-H]^-$ at *m/z* 269 and fragments at *m/z* 251 (18 u, OH group) and *m/z* 225 ( 44 u, $CO_2$), typical from this type of compound (MCNAB, et al., 2009; SHI et al., 2013); identity was confirmed by comparison with the authentic standard.

This is the first study in literature reporting the presence of the feruloyl derivative compounds, scutellarin and methyl apigenin glucuronide in *A. chica* leaves, together with the overall quantification of phenolic compounds by HPLC-DAD. Scutellarin was the major phenolic compound and accounted for ≈70% of the sum of the identified phenolic compounds. Other studies indicated the presence of two desoxyanthocyanidins (carajurin and carajurone) in *A. chica* leaves cultivated in Bogotá (Colombia) (DEVIA et al., 2002) and Costa Rica (ZORN et al., 2001). In *A. chica* leaves cultivated in Southern Brazil, the presence of isoscutellarein, 6-hydroxyluteolin, hispidulin and luteolin was reported (SIRAICHI et al., 2013). None of these compounds was detected in the present study. This can be attributed to several factors, such as environmental aspects of plant cultivation, complexity of phenolic compounds and methods of extraction and analysis (BALASUNDRAM; SUNDRAM; SAMMAN, 2006; TOMÁS-BARBERÁN; ESPÍN, 2001;).

The total sum of the identified phenolic compounds of *A. chica* leaves, measured by HPLC-DAD, in this study, was very high (21,520 μg/g, Table 1), which accounts for ≈ 2% of

the composition of the freeze-dried material. According to the literature, *A. chica* leaf extract presented 10,200 μg/g (FW leaves) of total phenolic compounds (expressed as gallic acid equivalent), as spectrophotometrically determined by the Folin-Ciocalteau method (SILVA, SOUZA, et al., 2007). Such result is higher that found in our study (≈ 6,456 μg/g, FW) probably due to the fact than many compounds in the extract, e.g. ascorbic acid and reducing sugars, also react with the Folin-Ciocalteau reagent.

## 3.2. CAROTENOID PROFILE OF *A. CHICA* LEAVES

Regarding the profile of carotenoids of *A. chica*, as observed in Table 2, the HPLC-DAD-APCI-MS/MS allowed the assignment of ten separated carotenoids (Figure 2). The major carotenoids were (all-*E*)-lutein (**peak 5**), followed by (all-*E*)-β-carotene (**peak 9**) and (all-*E*)-α-carotene (**peak 8**). For the identified peaks, the attribution of the protonated molecule $[M+H]^+$ was confirmed by means of the expected MS/MS fragments for both the polyene chain and functional groups of carotenoids, as well as by the UV-vis spectra. (all-*E*)-β-carotene (**peak 9**) was positively confirmed by co-elution and comparison with an authentic standard.

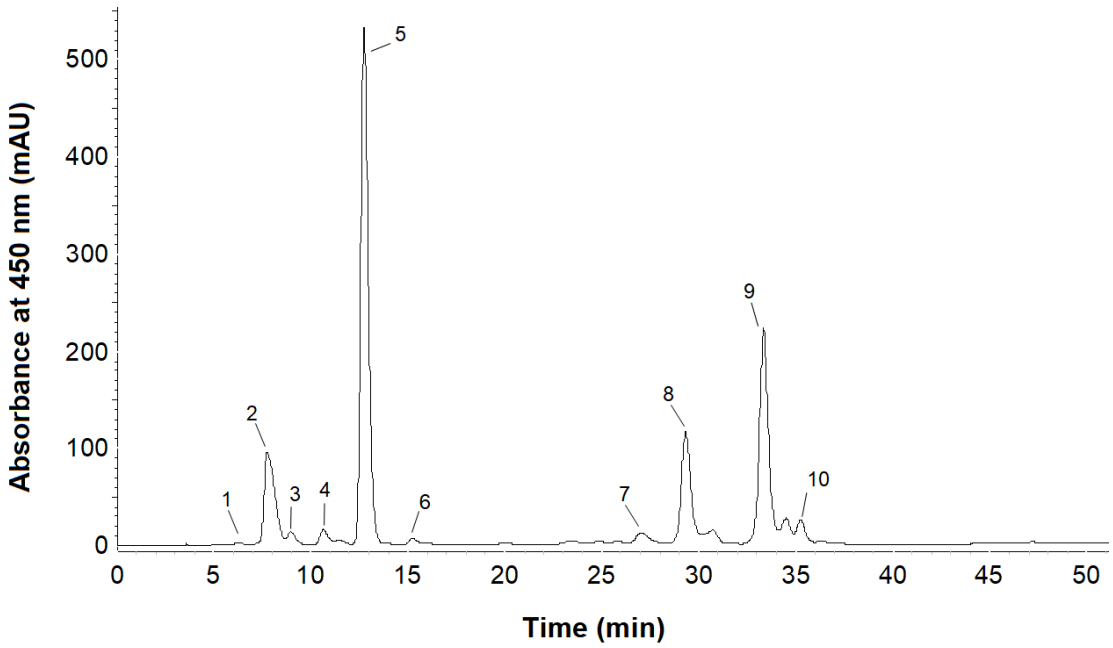
All the identified xanthophylls had three or two hydroxyl (OH) groups and, as expected for reverse phase columns, they eluted before the carotenes.

**Table 2**. Carotenoid profile of *Arrabidaea chica* leaves by HPLC-DAD-APCI-MS/MS.

| Peak | Carotenoid[a] | Concentration (μg/g leaves)[b] | $t_R$ (min)[c] | $\lambda_{max}$ (nm)[d] | %II I/ II | $\%A_B/A_{II}$ | $[M+H]^+$ (*m/z*) | MS/MS (+) (*m/z*) |
|---|---|---|---|---|---|---|---|---|
| 1 | (13*Z*)-Violaxathin[f] | 8.54 ± 0.10 | 6.1 | 313, 405, 427, 455 | 40 | 45 | 601 | 583, 565, 509, 491, 221 |
| 2 | (all-*E*)-Violaxanthin[f] | 39.06 ± 10.31 | 7.7 | 415, 438, 468 | 83 | 0 | 601 | 583, 565, 509, 491, 221 |
| 3 | (all-*E*)-Luteoxanthin[f] | 11.92 ± 0.54 | 8.9 | 398, 421, 448 | 100 | 0 | 601 | 583, 565, 491, 221 |
| 4 | Mix of *Z*-lutein + epoxycarotenoids[g] | 37.55 ± 0.96 | 10.6 | 330, 419, 440, 467 | 27 | 37 | 601 | 583, 565, 509, 491, 221 |
| | | | | | | | 585 | 567, 549, 493, 475, 221 |
| | | | | | | | 551[e] | 533, 459 |
| 5 | (all-*E*)-Lutein[g] | 204.28 ± 40.17 | 12.7 | 420, 444, 473 | 60 | 0 | 569 | 551, 533 477, 459 |
| 6 | (all-*E*)-Zeaxanthin[f] | 11.04 ± 1.16 | 15.2 | 420, 450, 476 | 25 | 0 | 569 | 551, 533 , 477 |
| 7 | (13*Z*)-β-Carotene[f] | 17.28 ± 1.42 | 26.9 | 338, 415, 444, 469 | 11 | 42 | 537 | 444 |
| 8 | (all-*E*)-α-Carotene[f] | 79.86 ± 5.62 | 29.3 | 420, 445, 473 | 63 | 0 | 537 | 481, 444, 413 |
| 9 | (all-*E*)-β-Carotene[f] | 129.54 ± 8.38 | 33.3 | 420, 450, 477 | 29 | 0 | 537 | 457, 444, 413, 400 |
| 10 | (9*Z*)-β-Carotene[f] | 21.40 ± 0.77 | 35.2 | 340, 420, 446, 472 | 14 | 8 | 537 | 457, 444, 413 |
| **Total carotenoids (μg/g leaves)** | | 560.46 ± 69.42 | | | | | | |
| **Vitamin A value (μg RAE/g leaves)** | | 15.73 ±1.02 | | | | | | |

[a]Tentative identification based on Uv-visible and mass spectra as well as retention times on $C_{30}$ column and published data. [b]Mean ± standard deviation (n = 3, dry basis). [c]Retention time on the $C_{30}$ column. [d]Linear gradient of methanol/MTBE. [e]In-source detected fragment. The peaks were quantified as equivalent of β-carotene[f] and lutein[g]. RAE = retinol activity equivalent.

**Figure 2 -** HPLC-DAD chromatogram at 450 nm of carotenoids of *Arrabidaea chica* leaf extract. Peaks: 1) (13*Z*)-violaxathin; 2) (all-*E*)-violaxanthin; 3) (all-*E*)-luteoxanthin;4) Mix of *Z*-lutein + epoxycarotenoids; 5) (all-*E*)-lutein; 6) (all-*E*)-zeaxanthin; 7) (13*Z*)-β-carotene; 8) (all-*E*)-α-carotene; 9) (all-*E*)-*β*-Carotene; 10) (9*Z*)-β-carotene. The peak characterization is given in Table 2.




**Peaks 1** and **2** were tentatively identified as (13*Z*)-violaxanthin and (all-*E*)-violaxanthin, respectively,, because the (*Z*) isomer presented lower values of $\lambda_{max}$, %III/II and high *cis* peak intensity (45%) compared to the corresponding (all-*E*)-isomer. Moreover, both peaks showed the $[M+H]^+$ at *m/z* 601 and fragments at *m/z* 583 $[M+H-H_2O]^+$ and *m/z* 565 $[M+H-2H_2O]^+$, due to neutral losses of two hydroxyl groups, at *m/z* 509 $[M+H-92]^+$ resulting from the loss of toluene and at *m/z* 221, showing the presence of an epoxy group in a hydroxylated β-ring (CHISTÉ; MERCADANTE, 2012; CRUPI et al., 2013). (all-*E*)-luteoxanthin (**peak 3**) presented $\lambda_{max}$ at 398, 421 and 448 nm, high percentage of %III/II (100%), $[M+H]^+$ at *m/z* 601, and two consecutive losses of OH groups were observed in the MS/MS spectrum at *m/z* 583 and *m/z* 565. As also observed for peaks 1 and 2, peak 3 presented the characteristic fragment at *m/z* 221 in the MS/MS spectrum.

**Peak 4** was assigned as a mixture of (*Z*)-lutein + epoxycarotenoids and showed three protonated molecules: *m/z* 601, *m/z* 585 and *m/z* 551 (in-source ionization). The $[M+H]^+$ at *m/z* 601 fragmented at *m/z* 583 $[M+H-H_2O]^+$, *m/z* 565 $[M+H-2H_2O]^+$, *m/z* 509

[M+H-92] $^{+}$ and at *m/z* 221 in the MS/MS spectrum. The $[M+H]^+$ at *m/z* 585 showed successive OH losses at *m/z* 567 and *m/z* 549, elimination of toluene from the polyene chain at *m/z* 493, loss of toluene combined with an OH (*m/z* 475 $[M+H-H_2O-92]^+$) and a fragment at *m/z* 221. The presence of a (Z)-carotenoid in peak 4 was noticed by the presence of a *cis*-peak (%$A_B/A_{II}$) at 330 nm in the UV-Visible spectrum, which was confirmed by the observation of an intense in-source fragment at *m/z* 551 in the MS spectrum, which is characteristic of lutein and its (Z)-isomers ($[M+H]^+$ at *m/z* 569), as well as the fragments at *m/z* 533 $[M+H-H_2O]^+$ and at *m/z* 459 [M+H-92] $^{+}$.

The (all-*E*)-lutein (**peak 5**) and (all-*E*)-zeaxanthin (**peak 6**) have the same chemical formula ($C_{40}H_{56}O_2$) and showed $[M+H]^+$ at *m/z* 569 and MS/MS fragments at *m/z* 551 $[M+H-H_2O]^+$, *m/z* 533 $[M+H-2H_2O]^+$ and at *m/z* 477 $[M+H-92]^+$; however, zeaxanthin has 11 conjugated double bonds (c.d.b.) and two β-rings, while lutein has 10 c.d.b, one β-ring and one ε-ring (DE FARIA et al., 2009). Both the compounds can be differentiated by the UV-vis spectrum, where lutein presented $\lambda_{max}$ values lower than those of zeaxanthin, and MS spectrum, which showed an intense fragment at *m/z* 551 when compared to the $[M+H]^+$ (*m/z* 569), in contrast to that observed for zeaxanthin (DE ROSSO; MERCADANTE, 2007).

The carotenes (13*Z*)-β-carotene (**peak 7**), (all-*E*)-α-carotene (**peak 8**), (all-*E*)-β-carotene (**peak 9**) and (9*Z*)-β-carotene (**peak 10**) showed $[M+H]^+$ at *m/z* 537 and the characteristic fragment at *m/z* 444 $[M-92]^+$ from the polyene chain. The (13*Z*)-β-carotene and (9*Z*)-β-carotene were identified by the presence of the *cis*-peak at the 338-340 nm range in their UV-vis spectra (Table 2). In addition, the assignment of these *Z* isomers assumes that the *cis* peak intensity (%$A_B/A_{II}$) increases and the spectral fine structure (%III/II) decreases as the (*Z*)-double bond approaches the center of the molecule (BRITTON; LIAAEN-JENSEN; PFANDER, 2004). The (all-*E*)-α-carotene has the same chromophore as lutein and, therefore, their UV-visible absorption spectra were similar. In addition, (all-*E*)-α-carotene showed the presence of intense fragments at *m/z* 481 and *m/z* 444 in the MS/MS spectrum, corresponding to the neutral losses of the ε-ring and toluene, respectively. In (all-*E*)-β-carotene MS spectrum, the elimination of β-ring with an additional methylene group, $[M+H-137]^+$ resulted in the formation of the fragment at *m/z* 400.

This is the first report concerning the carotenoid profile of *A. chica* leaves. Among the identified carotenoids, only β-carotene (and its *cis* isomer) and α-carotene were the compounds that contributed to vitamin A activity (15.73 μg RAE/g d.b. leaves). The

vitamin A activity of *A. chica* leaves was similar to that reported for *Allmania nodiflora* (16.05 μg RAE/g dw), and lower than *Alternanthera pungens* (57.76 μg RAE/g dw), which are other examples of green leafy vegetables, but higher than *Brassica oleracea* (6.41 μg RAE/g dw) and *Mentha spicata* L. (12.46 μg RAE/g dw) (RAJU, et al., 2007).

Regarding the lutein content of *A. chica* leaves, it was about thrice higher than the leaves of *Prunus armeniaca*, whose values (56.7-65.7 μg/g) varied during the maturation period (ZEB; KHADIM; ALI, 2017) and about twenty to twenty-seven times higher than the values found for different varieties of *Hippophae rhamnoides* leaves (8 -11 μg/g) (POP et al., 2014). In the same study, the contents of zeaxanthin (5-6 μg/g) were about twice lower that *A. chica* leaves. These data are of paramount importance, since high consumption of dark green leafy vegetables, specifically rich in lutein and zeaxanthin, are associated with a lower risk of cataract and age-related macular degeneration (MOELLER; JACQUES; BLUMBERG, 2000). In addition, according to the classification of good sources of carotenoids (low: 0-1 μg/g, moderate: 1-5 μg/g, high: 5-20 μg/g, very high> 20 μg/g), as suggested by Britton and Khachik (2009), the freeze-dried *A. chica* leaves can be considered as a high source of zeaxanthin and very high of lutein, β-carotene and α-carotene.

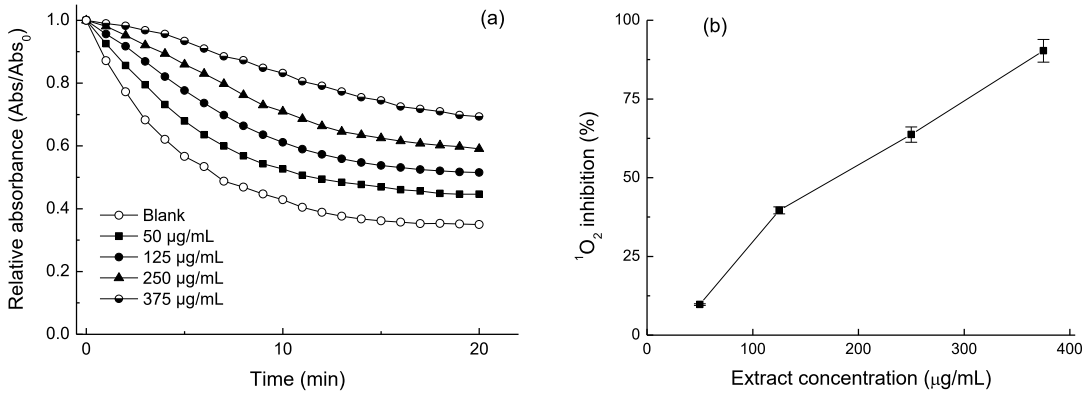
### 3.3. *IN VITRO* ANTIOXIDANT CAPACITY OF A. CHICA EXTRACT

The MeOH/water extract of *A. chica* leaves was able to scavenge ABTS radical with values (86.81 ± 1.5 μM TE/g, FW) higher than those previously reported for *A. chica* leaves (64 μM TE/g, FW) in an exploratory study with fifteen selected plants from Amazonian region (SILVA et al., 2007). Although ABTS radical is a stable and non-biological reactive species, it is a single electron transfer-based assay used by many research laboratories for studying antioxidant capacity due to its operational simplicity. However, when ABTS assay is considered, there are conflicting results on pure antioxidant compounds, associating their structure and ability to donate one or two electrons with their scavenging efficiency (HUANG; OU; PRIOR, 2005). As examples, in a study, scutellarin and scutellarein demonstrated $IC_{50}$ values at 3.53 and 3.00 μM, respectively (QIAN, 2011), and in another study, they exhibited 33.3 μM and 18.3 μM, respectively (LIU, 2018).

In this study, the $^1O_2$-quenching ability of *A. chica* leaf extract was assessed since $^1O_2$ is a highly reactive oxygen species (ROS) that can be generated in both physiological and food systems in the presence of light and photosensitizers. The photosensitization

system used in this study was efficient in the generation of $^1O_2$ with consequent degradation of tryptophan. The *A. chica* extract, at all the tested concentrations, was able to protect tryptophan against the oxidation damage of $^1O_2$ and this quenching effect was fitted to the first-order reaction with high values of determination coefficient ($R^2 = 0.99$), as shown in Figure 3a. This is the first report concerning the quenching ability of *A. chica* leaf extract against oxidative damage of $^1O_2$ and a concentration-dependent effect on $^1O_2$ inhibition was observed for the extract (Fig. 3b) whit an $IC_{50}$ at 177.2 ± 4.2 µg/mL. The extract of *A. chica* leaves at 375 µg/mL exhibited the highest percentage of protection (90.30%) against $^1O_2$, but lower quenching efficiency than that found for quercetin (positive control) ($IC_{50}$ = 1.88 ± 0.08 µg/mL). However, when compared to the literature, the *A. chica* extract showed higher efficiency as $^1O_2$ quencher than other leaf plant extracts, such as *Terminalia chebula* ($IC_{50}$ = 424.50 µg/mL), *Terminalia belerica* ($IC_{50}$ = 233.12 µg/mL) and *Emblica officinalis* ($IC_{50}$ = 490.42 µg/mL), obtained with MeOH/water (70:30, v/v) (HAZRA, et al., 2010), whereas lower than the ethanolic extract of *Vismia cauleflora* ($IC_{50}$ = 27 µg/mL), another Amazonian plant (RIBEIRO et al., 2015).

**Figure 3 -** (a) Decay of absorbance intensity of tryptophan in the presence of sensitizer (methylene blue) and *Arrabidaea chica* leaf extracts and (b) percentage of $^1O_2$ inhibition as a function of the *Arrabidaea chica* leaf extract concentration. Each point represents the values obtained in four concentrations, performed in triplicate (Mean ± standard deviation).




The hydromethanolic extract of *A. chica* leaves exhibited high levels of phenolic compounds, as well as a high antioxidant capacity in both the assays. As scutellarin content accounted for ≈ 70% of the sum of the identified phenolic compounds, it was

suggested that this compound might presented high contribution to the assessed antioxidant capacity since a high antioxidant capacity was already reported in the literature for scutellarin (HONG; LIU, 2004; LIU, et al., 2005). Ascorbic acid was not detected in this extract.

## 4. CONCLUSION

*A. chica* leaves can be seen as a promising source of bioactive compounds with high antioxidant capacity probably due to their high contents of phenolic compounds, which may explain the therapeutic use of these leaves in the folk medicine. For the first time, the presence of feruloyl derivative compounds, scutellarin, methyl apigenin glucuronide and ascorbic acid, and the carotenoid profile of *A. chica* leaves were reported, as well as its protective effect against $^1O_2$, which occurred in a concentration-dependent manner.

## REFERENCES

AOAC (Association of Analytical Chemists) (2000). Official methods of analysis (17th ed.). Gaithersburg, MD, USA: AOAC International.

ABUBAKAR, M., ABAS, F., WEI, S., SHAARI, K., SA, I., GHAZALI, H. M., & LAJIS, N. H. Chemical constituents and biological activities of Callicarpa maingayi leaves. **South African Journal of Botany**, *104*, 98–104, 2016.

ARO, A. A., SIMÕES, G. F., ESQUISATTO, M. A. M., FOGLIO, M. A., CARVALHO, J. E., OLIVEIRA, A. L. R., … PIMENTEL, E. R.. Arrabidaea chica extract improves gait recovery and changes collagen content during healing of the Achilles tendon. **Injury**, *44*(7), 884–892, 2013.

BALASUNDRAM, N., SUNDRAM, K., & SAMMAN, S. Phenolic compounds in plants and agri-industrial by-products : Antioxidant activity , occurrence , and potential uses, **Food Chemistry**, 99, 191–203, 2006.

BERGQUIST, S. Å. M., GERTSSON, U. E., & OLSSON, M. E. Influence of growth stage and postharvest storage on ascorbic acid and carotenoid content and visual quality of baby spinach (Spinacia oleracea L.). **Journal of the Science of Food and Agriculture**, 86(3), 346–355, 2006.

BERTO, A., RIBEIRO, A. B., DE SOUZA, N. E., FERNANDES, E., & CHISTÉ, R. C. Bioactive compounds and scavenging capacity of pulp, peel and seed extracts of the Amazonian fruit Quararibea cordata against ROS and RNS. **Food Research International**, *77*, 236–243, 2015.

BRITTON G, KHACHIK F. Carotenoid in Food. In: Carotenoids: Nutrition and Health. 5: 3. BRITTON, G., LIAAEN-JENSEN, F, PFANDER, H (Eds.). Birkhauser Verlag Basel, ISBN 978-3-7643-7500-3, 2009, pp. 45-66.

BRITTON, G.; LIAAEN-JENSEN, S.; PFANDER, H. Introduction and guidelines on the use of the Handbook. In: **Carotenoids**. Birkhäuser, Basel, 2004. p. 1-33.

CHANDRASEKARA, A., SHAHIDI, F. Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MSn. *Journal of Functional Foods*, *3*(3), 144–158, 2011.

CHISTÉ, R., BENASSI, M. T., & MERCADANTE, A. Z. Effect of solvent type on the extractability of bioactive compounds, antioxidant capacity and colour properties of natural annatto extracts. *International Journal of Food Science and Technology*, *46*(9), 1863–1870, 2011.

CHISTÉ, R. C., MERCADANTE, A. Z. Identification and quantification, by HPLC-DAD-MS/MS, of carotenoids and phenolic compounds from the Amazonian fruit Caryocar villosum. **Journal of Agricultural and Food Chemistry**, 60(23), 2012.

CHOE, E., MIN, D. B. Chemistry and Reactions of Reactive Oxygen Species in Foods. **Critical Reviews in Food Science and Nutrition**, *46:1*, 1–22, 2015.

ČÍŽ, M., ČÍŽOVÁ, H., DENEV, P., KRATCHANOVA, M., SLAVOV, A., & LOJEK, A.. Different methods for control and comparison of the antioxidant properties of vegetables. **Food Control**, 21(4), 518–523, 2010.

CRUPI, P., TOCI, A. T., MANGINI, S., WRUBL, F., RODOLFI, L., TREDICI, M. R., … ANTONACCI, D. Determination of fucoxanthin isomers in microalgae ( Isochrysis sp .) by high-performance liquid chromatography coupled with diode-array detector multistage mass spectrometry coupled with positive electrospray ionization, **Rapid Commun. Mass Spectrom**. (January), 1027–1035, 2013.

DE FARIA, A. F., DE ROSSO, V. V., MERCADANTE, A. Z.. Carotenoid composition of jackfruit (Artocarpus heterophyllus), determined by HPLC-PDA-MS/MS. **Plant Foods for Human Nutrition**, *64*(2), 108–115, 2009.

DE ROSSO, V. V., MERCADANTE, A. Z. Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from Amazonian fruits. **Journal of Agricultural and Food Chemistry**, *55*(13), 5062–5072, 2007.

DEVIA, B., LLABRES, G., WOUTERS, J., DUPONT, L., ESCRIBANO-BAILON, M. T., DE PASCUAL-TERESA, S., … TITS, M. New 3-deoxyanthocyanidins from leaves of Arrabidaea chica. **Phytochemical Analysis**, *13*(2), 2002.

DZIADEK, K., KOPEĆ, A., TABASZEWSKA, M. Potential of sweet cherry (*Prunus avium L.*) by-products: bioactive compounds and antioxidant activity of leaves and petioles. **European Food Research and Technology**, v. 245, n. 3, p. 763-772, 2019.

EKPA, O. D. Nutrient composition of three Nigerian medicinal plants. **Food Chemistry**, *57*(2), 229–232,1996.

EL-HELA, A. A., AL-AMIER, H. A., & IBRAHIM, T. A. Comparative study of the flavonoids of some Verbena species cultivated in Egypt by using high-performance liquid chromatography coupled with ultraviolet spectroscopy and atmospheric pressure chemical ionization mass spectrometry. **Journal of Chromatography A**, 1217(41), 6388–6393, 2010.

FAO/WHO . *Food energy—methods of analysis and conversion factors*. Report of a technical workshop. FAO Food and Nutrition Paper No. 77 Food and Agriculture Organization of the United Nations: Rome, 2003.

FERNANDES, F., VALENTÃO, P., SOUSA, C., PEREIRA, J. A., SEABRA, R. M., ANDRADE, P. B. Chemical and antioxidative assessment of dietary turnip ( Brassica rapa var . rapa L .). **Food Chemistry**, 105, 2007.

FERRERES, F., VALENTÃO, P., RAFAEL, L., PINHEIRO, C., CARDOSO, L., PEREIRA, J. A., … ANDRADE, P. B. Phenolic Compounds in External Leaves of Tronchuda Cabbage ( *Brassica oleracea L.* var . costata DC ). **Journal of Agricultural and Food Chemistry**, 53, 2901–2907, 2005.

HALLIWELL, B. Free radicals and antioxidants: updating a personal view. **Nutrition reviews**, v. 70, n. 5, p. 257-265, 2012.

HAZRA, B., SARKAR, R., BISWAS, S., & MANDAL, N. Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of Terminalia chebula , Terminalia belerica and Emblica officinalis. **BCM Complementary&Alternative Medicine**, *10*, 20, 2010.

HONG, H., & LIU, G. Q. Scutellarin Attenuates Oxidative Glutamate Toxicity in PC12 cells. **Planta Med**, *70*, 427–431, 2004.

HUANG, D., OU, B., & PRIOR, R. L. P. The Chemistry behind Antioxidant Capacity Assays. **Journal of Agricultural and Food Chemistry**, *53*(6), 1841–1856, 2005.

ICH. International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH). Guidance for industry, Q2B validation of analytical procedures: Methodology. Geneva: ICH Secretariat c/o IFPMA7–10, 2005.

KATOCH, N., KAUR, P., KASHYAP, P., GUPTA, S., & DAHIYA, R. S. Role of oxidative stress in cardiovascular diseases. **Research Journal of Pharmaceutical, Biological and Chemical Sciences**, 4(3), 870–881, 2013.

LIU, H., YANG, X., TANG, R., LIU, J., XU, H. Effect of scutellarin on nitric oxide production in early stages of neuron damage induced by hydrogen peroxide. **Pharmacology Research**, 51, 205–210, 2005.

LIU, Q., LI, X., OUYANG, X., CHEN, D. Dual Effect of Glucuronidation of a Pyrogallol-Type Phytophenol Antioxidant : A Comparison between. **Molecules**, 23*(12)*, 3225, 2018.

MAFIOLETI, L., DA SILVA JUNIOR, I. F., COLODEL, E. M., FLACH, A., DE OLIVEIRA, D. T. M. Evaluation of the toxicity and antimicrobial activity of

hydroethanolic extract of Arrabidaea chica (Humb. & Bonpl.) B. Verl. **Journal of Ethnopharmacology**, *150*(2), 576–582, 2013.

MCNAB, H., FERREIRA, E. S. B., HULME, A. N., QUYE, A. Negative ion ESI – MS analysis of natural yellow dye flavonoids — An isotopic labelling study. **International Journal of Mass Spectrometry**, 284, 57–65, 2009.

MOELLER, S. M., JACQUES, P. F., BLUMBERG, J. B. The potential role of dietary xanthophylls in cataract and age-related macular degeneration. **Journal of the American College of Nutrition**, v. 19, n. sup5, p. 522S-527S, 2000.

POP, M. R., WEESEPOEL, Y., SOCACIU, C., PINTEA, A., VINCKEN, J., GRUPPEN, H. Carotenoid composition of berries and leaves from six Romanian sea buckthorn (*Hippophae rhamnoides* L.) varieties. **Food Chemistry**, 147, 1–9, 2014.

QIAN, L., LI, N., TANG, Y., ZHANG, L., TANG, H., WANG, Z., … DING, A.-W. Synthesis and Bio-Activity Evaluation of Scutellarein as a Potent Agent for the Therapy of Ischemic Cerebrovascular Disease. **International Journal of Molecular Sciences**, 12, 8208–8216, 2011.

RAJU, M., VARAKUMAR, S., LAKSHMINARAYANA, R., PARTHASARATHY, T., BASKARAN, V. Carotenoid composition and vitamin A activity of medicinally important green leafy vegetables. **Food Chemistry**, 101, 1598–1605, 2007.

RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M., CATHERINE, R.-E. Antioxidant Activity Applying an Improved Abts Radical Cation Decolorization Assay. **Free Radical Biology & Medicine**, 26(98), 1231–1237,1999.

RIBEIRO, A. B., BERTO, A., CHISTÉ, R. C., FREITAS, M., VISENTAINER, J. V, FERNANDES, E. Bioactive compounds and scavenging capacity of extracts from different parts of Vismia cauliflora against reactive oxygen and nitrogen species. **Pharmaceutical Biology**, 0209, 1267–1276, 2015.

RIBEIRO, A. B., CHISTÉ, R. C., LIMA, J. L. F. C., FERNANDES, E. Solanum diploconos fruits: profile of bioactive compounds and in vitro antioxidant capacity of different parts of the fruit. **Food & Function**, 7(5), 2249–2257, 2016.

RIBEIRO, A. F. C., TELLES, T. C., FERRAZ, V. P., SOUZA-FAGUNDES, E. M., CASSALI, G. D., CARVALHO, A. T., MELO, M. M. Effect of Arrabidaea chica extracts on the Ehrlich solid tumor development. **Brazilian Journal of Pharmacognosy**, 22(2), 364–373, 2012.

RIBEIRO, S. M., DE QUEIROZ, H. J., DE QUEIROZ, M. E. L. R., CAMPOS, F. M., SANT'ANA, H. M. P. Antioxidant in Mango ( *Mangifera indica L* .) Pulp. **Plant Foods for Human Nutrition**, 62, 13–17, 2007.

SHARIFIFAR, F., DEHGHN-NUDEH, G., MIRTAJALDINI, M. Major flavonoids with antioxidant activity from Teucrium polium L . **Food Chemistry**, 112, 885–888, 2009.

SHI, Y., WU, C., CHEN, Y., LIU, W., FENG, F., XIE, N. Comparative analysis of three Callicarpa herbs using high performance liquid chromatography with diode array detector and electrospray ionization-trap mass spectrometry method. **Journal of Pharmaceutical**

**and Biomedical Analysis**, 75, 239–247, 2013.

SILVA, B. A., FERRERES, F., MALVA, O., DIAS, A. C. P. Phytochemical and antioxidant characterization of Hypericum perforatum alcoholic extracts. **Food Chemistry**, 90, 157–167, 2005.

SILVA, E. M., SOUZA, J. N. S., ROGEZ, H., REES, J. F., LARONDELLE, Y. Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. **Food Chemistry**, 101(3), 1012–1018, 2007.

SIRAICHI, J. T. G., FELIPE, D. F., BRAMBILLA, L. Z. S., GATTO, M. J., TERRA, V. A., CECCHINI, A. L., … CORTEZ, D. A. G. Antioxidant Capacity of the Leaf Extract Obtained from Arrabidaea chica Cultivated in Southern Brazil. **Plos One**, 8(8), 1–10, 2013.

SUN, H., MU, T., XI, L., ZHANG, M., CHEN, J. Sweet potato ( Ipomoea batatas L .) leaves as nutritional and functional foods. **Food Chemistry**, 156, 380–389, 2014.

SUROWIEC, I., SZOSTEK, B., TROJANOWICZ, M. HPLC-MS of anthraquinoids, flavonoids, and their degradation products in analysis of natural dyes in archeological objects. **Journal of Separation Science**, 30(13), 2070–2079, 2007.

TOMÁS-BARBERÁN, F. A., ESPÍN, J. C. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables, **Journal of the Science of Food and Agricuture**, 81(February 2001), 853–876, 2001.

VALLEJO, F., TOMÁS-BARBERÁN, F., GARCIA-VIGUERA, C. Health-Promoting Compounds in Broccoli as Influenced by Refrigerated Transport and Retail Sale Period. **Journal of Agricultural and Food Chemistry**, 51, 3029–3034, 2003.

ZEB, A., KHADIM, N., & ALI, W. Changes in the Polyphenolic Profile , Carotenoids and Antioxidant Potential of Apricot ( Prunus armeniaca L .) Leaves during Maturation. **Agriculture**, 7(9), 2017.

ZORN, B., GARCÍA-PIERES, A. J., CASTRO, V., MURILLO, R., MORA, G., MERFORT, I. 3-Desoxyanthocyanidins from Arrabidaea chica. **Phytochemistry**, 56(8), 831–835, 2001.

# CAPÍTULO III

## SCAVENGING CAPACITY OF EXTRACTS OF *ARRABIDAEA CHICA* LEAVES FROM THE AMAZONIA AGAINST ROS AND RNS OF PHYSIOLOGICAL AND FOOD RELEVANCE

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A versão publicada pode ser encontrada no Anexo B.

**Scavenging capacity of extracts of *Arrabidaea chica* leaves from the Amazonia against ROS and RNS of physiological and food relevance**


## ABSTRACT

*Arrabidaea chica*, a medicinal plant found in the Amazon rainforest, is a promising source of bioactive compounds, which can be used to inhibit oxidative damage in both food and biological systems. In this study, the *in vitro* scavenging capacity of characterized extracts of *A. chica* leaves, obtained with green solvents of different polarities [water, ethanol and ethanol/water (1:1, v/v)] through ultrasound-assisted extraction, was investigated against reactive oxygen (ROS) and nitrogen (RNS) species, namely superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl), and peroxynitrite anion ($ONOO^-$). The extract obtained with ethanol/water presented about three times more phenolic compound contents (11.8 mg/g) than ethanol and water extracts (3.8 and 3.6 mg/g, respectively), being scutellarein the major compound (6,76 mg/g). All extracts showed high scavenging efficiency against the tested ROS and RNS, in a concentration-dependent manner, with low $IC_{50}$ values, and ethanol/water extract was the most effective one. In addition, all the extracts were five times more efficient against $ROO^{\bullet}$ than Trolox. Therefore, the extracts from *A. chica* leaves exhibited high promising antioxidant potential to be used against oxidative damage in food and physiological systems.


**Keywords:** Antioxidant capacity; Amazonian plant; reactive oxygen species; reactive nitrogen species; green solvents; phenolic compounds.

## 1. INTRODUCTION

In biological systems, reactive oxygen (ROS) and nitrogen (RNS) species play an essential role in maintaining the body's normal physiological condition (HUYUT et al., 2017; OZTASKIN et al. 2017). However, the overproduction of ROS/RNS, as a result of physiological disorders, combined with the deficiency of endogenous and exogenous antioxidants in the human body induces oxidative and nitrosative stress, damaging basic components for function and cell survival, which may be related to the occurrence of several chronic degenerative diseases, such as cardiovascular diseases, diabetes, Alzheimer's and cancer (ANRAKU et al., 2018; RIBEIRO et al. 2016; RIBEIRO et al., 2018).

In addition to the deleterious effect caused to physiological systems, oxidative reactions induced by the presence of ROS and RNS can also be observed in food systems. The oxidative effects of ROS can occur during food harvesting, storage and processing, producing off-flavors, changes in color and texture, which adversely affect the overall quality and food safety due to the degradation of essential fatty acids, amino acids and vitamins, and might promote the formation of toxic and carcinogenic compounds (CHOE; MIN, 2006; PATEIRO et al., 2021 SHAHIDI; ZHONG, 2010; SHAHIDI; ZHONG 2015).. Due to these harmful effects, the inhibition of ROS and RNS in food systems is highly desirable and, therefore, the use of antioxidants as food additive (natural or artificial) can be seen as a very efficient strategy to delay oxidative reactions that decrease the shelf life of processed foods.

Scientific evidences suggest that antioxidants obtained from natural sources, such as fruits and other vegetables, are efficient replacements to synthetic antioxidants with the same purpose of preventing the formation of undesirable oxidation products, improving the quality of food products, and as consequence improving the life-quality of consumers (CHONG et al., 2015; MEZZA et al, 2018; RIBEIRO et al, 2015; SILVA FARIA et al, 2020; WANG et al, 2018). Scientific data combined with the perception of modern consumers about the need for foods with natural and healthier ingredients in the formulation resulted in a growing trend at the scientific level and in the food industry for the incorporation of natural additives.

In this context, the Amazon is known as one of the richest biome in the world concerning biodiversity, including several claimed medicinal plants, which present a number of bioactive molecules in their composition, with promising biological activities (OLIVEIRA et al., 2013). Among these plants, *Arrabidaea chica* (Humb. & Bonpl.) B. Verl. (pariri), from the Bignoniaceae family, is potentially rich in phenolic compounds that has been used to treat

various diseases (MAFIOLETI et al., 2013; RODRIGUES et al., 2014; ZORN et al., 2001). Some studies have also reported anti-inflammatory, antimicrobial, and antifungal properties for the leaves of *A. chica* (BARBOSA et al., 2008; FERREIRA, et al, 2013; OLIVEIRA, et al., 2009).

According to a recent study published by our research group, *A. chica* leaves were considered as promising sources of phenolic compound contents, being feruloyl hexose, scutellarin, apigenin glucuronide, flavone-glucuronyl derivative, scutellarein, methyl apigenin glucuronide and apigenin the tentatively identified compounds, in addition to exhibit high quenching capacity against singlet oxygen ($^1O_2$) (DE SIQUEIRA et al. 2019), a highly reactive ROS derived from molecular oxygen.

Other studies also showed that the extract of *A. chica* leaves has higher antioxidant capacity when compared to scutellarein and apigenin isolated from the leaf, as evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl), β-carotene/ linoleic acid, TRAP (total reactive antioxidant potential) and peroxyl radicals ($ROO^{\bullet}$) assays (SILVA et al. 2007; SIRAICH et al. 2013). Furthermore, in an *in vitro* cellular study, post-treatment with *A. chica* in L929 fibroblasts decreased oxidative damage by inhibiting intracellular ROS and mitochondrial superoxide anion radical ($O_2^{\bullet-}$) induced by UV-A and UV-B irradiation (RIBEIRO et al., 2018). However, to the best of our knowledge, there are no reports in the literature regarding the direct antioxidant effect of *A.chica* extracts against other ROS and RNS commonly found in physiological and food systems, namely $O_2^{\bullet-}$, hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl) and peroxynitrite anion ($ONOO^-$). Such information may stimulate relevant applicability to extracts obtained from *A. chica* leaves to confer antioxidant action in several applications of interest to the food, pharmaceutical and cosmetic industries.

On the other hand, it should be taken into account that several factors can significantly affect the phytochemical content and antioxidant capacity of natural extracts, including the type and polarity of the solvent used (CHISTÉ; DE TOLEDO BENASSI.; MERCADANTE, 2014; LOU; HS; HO, 2014). In addition, there is a greater appeal for the use of solvents that favor green chemistry, associated with the use of emergent extraction techniques, for example, ultrasound-assisted extraction (UAE), which is less time-consuming, safer, promotes high-efficiency extractions, and can minimize environmental impacts (CVJETKO BUBALO et al., 2018; FILIPPI et al. 2015).

Therefore, our study investigated the *in vitro* antioxidant capacity of *A. chica* extracts, obtained through UAE technique using three green solvents of different polarities [water,

ethanol and ethanol/water mixture (1:1, v/v)] against ROS and RNS of food and physiological relevance, namely $O_2^{\bullet-}$, $H_2O_2$, HOCl, $^1O_2$, $ROO^{\bullet}$ and $ONOO^-$ (in presence and absence of $NaHCO_3$ to simulate physiological conditions).

## 2. MATERIALS AND METHODS

### 2.1. CHEMICALS

Scutellarin, apigenin, quercetin, L-ascorbic acid, quercetin 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), lucigenin, fluorescein Dihydrorodamine (DHR), phenazine methosulfate (PMS), nitroblue tetrazolium chloride (NBT), α,α′-Azodiisobutyramidine dihydrochloride (AAPH), dimethyl sulfoxide (DMSO), sodium hypochlorite solution (NaClO, with 4% available chlorine), β-nicotinamide adenine dinucleotide (NADH), Tris-buffer HCl, methylene blue (MB), hydrogen peroxide (30%), ethanol (EtOH), methanol (MeOH), acetonitrile, formic acid, methylene blue (MB), sulfuric acid, formic acid, sodium chloride, tribasic sodium phosphate dodecahydrate, potassium chloride, sodium nitrite, sodium bicarbonate, monobasic potassium phosphate and dibasic sodium phosphate were purchased from Sigma-Aldrich (St. Louis, USA). L-tryptophan was obtained from Fisher Scientific (Pittsburgh, PA, USA). For all chromatographic analyses, samples and solvents were filtered using, respectively, 0.22 and 0.45 μm membranes, both from Millipore (Billerica, MA, USA). Ultrapure water was obtained from the Milli-Q system (Millipore Corp., Milford, MA, USA).

### 2.2. ARRABIDAEA CHICA LEAVES

The leaves of *A. chica* (≈ 250 g), collected from five different plants belonging to the Active Germplasm Bank of *EMBRAPA Amazonia Oriental*, located in Belém, Pará, Brazil (01°26'14.7"S and 48°26'52.2"W), were freeze-dried (Liotop, L101, São Paulo, Brazil), ground in a knife mill, vacuum packed in plastic bags and stored at -18 °C until analysis.

The access to the selected leaves was registered in the Brazilian National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen #A89EDD3).

## 2.3. EXTRACTS OF ARRABIDAEA CHICA LEAVES

Three extracts from the leaves of *A. chica* were prepared, according to the methodology described by Chisté et. al. (2011) with some modifications, using the following green solvents: water, ethanol and ethanol/water mixture (1:1, v/v). The choice for these solvents considered the permissibility of residues in the extracts after evaporation, in accordance with Directive 95/45/EC of the Commission of the European Communities (DIRECTIVE 95/45 / CE da Comissão, 1995).

The freeze-dried leaves of *A. chica* (3 g) were subjected to UAE with each green solvent in an ultrasonic bath (QUIMIS - model 03350, Diadema-SãoPaulo /Brazil) for 5 min at room temperature (25 °C), and fixed ultrasonic frequency at 25 KHz, at the solid-liquid ratio of 1:10 (w/v). After the UAE procedure, the extracts were centrifuged (Heraeus multifuge x 1R Thermo Electron Led GMBH) at 11,648 x *g* for 5 min. The extraction procedure was repeated seven times for each solvent and the supernatants were combined after vacuum filtration. The extracts containing ethanol in the composition were subjected to evaporation at reduced pressure in a rotary evaporator (T< 38 °C). Water extract and the remaining water in the ethanol/water extract were frozen and freeze-dried. All the dried extracts were sealed under $N_2$ flow and stored at -18 °C, under light-free conditions, until analysis. The extractions were carried out in triplicate (n = 3).

### *2.4. HPLC-DAD determination of phenolic compounds in the A. chica extracts*

The phenolic compounds composition of *A. Chica* extracts were determined by high performance liquid chromatography (HPLC) coupled to a diode array detector (DAD) on an Agilent HPLC (model Agilent 1260 Infinity, Santa Clara, CA, USA) equipped with a quaternary pump (G1311C), an automatic injector (G7129), an oven (G1316A) and a DAD detector (G1328C).

The *A. chica* extracts were analyzed after solubilizing 10 mg of each dried extract in methanol/water (80:20, v/v) and the phenolic compounds were separated on a $C_{18}$ Synergi Hydro column (Phenomenex, 4 μm, 250 × 4.6 mm), at a temperature of 29 °C at 0.9 mL/min, with a linear gradient consisting of water/formic acid (99.5: 0.5, v/v) and acetonitrile/ formic acid (99.5 : 0.5, v/v) (CHISTÉ & MERCADANTE, 2012).

The UV-visible (UV-vis) spectra were recorded from 200 to 600 nm, and chromatograms were processed at 270, 320 and 360 nm. The phenolic compounds were identified by combining the following information: elution order, retention time in the $C_{18}$ column, comparison with

authentic standards analyzed under the same conditions, and UV-Vis spectra compared to the phenolic compounds previously identified for *A. chica* leaves, by LC-MS, by our research group (DE SIQUEIRA et al, 2019). The phenolic compounds were quantified using six-point analytical curves (3 to 100 µg/mL, in duplicate), of scutellarin [$r^2$ = 0.99, limit of detection (LOD) = 0.14µg/mL and limit of quantification (LOQ) = 0.41 µg/mL], scutellarein ($r^2$ = 0.98, LOD = 0.23 µg/mL and LOQ = 0.69 µg/mL) and apigenin ($r^2$= 0.99, LOD = 0.17 µg / mL and LOQ = 0.51 µg/mL). The parameters of the analytical curves (standard deviation and the slope) were used to calculate the LOD and LOQ values (ICH, 2005). The phenolic compound contents were expressed as mg/g of dried extracts (dry basis, d.b.), considering three independent extraction procedures (n = 3).

## 2.5. IN VITRO SCAVENGING CAPACITY DETERMINATION AGAINST ROS AND RNS

The ROS- and RNS-scavenging assays were carried out at 37 °C, in a microplate reader (Synergy HT, BioTek, Vermont, USA) equipped with a thermostat using fluorescence, absorbance or chemiluminescence modes of detection. Each antioxidant assay corresponds to, at least, four individual experiments, in triplicate, using five concentrations (0.03 to 500 µg/mL). The dried extracts of *A. chica* were dissolved in ethanol/water (1:1, v/v) for all the assays, and analyzed immediately to avoid degradation of the bioactive compounds. Quercetin (0.001 to 30 µg/mL) and scutellarein (0.10 – 500 µg/mL) were used as positive controls in the $O_2^{\bullet-}$, HOCl, $^1O_2$ and $ONOO^-$scavenging assays, while scutellarein and ascorbic acid (15 to 500 µg/mL) were used for $H_2O_2$. $IC_{50}$ values (µg/mL) were calculated from curves of antioxidant concentrations *versus* the inhibition percentage using Origin Pro 8 software (OriginLab Corporation, Northampton, MA, USA). Before carrying out each assay, additional tests to check interference effects among the *A. chica* extracts and the solvents with the used probes or selected wavelengths were done and none interference was observed for the assay conditions.

### 2.5.1. SUPEROXIDE ANION RADICAL ($O_2^{\bullet-}$) SCAVENGING ASSAY

*A. chica* extracts were evaluated in relation to their capacity to scavenge $O_2^{\bullet-}$, using a non-enzymatic system containing NADH/PMS/$O_2$ (CHISTÉ et al., 2011). This system is able to produce $O_2^{\bullet-}$, which reduces NBT to a purple diformazan. The reaction mixtures in the microplate wells contained the following reagents at final concentrations (in a final volume of 300 µL): NADH (166 µM), NBT (43.3 µM), PMS (2.7 µM), the extracts dissolved in EtOH:$H_2O$ solution (1:1, v/v) and quercetin dissolved in DMSO. NADH, NBT and PMS were

dissolved in 19 mM phosphate buffer, pH 7.4. The percentage of inhibition of NBT reduction to diformazan, by the extract/standards, was monitored by spectrophotometry at 560 nm after 10 min of plate introduction.

### 2.5.2. HYDROGEN PEROXIDE ($H_2O_2$) SCAVENGING ASSAY

The scavenging capacity of *A.chica* extracts against $H_2O_2$ was determined by monitoring the inhibition of chemiluminescence resulting from $H_2O_2$-induced oxidation of lucigenin (CHISTÉ et al., 2011). The reaction mixtures in the wells contained the following reagents at final concentrations (in a final volume of 250 µL): 50 mM Tris-HCl buffer (PH 7.4), lucigenin (0.8 mM) dissolved in Tris-HCl buffer, extracts of *A. chica,* and 1% (w/w) $H_2O_2$. The chemiluminescence signal was detected in the microplate reader after 5 min of plate introduction. Results were expressed as percentage of inhibition of $H_2O_2$-induced oxidation of lucigenin.

### 2.5.3. HYPOCHLOROUS ACID (HOCL) SCAVENGING ASSAY

The scavenging capacity of *A. chica* extracts against HOCl was determined by monitoring the inhibition of HOCl-induced oxidation of DHR to rhodamine 123 (CHISTÉ et al., 2011). HOCl was prepared by adjusting a 1% (v/v) NaClO solution to pH 6.2 with $H_2SO_4$ 10% (v/v, followed by HOCl quantification by spectrophotometry at 235 nm, using the molar absorption coefficient of 100 $M^{-1}.cm^{-1}$ [32]. The reaction mixture was composed of the following reagents at the indicated final concentrations (final volume of 300 µL): DHR (5 µM), HOCl (5 µM), and *A. chica* extracts. The fluorescence signal, at an emission wavelength at 528 ± 20 nm and excitation at 485 ± 20 nm, was detected in the microplate reader immediately after plate insertion. Results were expressed as percentage of inhibition of HOCl-induced oxidation of DHR.

### 2.5.4. PEROXYNITRITE ANION ($ONOO^-$) SCAVENGING ASSAY

The scavenging capacity of the *A. chica* extracts against $ONOO^-$ was determined by monitoring the inhibition of $ONOO^-$ induced oxidation of DHR to fluorescent rhodamine 123 [32]. $ONOO^-$ was synthesized as described by (GOMES et al., 2007). The reaction mixture was composed of the following reagents at the indicated final concentrations (final volume of 300 µL): DHR (5 µM), extract or standard and $ONOO^-$ (600 nM). The fluorescence signal, at an emission wavelength at 528 ± 20 nm and excitation at 485 ± 20 nm, was detected in the

microplate reader after a 2 min incubation period. In a set of parallel experiments, assays were conducted in the presence of 25 mM $NaHCO_3$ to simulate physiological conditions of $CO_2$ concentration. The results were expressed as percentage of inhibition of $ONOO^-$ induced oxidation of DHR.

### 2.5.5. PEROXYL RADICAL SCAVENGING ASSAY (ROO•) (ORAC)

$ROO^{\bullet}$ was generated by the thermodecomposition of AAPH at 37 °C, and the $ROO^{\bullet}$ scavenging capacity was measured by monitoring the effect of *A. chica* extract on the inhibition of fluorescence decay, due to fluorescein oxidation, induced by $ROO^{\bullet}$ (OU; HAMPSCH-WOODILL; PRIOR, 2011; RODRIGUES et al., 2012). The reaction mixtures consisted of the following reagents at the final concentrations (final volume of 200 µL): fluorescein (61.2 nM), AAPH solution (19.1 mM), different concentrations of *A. chica* extracts dissolved in 75 mM phosphate buffer (pH 7.4). Trolox was used as positive control. The fluorescence signal, at the emission wavelength of 528 nm with excitation at 485 nm, was monitored every minute until the total decay of fluorescence. The relative ability to capture $ROO^{\bullet}$ was expressed as the ratio between the slope of the curve of each extract or positive control and the slope obtained for trolox, as proposed by Rodrigues, et al. (2012).

### 2.5.6. SINGLET OXYGEN ($^1O_2$) QUENCHING ASSAY

The scavenging capacity of *A. chica* extracts and positive controls (quercetin and scutellarein) to inhibit $^1O_2$ was evaluated according to the method described by De Siqueira et al. (2019). The $^1O_2$ was generated, at room temperature (25 °C) and under atmospheric air, by direct sensitization of methylene blue (MB) by a 75W incandescent lamp, used as an excitation source, and two filters (red and orange) were placed between the excitation source and the cuvette containing the reactants (*A. chica* extratcs, L-tryptophan and MB), to excite MB only. The reaction was monitored by spectrophotometry, in the range of 200-800 nm, for 20 min and the absorbance of tryptophan was recorded at 219 nm. The kinetic data obtained from the tryptophan absorbance decay were fitted to a first-order reaction to calculate the rate constants. The percentage of protection of the *A. chica* extract, or the positive controls, against the oxidative damage of $^1O_2$ was calculated through Equation (1).

$$Protection\ (\%) = \frac{K_{obs}^{TRP} - K_{obs}^{TRP+antioxidant}}{K_{obs}^{TRP}} \text{x } 100 \qquad (1)$$

where, $K_{obs}^{TRP}$ is the rate constant for the observed pseudo-first order reaction fitted to the TRP decay curve (obtained in the blank experiment); and $K_{obs}^{TRP+antioxidant}$ is the rate constant for the observed pseudo-first order reaction fitted to the decay curve *of TRP* in the presence of the antioxidant compound.

### 2.6. STATISTICAL ANALYSIS

The $IC_{50}$ values (mean ± standard deviation) were subjected to ANOVA analysis of variance and the means were classified by Tukey's test at the 95% significance level using Statistica 7.0 software (Statsoft Inc.). Analytical curves were plotted by linear regression ($p<0.05$) using Origin 8 Software (OriginLab Corporation, Northampton, MA, USA).

## 3. RESULTS AND DISCUSSION

### 3.1. PHENOLIC COMPOUNDS COMPOSITION OF ARRABIDAEA CHICA EXTRACTS

Any modification in the composition of a selected solvent alters its polarity, and consequently promotes changes in the phenolic compounds composition during extraction procedures (CHISTÉ et al., 2011). Considering the low toxicity, good extraction yield, safety for human consumption and its application in the food industry, ethanol, ethanol/water mixture (1:1, v/v) and water were used as green solvents. Thus, in addition to the cavitation promoted by the UAE procedure that facilitate bioactive compounds extraction from plant tissues, the composition of phenolic compounds identified in the extracts of *A. chica* leaves were result of their solubility in each solvent (Table 1).

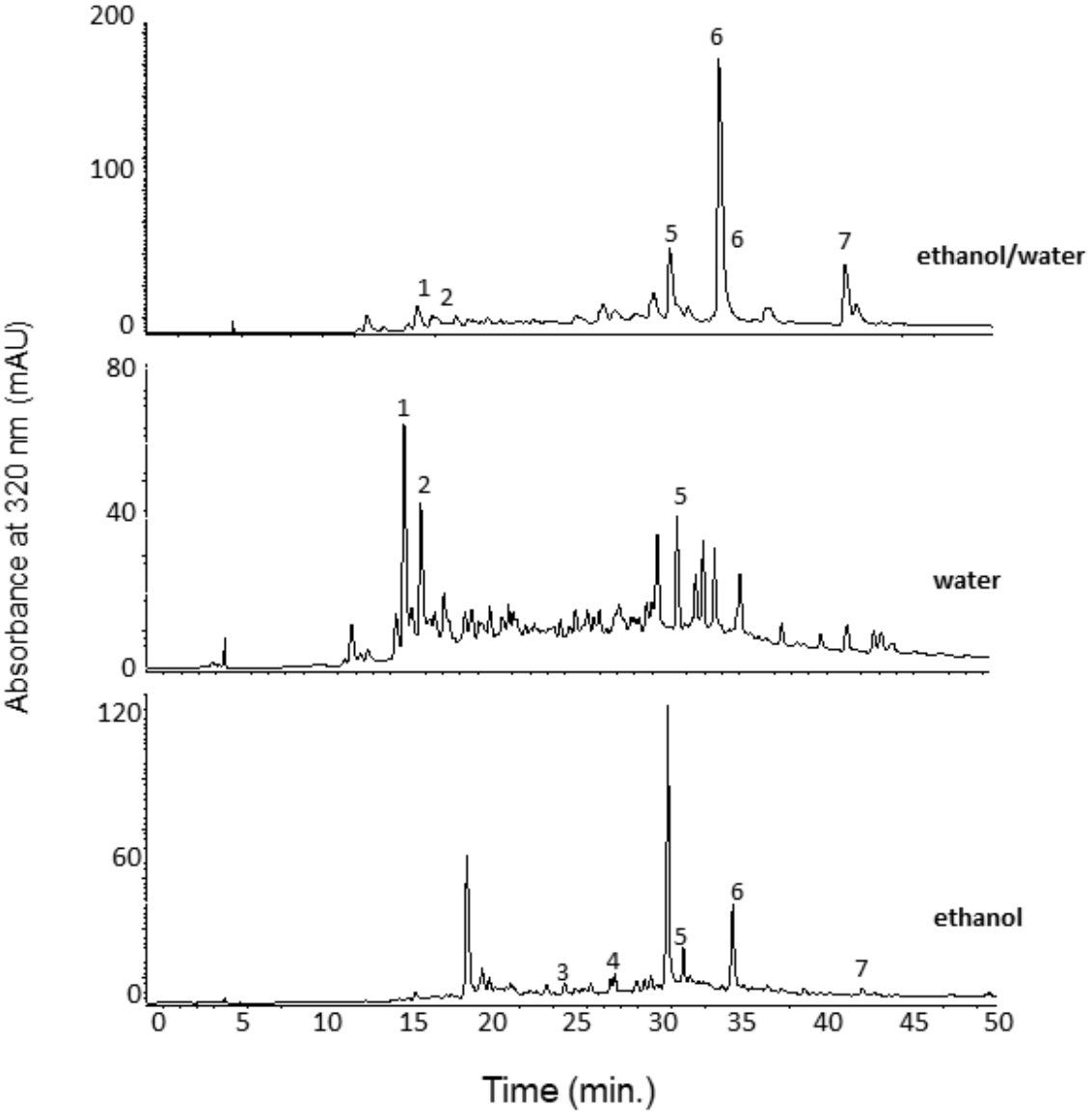
HPLC analysis of the freeze-dried extracts of *A. chica* leaves allowed the separation and quantification of seven phenolic compounds (Figure 1). However, variations were observed among the extracts regarding the individual compounds. The highest levels of phenolic compounds were found in the ethanol/water (1:1, v/v) extract (Table 1), about 3 times higher than the extracts obtained with ethanol and water separately.

**Table 1.** Profile of phenolic compounds from extracts of *A. chica* determined by HPLC-DAD.

| Peak | Phenolic compound* | $t_R$ (min)[a] | $\lambda_{max}$ (nm)[b] | Concentration (mg/g extract)[c] | | |
|---|---|---|---|---|---|---|
| | | | | **EtOH/$H_2O$** | **$H_2O$** | **EtOH** |
| 1 | Feruloyl hexose (isomer 1)[d] | 15 -16,3 | 313 | 0.85 ± 0.03 | 1.71 ± 0.06 | nd |
| 2 | Feruloyl hexose (isomer 2)[d] | 16,6 - 17,5 | 309 | 0.49 ± 0.05 | 1.28 ± 0.03 | nd |
| 3 | Feruloyl derivative [d] | 24,7 | 273, 327 | nd | nd | 0.45 ± 0.01 |
| 4 | Scutellarin [e] | 27,7 | 282, 334 | nd | nd | 0.67 ± 0.09 |
| 5 | Flavone-glucuronyl derivative [d] | 31,6 - 32,6 | 275, 328 | 2.18 ± 0.36 | 0.82 ± 0.05 | 0.88 ± 0.07 |
| 6 | Scutellarein[d] | 34,6 - 35,5 | 282, 337 | 6.79 ± 0.59 | nd | 1.51 ± 0.13 |
| 7 | Apigenin[f] | 42,3 - 43,3 | 267,293, 337 | 1.49 ± 0.09 | nd | 0.09 ± 0.02 |
| | **Total sum (mg/g)** | | | **11.80 ± 1.13** | **3.81 ± 0.15** | **3.62 ± 0.25** |

*Tentative identification based on the retention time on $C_{18}$ column, UV-visible spectra and comparison with the identification previously carried out by our research group for *A. chica* leaves (De Siqueira et al 2019). [a]Retention time ($t_R$) on $C_{18}$ column. [b]Solvent: gradient of water with 0.5% formic acid and acetonitrile with 0.5% formic acid. [c]Mean ± standard deviation (n=3, dry basis). The peaks were quantified as equivalent of [d]scutellarein, [e]scutellarin and [f]apigenin. Abbreviations: nd, not detected; EtOH/$H_2O$, ethanol/water (1:1, v/v); $H_2O$, water; EtOH, ethanol.

**Figure 1.** HPLC-DAD chromatogram at 320 nm of phenolic compounds of the extracts of *Arrabidaea chica* leaves. Peak characterization is given in Table 1.




The mixture of solvents of different polarities, such as ethanol, which has medium polarity, and water, which is a strong polar solvent, promote high efficiency in the extraction of a range of phenolic compounds of different degrees of polarity (ALVES ALCÂNTARA, 2018; ILAIYARAJA et al. 2015; PEREIRA et al., 2017)

In addition, mixing water with organic solvents was reported to increase the efficiency of phenolic compounds extraction from dry samples, since it allows the hydration of dry particles and swelling of plant tissues, favoring the penetration of the organic solvent into the

plant matrix (SHI et al. 2014) and, consequently, increase mass transfer by molecular diffusion (GHITESCU et al. 2015). Furthermore, ethanol breaks the bonds between the sample matrix and phenolic compounds, which increases the recovery of these compounds (GHITESCU et al. 2015).

Unlike the extract of *A. chica* leaves using methanol/water (8:2, v/v), as previously published by our research group (DE SIQUEIRA et al, 2019), which showed scutellarin as the major compound, in the ethanol/water extract (1:1, v/v) (Figure 1) only its aglycone form (scutellarein) was identified, comprising about 57% of the total area of the phenolic compounds (Table 1). Both the compounds were found in the extract obtained with ethanol, being scutellarein found at the highest concentration (41%).

On the other hand, in the extract obtained with water, the flavonoids concentration were lower (glucuronyl-flavone derivative) or not detected (scutellarin, scutellarein and apigenin). However, water extract presented the highest levels of phenolic acids (isomers of ferulic acid glycosides), confirming the selectivity of water for compounds of high polarity in the composition of *A. chica* leaves.

The ethanol/water extract of *A. chica* leaves showed total phenolic compound contents ≈ 10 times lower than that found in the hydromethanolic extract of the same plant (21.5 mg/g) (DE SIQUEIRA et al, 2019). By comparing with extracts obtained from other vegetables, it showed lower values than the hydroalcoholic extract of artichoke leaves (*Cynara cardunculus*) (73 mg/g) (PISTÓN et al, 2014), similar to *Eryngium foetidum* (9.99 mg/g) (LEITÃO et al., 2020) and higher than extracts obtained from the peel (5.4 mg/g) and pulp (1.8 mg/g) of *Antrocaryon amazonicum* fruits (Barbosa et al, 2021).

## 3.2. ROS- AND RNS- SCAVENGING CAPACITY OF THE *ARRABIDAEA CHICA* EXTRACTS

*A. chica* extracts and scutellarein (authentic standard) were able to scavenge all the ROS and RNS tested, with $IC_{50}$ values at low µg/mL ranges (Table 2) and in a concentration-dependent manner (Figure 2).

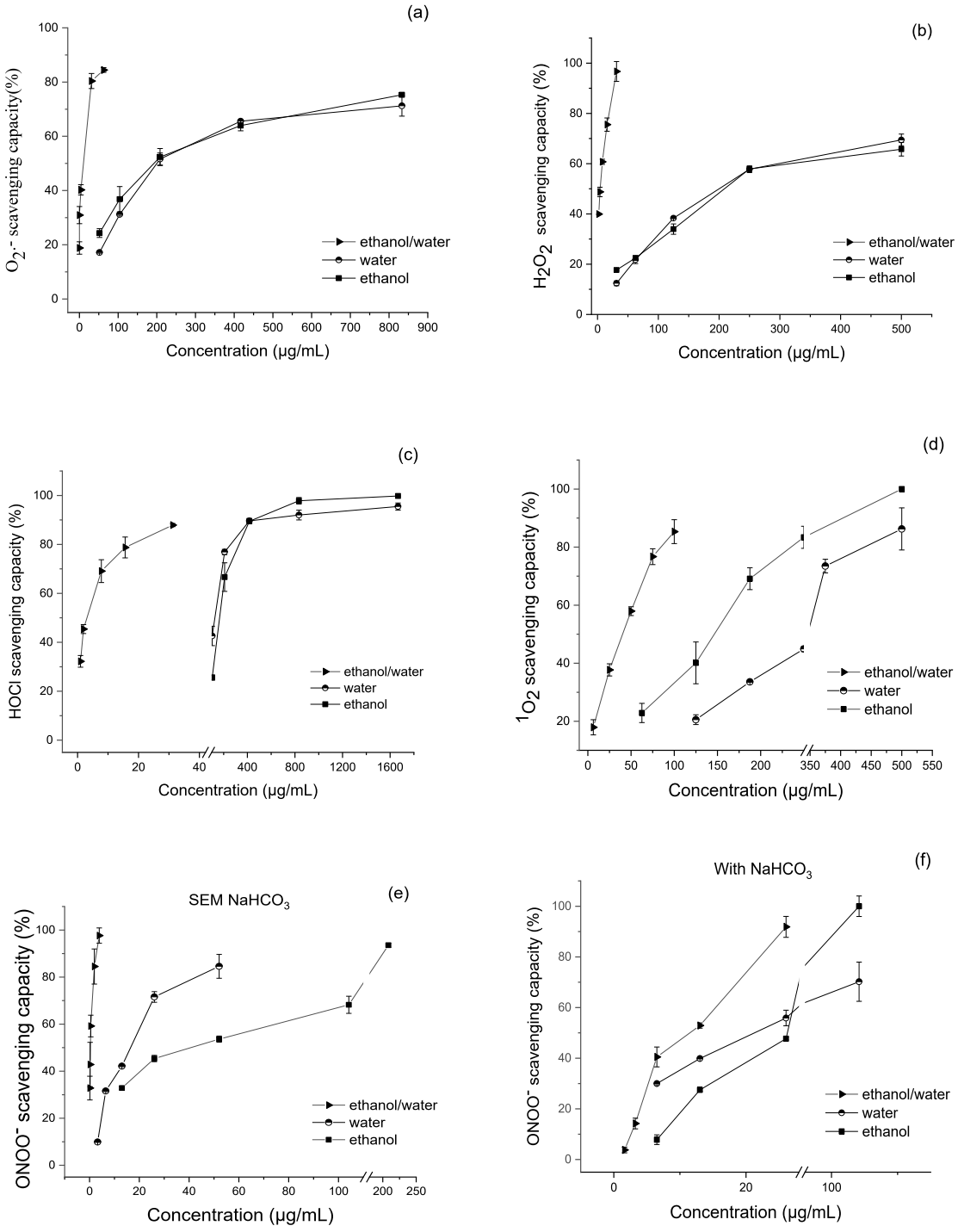
In general, the extract obtained with ethanol/water (1:1, v/v), which presented the highest contents of phenolic compounds, exhibited higher antioxidant efficiency against all the ROS and RNS tested, with lower $IC_{50}$ values, (Table 2). Interestingly, all the extracts showed the same scavenging capacity against $ROO^{\bullet}$, with values five times higher than trolox (positive control).

**Table 2. –** Scavenging capacity of extracts of *A. chica* leaves and standard compounds against reactive oxygen species (ROS) and reactive nitrogen species (RNS).

| Extract/ compound | ROS | | | | | RNS | |
|---|---|---|---|---|---|---|---|
| | $IC_{50}$ (µg.mL$^{-1}$)* | | | | $S_{sample}/S_{trolox}$ | $IC_{50}$ (µg.mL$^{-1}$) | |
| | $O_2^{\bullet-}$ | $H_2O_2$ | HOCl | $^1O_2$ | ROO$^{\bullet}$ | $ONOO^-$ | |
| | | | | | | Absence of $NaHCO_3$ | Presence of $NaHCO_3$ |
| $H_2O$ | 204 ± 13$^a$ | 198 ± 4$^a$ | 127 ± 9$^b$ | 271 ± 1$^a$ | 5.00 ±<0.01$^a$ | 16.5 ± 0.2$^b$ | 21 ±1$^b$ |
| EtOH/$H_2O$ | 10 ± 1$^c$ | 4.3 ± 0.5$^d$ | 2.9 ± 0.3$^c$ | 35 ± 6$^c$ | 5.00 ±<0.01$^a$ | 0.34 ± 0.07$^d$ | 11.1 ± 0.7$^c$ |
| EtOH | 196 ± 2$^a$ | 210 ± 9$^a$ | 166 ± 14$^a$ | 143 ± 8$^b$ | 5.00 ±<0.01$^a$ | 40.3 ± 0.6$^a$ | 28.3 ± 0.9$^a$ |
| **Positive control** | | | | | | | |
| Scutellarein | 107 ± 9$^b$ | 151 ± 5$^b$ | 4.40 ± 0.10$^c$ | 7.8 ± 0.2$^d$ | 0.15 ±< 0.01$^b$ | 7 ± 0.4$^c$ | 4.7 ± 0.4$^d$ |
| Quercetin | 12.9 ± 0.5$^c$ | - | 13 ± 1$^c$ | 1.9 ± 0.1$^d$ | - | 0.01± < 0.01$^d$ | 0.01± < 0.01$^e$ |
| Ascorbic acid | - | 41 ± 7$^c$ | - | - | - | - | - |
| Trolox | | | | | 1.00 | | |

*$IC_{50}$, inhibitory concentration, *in vitro*, to decrease the oxidizing effect of each reactive species by 50% (mean ± standard deviation) (n = 3, dry basis). Ssample = slope for the curve of *A. chica*. $S_{Trolox}$ = slope for the trolox curve. $H_2O$ = water; EtOH/$H_2O$ = ethanol/water; EtOH = ethanol. Means at the same column with the same lowercase superscript letters are statistically equals, at 95% significance (Tukey's test).

**Figure 2.** – Concentration-dependent behavior of extracts of *Arrabidaea chica* leaves against (a) superoxide anion radical ($O_2^{\bullet-}$), (b) hydrogen peroxide ($H_2O_2$), (c) hypochlorous acid (HOCl), ( d) singlet oxygen ($^1O_2$) and (e) peroxynitrite anion ($ONOO^-$) in the absence and (f ) in the presence of $NaHCO_3$. Each point represents the values obtained by four experiments, at five concentrations, carried out in triplicate (mean ± standard deviation).



In relation to $O_2^{\bullet-}$, the extract of *A. chica* obtained with ethanol/water (1:1, v/v) was significantly more effective than those obtained with ethanol and water, and it showed higher scavenging capacity than quercetin (positive control) (Table 2), other medicinal plants, such as *Castanea sativa* (13.60 µg/mL) and *Quercus robur* (11.00 µg/mL) (ALMEIDA; FERNANDES, 2008), and about 5 times superior than the leaves of *Vismia cauliflora* (medicinal plant from the Amazonia) (54.00 µg/mL) (RIBEIRO et al., 2015). . The high efficiency of the ethanol/water extract (1:1, v/v) may be associated, in addition to the presence of scutellarein (major compound), to the synergy with other phenolic compounds, given the fact that this extract was considered a more effective scavenger of $O_2^{\bullet-}$ when compared with scutellarein (about 10 times).

$O_2^{\bullet-}$ can be formed enzymatically and chemically from triplet oxygen (molecular oxygen, $^3O_2$). This ROS is very important in the reduction of oxygen to generate other reactive species, such as $H_2O_2$, hydroxyl radical ($HO^{\bullet}$) and $^1O_2$. In food systems, it can be generated, in addition to the action of enzymes, such as xanthine oxidase, by ohmic food processing, gamma irradiation, microwaves and pulsed electric field and by the reaction of $^3O_2$ with the decomposition products of some azo compounds, such as azo dyes (CHOE; Min, 2006; PASTOR et al., 2004).

In most organisms, $O_2^{\bullet-}$ is converted into $H_2O_2$ by the action of the enzyme superoxide dismutase (SOD). Although $H_2O_2$ is not a free radical, it has a reactive potential, and in the presence of metal ions, it produces $HO^{\bullet}$, which is an oxidizing species with very high reactivity (BERTO et al., 2015; VALKO et al., 2007). Likewise, in foods, $H_2O_2$ can indirectly impart the loss of quality, since $HO^{\bullet}$ can act as an initiator of lipid peroxidation (CHOE; MIN, 2006). The ethanol/water extract (1:1, v/v) of *A. chica* was the most efficient in scavenging $H_2O_2$ among the tested extracts, with higher efficiency than scutellarein and ascorbic acid, both used as positive controls (Table 2). On the other hand, all the tested extracts also showed high efficiency when compared to other plant extracts, with $IC_{50}$ values about 1 to 66 times lower than those of *Vismia cauliflora* leaves (289.00 µg/mL) (Ribeiro et al., 2015) and 2 to 88 times of extracts of *Juglans regia* (383 µg/mL) (FREITAS; LIMA.; FERNANDES, 2009).

Another important ROS, HOCl can be generated in the presence of $H_2O_2$, produced by activated neutrophils and monocytes, where the enzyme myeloperoxidase (MPO) catalyzes the oxidation of chloride ion ($Cl^-$). HOCl is highly harmful and causes oxidation and chlorination reactions in biological systems (FREITAS; LIMA.; FERNANDES, 2009). It is considered a potent pro-inflammatory agent and, consequently, associated with a number of diseases resulting from

chronic, degenerative inflammation and various types of cancer (MALLE, et al, 2006; HO, et al., 2013). In this study, the ethanol/water extract (1:1, v/v) was also the most potent HOCl scavenger, followed by the water and ethanol extracts; being even more efficient than the quercetin and scutellarein (Table 2). Likewise, it showed higher scavenging efficiency than other hydrophilic extracts of Amazonian fruits, such as *Byrsonima crassifolia* (10 µg / mL) (MARIUTTI et al, 2014) and *Solanum sessiliflorum* (13 µg/mL) (RODRIGUES; MARIUTTI; MERCADANTE, 2013), and the $IC_{50}$ value was close to the values reported for extracts of artichoke (*Cynara cardunculus*) leaves (3.7 to 4.7 µg/mL) (PISTON et al., 2014).

Regarding the ability to quench $^1O_2$, a highly reactive ROS that is frequently generated in both physiological and food systems, the ethanol/water extract (1:1, v/v) of *A. chica* also showed the highest antioxidant capacity, yet less effective than quercetin (1.88 µg/mL) and scutellarein (7.84 µg/mL). The ethanol/water extract presented an $IC_{50}$ value close to the values reported for extracts of *Vismia cauliflora* leaves (27.00 µg/mL) (RIBEIRO et al., 2015) and *Cynara cardunculus* (29.00 µg/mL) (PISTON et al., 2014), and about seven times more efficient than *Solanum diploconos* (269 µg/mL), a native Brazilian fruit (RIBEIRO et al., 2016). Natural extracts that exhibit high $^1O_2$-quenching ability are highly desirable, since this ROS, induced by light and in the presence of photosensitizers such as chlorophyll, phaeophytins, riboflavin, myoglobin and heavy metals, may react with unsaturated fatty acids, by non-radical pathways, as in photo-oxidation to speed up lipid peroxidation (CHOE; MIN, 2006; SHAHID; ZONG, 2010). As an example, the incidence of light on foods, such as beer, milk and cheese, in the presence of riboflavin and other photosensitizers can promote the formation of off-flavors due to the degradation of lipid and protein molecules (HALLIWELL; GUTTERIDGE, 2015). Likewise, in biological organisms, photo-oxidation also contributes to oxidative damage, such as in skin diseases such as photoaging and photocarcinogenesis (SHAHID; ZONG, 2010). Thus, the incorporation of extracts with high scavenging capacity against ROS/RNS in food systems or even in cosmetic formulations is frequently seen as an effective strategy to delay direct oxidative reactions.

Another very important ROS highly associated with oxidative damages both in food and biological systems, $ROO^{\bullet}$ are originated in the propagation step during the chain reactions of lipid peroxidation. The presence of double bonds in unsaturated and polyunsaturated fatty acids of edible oils and other animal derived products, make them highly susceptible to oxidative damage, leading to the degradation of essential fatty acids and fat-soluble vitamins, promoting sensory changes and formation of chemical compounds with harmful effects on health, which

compromises the nutritional quality and safety of foods (SHAHID; ZONG, 2010; ZHANG, et al. 2010). Lipid autoxidation is observed in the presence of molecular oxygen and reactive species, and it involves a chain reaction that includes initiation, propagation and termination steps. After initiation, where lipid radicals are formed from lipid molecules, propagation reactions take place, where the lipid free radicals quickly react with molecular oxygen generating $ROO^{\bullet}$, which produce hydroperoxides (ROOH) after abstracting hydrogen from another intact molecule of unsaturated fatty acid (APAK.; ÖZYÜREK; GÜÇLÜ; ÇAPANOLU, 2016) Furthermore, in the presence of transition metals, such as Fe and Cu, light or high temperatures, ROOH can be decomposed into alkoxyl radicals ($RO^{\bullet}$), and then form by-products of lipid oxidation, such as ketones, aldehydes, acids, esters and other derived compounds, such as malonaldehyde (CHOE; MIN, 2006).

A common practice in the oilseed industries is the addition of synthetic antioxidant compounds, such as butylhydroxytoluene (BHT), butylhydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ), and propyl galatte (PG), to delay or inhibit the oxidative processes of edible oils (CASTRO-VARGAS; BAUMANN; PARADA-ALFONSO, 2016; SOUZA.; MARTÍNEZ; FERREIRA; KAISER, 2017). However, there is an increasing demand to the food industry to incorporate antioxidants of natural origin in the processing of food products, as replacements to the artificial ones, to accomplish the increased world tendency for the development of healthier products as part of healthy habits. In our study, all the *A. chica* extracts presented high potential to be used as a natural alternative to increase the stability of food systems. All the tested extracts exhibited the same scavenging capacity against $ROO^{\bullet}$ (Table 2), and they were 5 times more efficient than trolox (positive control) and about 33 times higher than scutellarein, which can raise the hypothesis of synergy between the other phenolic compounds in the extracts. When compared to other plant species, the *A. chica* extracts were considered effective scavengers of $ROO^{\bullet}$, about 26 times more effective than the extracts of pulp+skin (0.19, $S_{sample}/S_{trolox}$) and seeds (0.16, $S_{sample}/S_{trolox}$) of *Citharexylum solanaceum* (Barizão et al, 2016); and 16 times superior than the hydroalcoholic extract of artichoke leaves (*Cynara cardunculus*) (0.31, $S_{sample}/S_{trolox}$) (PISTÓN et al, 2014).

Regarding RNS, $ONOO^-$ is a strong oxidizing and nitrosative agent, which can affect the quality of foods, whose $O_2^{\bullet -}$ and nitric oxide ($^{\bullet}NO$) are the precursors. $ONOO^-$ can initiate lipid oxidation in foods, with the formation of free radicals that can lead to the loss of essential fatty acids, vitamins and proteins (BRANNAN et al, 2001; SKIBSTED, 2011). In biological systems, $ONOO^-$ can also cause nitrosative damages to biomolecules, including DNA, proteins

and lipids, and enzymatic inactivation, among other reactions (BRANNAN et al, 2001; SKIBSTED, 2011). In scavenging capacity assays of $ONOO^-$, it is also important to determine the ability to scavenge $ONOO^-$ in the presence of $NaHCO_3$ as a function of the predominance of the reaction between $ONOO^-$ and $CO_2$ , under physiological conditions (GOMES et al., 2006).

Regarding the scavenging capacity of *A. chica* extracts against $ONOO^-$, the ethanol/water extract also showed higher efficiency than the other extracts, in the presence and absence of $NaHCO_3$, followed by the water extract (Table 2). The ethanol/water extract was less efficient than quercetin (positive control), but was more efficient than other plant extracts, such as the extracts from the whole fruit of *Solanum diploconos* (RIBEIRO et al., 2016), presenting $IC_{50}$ values about eighty times lower in the absence of $NaHCO_3$ (27.8 µg/mL) and twice, in the presence (27.3 µg/mL). Additionally, the scavenging capacity of ethanol/water extract were about seventeen times higher than the extracts of *Vismia cauliflora* leaves (5.8 µg/mL), in the absence of $NaHCO_3$ (RIBEIRO et al., 2015).

In this study, the *A. chica* extracts showed high levels of phenolic compounds, and one of the advantages of using plant extracts as natural antioxidants might be related to the synergistic effects between the constituents of the extracts. The fact that the ethanol/water extract (1:1, v/v) of *A. chica* was as effective as the positive controls against ROS and RNS reinforces the interest in its use as alternative components in food formulations. In addition, another advantage that lies with the use of natural antioxidants is the replacement of synthetic ones, which express safety concerns due to their suspected carcinogenic effects (RAMIFUL et al., 2011).

## 4. CONCLUSIONS

This is the first report concerning the ability of *A. chica* leaf extracts to scavenge physiologically relevant ROS and RNS, namely HOCl, $H_2O_2$, $O_2^{\bullet-}$ and $ONOO^-$. All the *A. chica* extracts and scutellarein were able to scavenge all the reactive species tested in a concentration-dependent manner. Among the extracts, the one produced with ethanol/water as green solvent presented the highest content of phenolic compounds, where scutellarein was the major compound, and exhibited the highest scavenging capacity against all the ROS and RNS tested. The antioxidant potential herein investigated may be useful to delay or minimize the oxidative damages induced by the overproduction of ROS/RNS in both physiological and food systems.

**REFERENCES**

ALCÂNTARA, M.A.; DE LIMA BRITO POLARI, I.; DE ALBUQUERQUE MEIRELES, B.R.L.; DE LIMA, A.E.A.; DA SILVA JUNIOR, J.C.; DE ANDRADE VIEIRA, É.; DOS SANTOS, N.A.; DE MAGALHÃES CORDEIRO, A.M.T. Effect of the Solvent Composition on the Profile of Phenolic Compounds Extracted from Chia Seeds. **Food Chem**, 275, 489–496,2018.

ALMEIDA, I.F.; FERNANDES, E. Protective Effect of Castanea Sativa and Quercus Robur Leaf Extracts against Oxygen and Nitrogen Reactive Species. **Journal Photochem. Photobiol. B, Biol**, 91, 87–95,2008.

ANRAKU M, GEBICKI JM, IOHARA D, TOMIDA H, UEKAMA K, MARUYAMA T, HIRAYAMA F, OTAGIRI M. Antioxidant activities of chitosans and its derivatives in in vitro and in vivo studies. **Carbohydr Polym** 199:141–149, 2018.

APAK, R.; ÖZYÜREK, M.; GÜÇLÜ, K.; ÇAPANOĮLU, E. Antioxidant Activity/Capacity Measurement. 3. Reactive Oxygen and Nitrogen Species (ROS/RNS) Scavenging Assays, Oxidative Stress Biomarkers, and Chromatographic/Chemometric Assays. **Journal Agriculture Food Chem.** *64*, 1046–1070, 2016.

BARBOSA, W. L. R.; PINTO, L. D. N.; QUIGNARD, E.; VIEIRA, J. M. D. S.; SILVA, J. O. C.; ALBUQUERQUE, S. *Arrabidaea chica* (HBK) Verlot: Phytochemical approach, antifungal and trypanocidal activities. **Brazilian Journal of Pharmacognosy**, 18(4), 544–548, 2008.

BARIZÃO, E. O; VISENTAINER, J. V.; ALMEIDA, V. DE C.; RIBEIRO, D.; CHISTÉ, R.C., FERNANDES, E. *Citharexylum solanaceum* fruit extracts: Profiles of phenolic compounds and carotenoids and their relation with ROS and RNS scavenging capacities. **Food Research International** v. 86, p. 24-33, 2016.

BERTO, A.; RIBEIRO, A. B.; DE SOUZA, N. E.; FERNANDES, E.; CHISTÉ, R. C. Bioactive compounds and scavenging capacity of pulp, peel and seed extracts of the Amazonian fruit Quararibea cordata against ROS and RNS. **Food Research International**, 77, 236–243, 2015.

BRANNAN R.G., CONNOLLY B.J., DECKER E.A. Peroxynitrite: a potential initiator of lipid oxidation in food. **Trends in Food Sci & Technol**. 12: 164–173, 2001.

CASTRO-VARGAS, H. I., BAUMANN, W.;PARADA-ALFONSO, F. Valorization of agroindustrial wastes: Identification by LC-MS and NMR of benzylglucosinolate from papaya (*carica papaya L.*) seeds, a protective agent against lipid oxidation in edible oils. **Electrophoresis,** *37*(13), 1930–1937, 2016.

CHISTÉ, R.C.; BENASSI, M.T.; MERCADANTE, A.Z. Effect of Solvent Type on the Extractability of Bioactive Compounds, Antioxidant Capacity and Colour Properties of Natural Annatto Extracts. **Int. J. Food Sci**, 46, 1863–1870, 2011.

CHISTÉ, R.C.; DE TOLEDO BENASSI, M.; MERCADANTE, A.Z. Efficiency of Different Solvents on the Extraction of Bioactive Compounds from the Amazonian Fruit *Caryocar Villosum* and the Effect on Its Antioxidant and Colour Properties. **Phytochem. Anal**., 25, 364–372, 2014.

CHISTÉ, R. C.; MERCADANTE, A. Z. Identification and quantification, by HPLC-DAD-MS/MS, of carotenoids and phenolic compounds from the Amazonian fruit *Caryocar villosum*. **Journal of Agricultural and Food Chemistry**, 60(23), 5884–5892, 2012.

CHISTÉ R.C; MERCADANTE A.Z; GOMES A.; FERNANDES E.; LIMA J.L.F.D; BRAGAGNOLO N. In vitro scavenging capacity of annatto seed extracts against reactive oxygen and nitrogens pecies. **Food Chemistry**, 127(2), 419–26, 2011.

CHOE, E., MIN, D.B. Chemistry and Reactions of Reactive Oxygen Species in Foods. **Critical reviews in food science and nutrition**, v. 46, n. 1, p. 1-22, 2006.

CHONG, Y. M., CHANG, S. K., SIA, W. C. M., YIM, H. S. Antioxidant efficacy of mangosteen (*Garcinia mangostana Linn*.) peel extracts in sunflower oil during accelerated storage. **Food Bioscience**, 12, 18–25, 2015.

COMMISSION DIRECTIVE 95/45/EC (1995). Laying down specific purity criteria concerning colours for use in foodstuffs. **Official Journal of the European** Community, p,1–45.

CVJETKO BUBALO, M.; VIDOVIĆ, S.; RADOJČIĆ REDOVNIKOVIĆ, I.; JOKIĆ, S. New perspective in extraction of plant biologically active compounds by green solvents. **Food and Bioproducts Processing**, 109, 52–73, 2018.

DE SIQUEIRA, F. C; Leitão, D. D. S. T. C.; Mercadante, A. Z.; Chiste, R. C.; Lopes, A. S et al. Profile of phenolic compounds and carotenoids of *Arrabidaea chica* leaves and the in vitro singlet oxygen quenching capacity of their hydrophilic extract. **Food Research International**, v. 126, p. 108597, 2019.

FILIPPI, D.; BILIBIO, D.; BENDER, J.P.; CARNIEL, N.; PRIAMO, W.L. Kinetic Extraction of Total Polyphenols from Pitanga (*Eugenia Uniflora L.*): Effect of Ultrasonic Treatment, Modeling and Antioxidant Potential. **Journal of Food Process Engineering,** 38, 320–328, 2015.

FREITAS, M.; LIMA, J. L.; FERNANDES, E. Optical probes for detection and quantification of neutrophils' oxidative burst. A review. **Analytica Chimica Acta**, 649(1), 8–23, 2009.

GHITESCU, R.E.; VOLF, I.; CARAUSU, C.; BÜHLMANN, A.M.; GILCA, I.A.; POPA, V.I. Optimization of Ultrasound-Assisted Extraction of Polyphenols from Spruce Wood Bark. **Ultrasonics sonochemistry**, 22, 535–541, 2015.

GOMES, A., FERNANDES, E., LIMA, J.L.F.C. Use of fluorescence probes for detection of reactive nitrogen species: A review. **Journal of fluorescence**., *16*, n.1, p. 119-139, 2006**.**

GOMES, A.; FERNANDES, E.; SILVA, A. M. S.; SANTOS, C. M. M.; PINTO, D. C. G. A.; CAVALEIRO, J. A. S.; et al. 2-Styrylchromones: Novel strong scavengers of reactive oxygen and nitrogen species. **Bioorganic and Medicinal Chemistry**, 15(18), 6027–6036, 2007.

HALLIWELL, B.; GUTTERIDGE, J.MC. **Free radicals in biology and medicine**, 5ª ed.; OUP **Oxford university press**, Oxford USA, 2015.

HALLIWELL, B.; GUTTERIDGE, J.MC. **Free radicals in biology and medicine**. Oxford university press, USA, 2015.

HO, E.; GALOUGAHI, K.K.; LIU, C.C.; BHINDI, R.; FIGTREE, G.A. Biological markers ofox- idative stress: Applications to cardiovascular research and practice. **Redox Biology**, 1(1), 483–491, 2013.

HUYUT, Z.; BEYDEMIR, Ş.; GÜLÇIN, I. Antioxidant and antiradical properties of selected flavonoids and phenolic compounds. **Biochemistry research international**, v. 2017, 1–10, 2017.

ICH. 2005. International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use. Guidance for Industry, Q2B Validation of Analytical Procedures: Methodology. International Conference on Harmonization Secretariat, c/o **International Federation of Pharmaceutical Manufacturers and Associations**: Geneva; 7–10

ILAIYARAJA, N.; LIKHITH, K.R.; SHARATH BABU, G.R.; KHANUM, F. Optimisation of Extraction of Bioactive Compounds from *Feronia Limonia* (Wood Apple) Fruit Using Response Surface Methodology (RSM). **Food Chemistry***, 173*, 348–354, 2015.

LEITÃO, Deusa do Socorro Teixeira Costa et al. Amazonian *Eryngium foetidum* leaves exhibited very high contents of bioactive compounds and high singlet oxygen quenching capacity. **International Journal of Food Properties**, v. 23, n. 1, p. 1452-1464, 2020.

LOU, S.-N.; HSU, Y.-S.; HO, C.-T. Flavonoid compositions and antioxidant activity of calamondin extracts prepared using different solvents. **Journal of Food and Drug Analysis**, 22, 290–295, 2014.

MAFIOLETI, L.; DA SILVA JUNIOR, I. F.; COLODEL, E. M.; FLACH, A.; DE OLIVEIRA, D. T. M. Evaluation of the toxicity and antimicrobial activity of hydroethanolic extract of *Arrabidaea chica* (Humb. & Bonpl.) B. Verl. **Journal of Ethnopharmacology**, 150(2), 576–582, 2013.

MALLE, E.; MARSCHE, G.; ARNHOLD, J; DAVIES, M. J. Modification of low-density lipoprotein by myeloperoxidase-derived oxidants and reagent hypochlorous acid. **Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids**, v. 1761, n. 4, p. 392-415, 2006.

MARIUTTI, L. R. B.; RODRIGUES, E.; MERCADANTE, A. Z. Carotenoids from Byrsonima crassifolia: Identification, quantification and in vitro scavenging capacity against peroxyl radicals. **Journal of Food Composition and Analysis**, 31, 155–160, 2013.

MARIUTTI, L. R.; RODRIGUES, E.; CHISTÉ, R. C.; FERNANDES, E.; MERCADANTE, A. Z. The Amazonian fruit *Byrsonima crassifolia* effectively scavenges reactive oxygen and nitrogen species and protects human erythrocytes against oxidative damage. **Food research international**, v. 64, p. 618-625, 2014.

MEZZA, Gabriela N. et al. Antioxidant activity of rosemary essential oil fractions obtained by molecular distillation and their effect on oxidative stability of sunflower oil. **Food chemistry**, v. 242, p. 9-15, 2018.

OLIVEIRA, A.A.; SEGOVIA, V.Y.K.; SOUSA, E.C.G.; MATA, M.C.A.; GONÇALVES, R.M.; BEZERRA, P.O.M; JUNIOR, L.I.B. K. Antimicrobial activity of amazonian medicinal

plants, **Springerplus**, 2(1), 371–376, 2013.

OU, B.; HAMPSCH-WOODILL, M.; PRIOR,R. L. Development and validationofan improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. **Journal of Agricultural and Food Chemistry**, 49 (10), 4619–4626, 2001

ÖZTASKIN, Necla et al. Novel antioxidant bromophenols with acetylcholinesterase, butyrylcholinesterase and carbonic anhydrase inhibitory actions. **Bioorganic chemistry**, v. 74, p. 104-114, 2017.

PASTOR, I.; ESQUEMBRE, R.; MICOL, V.; MALLAVIA, R.; MATEO, C. R. A ready-to-use fluorimetric biosensor for superoxide radical using superoxide dismutase and peroxidase immobilized in sol–gel glasses. **Analytical biochemistry**, v. 334, n. 2, p. 335-343, 2004.

PATEIRO, M., GÓMEZ-SALAZAR, J. A., JAIME-PATLÁN, M., SOSA MORALES, M. E.; LORENZO, J. M. Plant extracts obtained with green solvents as natural antioxidants in fresh meat products. **Antioxidants**, v. 10, n. 2, p. 181, 2021.

PEREIRA BARBOSA-CARVALHO, Anna Paula et al. *Antrocaryon amazonicum*: An unexploited Amazonian fruit with high potential of scavenging reactive oxygen and nitrogen species. **Journal of Food Science**, v. 86, n. 9, p. 4045-4059, 2021.

PEREIRA, Gustavo Araujo et al. Optimizing the homogenizer-assisted extraction (HAE) of total phenolic compounds from banana peel. **Journal of Food Process Engineering**, v. 40, n. 3, p. e12438, 2017.

PISTÓN, M., MACHADO, I., BRANCO, C.S., CESIO, V., HEINZEN, H., RIBEIRO, D., et al. Infusion, decoction and hydroalcoholic extracts of leaves from artichoke (*Cynara cardunculus L. subsp. cardunculu*s) are effective scavengers of physiologically relevant ROS and RNS. **Food Research International**, 64(10), 150–156, 2014.

RAMFUL, D., AUMJAUD, B., NEERGHEEN, V. S., SOOBRATTEE, M. A., GOOGOOLYE, K., ARUOMA, O. I., & BAHORUN, T. Polyphenolic content and antioxidant activity of Eugenia pollicina leaf extract in vitro and in model emulsion systems. **Food Research International**, 44(5), 1190–1196, 2011.

Ribeiro, A. B.; Berto, A.; Chisté, R. C.; Freitas, M.; Visentainer, J. V; Fernandes, E. Bioactive compounds and scavenging capacity of extracts from different parts of *Vismia cauliflora* against reactive oxygen and nitrogen species. **Pharmaceutical Biology**, 53(9), 1267–1276, 2015.

RIBEIRO, A.B.; CHISTÉ, R.C.; LIMA, J.L.F.C.; FERNANDES, E. *Solanum Diploconos* Fruits: Profile of bioactive compounds and in vitro antioxidant capacity of different parts of the fruit. **Food & Function**, 7(5), 2249–2257, 2016.

RIBEIRO, F.M.; VOLPATO, H.; LAZARIN-BIDÓIA, D.; DESOTI, V.C.; DE SOUZA, R.O.; FONSECA, M.J.V.; UEDA-NAKAMURA, T.; NAKAMURA, C.V.; SILVA, S. DE O. The extended production of UV-Induced Reactive Oxygen Species in L929 Fibroblasts Is Attenuated by Posttreatment with *Arrabidaea Chica* through Scavenging Mechanisms. Journal of Photochemistry and Photobiology B: Biology, 178, 175–181, 2018.

RODRIGUES, E.; MARIUTTI, L.R.B.; FARIA, A.F.; MERCADANTE, A.Z. Microcapsules

Containing Antioxidant Molecules as Scavengers of Reactive Oxygen and Nitrogen Species. Food Chemistry, 134, 704–71.1, 2012.

RODRIGUES, E.; MARIUTTI, L.R.B.; MERCADANTE, A.Z. Carotenoids and Phenolic Compounds from *Solanum Sessiliflorum*, an Unexploited Amazonian Fruit, and Their Scavenging Capacities against Reactive Oxygen and Nitrogen Species. **Journal of Agricultural and Food Chemistry**, v. 61, n. 12, p. 3022-3029, 2013.

RODRIGUES, I.A.; AZEVEDO, M.M.B.; CHAVES, F.C.M.; ALVIANO, C.S.; ALVIANO, D.S.; VERMELHO, A.B. *Arrabidaea Chica* Hexanic Extract Induces Mitochondrion Damage and Peptidase Inhibition on Leishmania Spp. **BioMed Research Internationa**l, 1–7. 2014.

SHAHIDI, F.; ZHONG, Y. Lipid Oxidation and Improving the Oxidative Stability. **Chemical Society Reviews**, v. 39, n. 11, p. 4067-4079, 2010.

SHAHIDI, F.; ZHONG, Y. Measurement of Antioxidant Activity. **Journal of functional foods**, v. 18, p. 757-781, 2015.

SHI, D.; DING, H.; XU, S. Optimization of Microwave-Assisted Extraction of Wedelolactone from Eclipta Alba Using Response Surface Methodology. **Frontiers of Chemical Science and Engineering**, v. 8, n. 1, p. 34-42, 2014.

SILVA FARIA, W.C.; OLIVEIRA, M.G. DE; CARDOSO DA CONCEIÇÃO, E.; SILVA, V.B.; VEGGI, N.; CONVERTI, A.; MIGUEL DE BARROS, W.; FERNANDES DA SILVA, M.; BRAGAGNOLO, N. Antioxidant Efficacy and in Silico Toxicity Prediction of Free and Spray-Dried Extracts of Green Arabica and Robusta Coffee Fruits and Their Application in Edible Oil. **Food Hydrocolloids**, v. 108, p. 106004, 2020.

SILVA, E.M.; SOUZA, J.N.S.; ROGEZ, H.; REES, J.F.; LARONDELLE, Y. Antioxidant Activities and Polyphenolic Contents of Fifteen Selected Plant Species from the Amazonian Region. **Food Chemistry**, 101(3), 1012–1018, 2007.

SIRAICHI, J.T.G.; FELIPE, D.F.; BRAMBILLA, L.Z.S.; GATTO, M.J.; TERRA, V.A.; CECCHINI, A.L.; CORTEZ, L.E.R.; RODRIGUES-FILHO, E.; CORTEZ, D.A.G. Antioxidant Capacity of the Leaf Extract Obtained from Arrabidaea Chica Cultivated in Southern Brazil. **Plos One**, 8(8), 1–10, 2013.

SKIBSTED, L.H. Nitric Oxide and Quality and Safety of Muscle Based Foods. **Nitric Oxide**, 24, 176–183, 2011.

SOUZA, A.L.; MARTÍNEZ, F.P.; FERREIRA, S.B.; KAISER, C.R. A Complete Evaluation of Thermal and Oxidative Stability of Chia Oil: The Richest Natural Source of α-Linolenic Acid. **Journal of Thermal Analysis and Calorimetry**, v. 130, n. 3, p. 1307-1315, 2017.

VALKO, M.; LEIBFRITZ, D.; MONCOL, J.; CRONIN, M.T.D.; MAZUR, M.; Telser, J. Free Radicals and Antioxidants in Normal Physiological Functions and Human Disease. **The International Journal of Biochemistry and Cell Biology**, 39(1), 44–84, 2007.

WANG, Y.Z.; FU, S.G.; WANG, S.Y.; YANG, D.J.; WU, Y.H.S.; CHEN, Y.C. Effects of a Natural Antioxidant, Polyphenol-Rich Rosemary (Rosmarinus Officinalis L.) Extract, on Lipid Stability of Plant-Derived Omega-3 Fatty-Acid Rich Oil. **LWT- Food Science and**

**Technology**, 89, 210–216, 2018.

ZHANG, Y.; YANG, L.; ZU, Y.; CHEN, X.; WANG, F.; LIU, F. Oxidative Stability of Sunflower Oil Supplemented with Carnosic Acid Compared with Synthetic Antioxidants during Accelerated Storage. **Food Chemistry**. *118*, 656–662, 2010.

ZORN, B.; GARCÍA-PIERES, A.J.; CASTRO, V.; MURILLO, R.; MORA, G.; MERFORT, I. 3-Desoxyanthocyanidins from *Arrabidaea Chica*. **Phytochemistry**, *56*, 831–835, 2001.

# CONCLUSÃO GERAL

As folhas da planta Amazônica *A. chica* apresentaram elevados teores de compostos antioxidantes como, ácido ascórbico, carotenoides e compostos fenólicos. Pela primeira vez, a presença dos compostos derivados de feruloíl hexose, flavona-glicuronil derivativa, escutelarina, glicuronídeo de apigenina metílica, ácido ascórbico, e o perfil de carotenoides das folhas de *A. chica* foram relatados. O extrato etanol/água (1:1, v/v) da *A. chica* foi capaz de desativar, *in vitro,* o efeito oxidante das principais ROS e RNS de importância para os sistemas fisiológicos e alimentícios. Em geral, os valores de $IC_{50}$ para as ROS e RNS mostraram que os extratos de *A. chica* apresentaram melhor eficiência na desativação da espécie ONOO- seguido do HOCl, $H_2O_2$, $O2^{\cdot-}$ e $^1O_2$. Tais informações são de grande importância para a indústria de produtos naturais, uma vez que a *A. chica* é uma fonte promissora de compostos bioativos antioxidantes a ser utilizada pela indústria alimentícia, farmacêutica e cosmética.

# ANEXO A

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# Profile of phenolic compounds and carotenoids of *Arrabidaea chica* leaves and the *in vitro* singlet oxygen quenching capacity of their hydrophilic extract

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ABSTRACT

*Arrabidaea chica* (Brazilian name = *pariri*) is a plant species that belongs to the Bignoniaceae family, occurring in tropical America and widespread in the Amazonian region of Brazil. In this study, the phenolic compound and carotenoid profiles of *A. chica* leaves were determined by HPLC-DAD-MS. Scutellarin was identified as the main phenolic compound (15,147.22 μg/g, dry basis, d.b.) and lutein (204.28 μg/g, d.b.), β-carotene (129.5 μg/g, d.b.) and α-carotene (79.86 μg/g, d.b.) as the major carotenoids. Moreover, *A. chica* leaves presented 152.7 μg/g of ascorbic acid (fresh weight). For the first time, the carotenoid profile and ascorbic acid contents were reported for *A. chica* leaves. Regarding the antioxidant capacity, *A. chica* extract was able to scavenge ABTS radical (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), and for the first time, the protective effect against singlet oxygen ($^1O_2$) was reported. The *A. chica* extract was efficient as ABTS radical scavenger (86.81 μM Trolox/g of fresh leaves) and inhibited the degradation of tryptophan by $^1O_2$ in a concentration-dependent manner with an $IC_{50}$ at 177 μg/mL. Thus, *A. chica* leaves can be exploited as a promising source of bioactive compounds that may be useful to human health or food systems against oxidative damage.

## 1. Introduction

High levels of reactive species in living systems can oxidize biomolecules, leading to tissue damage, cell death or the onset of various chronic-degenerative diseases, such as cancer, diabetes, kidney problems, hypertension, cardiovascular diseases, and atherosclerosis (Halliwell, 2012; Katoch, Kaur, Kashyap, Gupta, & Dahiya, 2013). In food systems, the occurrence of reactive species is also responsible for the onset of food oxidation reactions (Choe & Min, 2015). Studies have shown a growing interest in scientific researches on bioactive compounds, such as phenolic compounds, carotenoids and ascorbic acid, from medicinal and dietary plants that may help to minimize the effects of oxidative damages both in foods and *in vivo* (Dziadek, Kopeć, & Tabaszewska, 2018; Ribeiro et al., 2015; Sharififar, Dehghn-nudeh, & Mirtajaldini, 2009; Silva, Ferreres, Malva, & Dias, 2005).

Brazilian flora, especially the Amazonian biome, is one of the richest sources in the world of species with bioactive substances, and among these species, *Arrabidaea chica* (Humb. & Bonpl.) B. Verlot, also known as "crajiru", "pariri" or "chica", is a tree plant belonging to the Bignoniaceae family, widely used in folk medicine as tea to treat gastric ulcers, anemia, infections, inflammation, herpes, among others (Mafioleti, da Silva Junior, Colodel, Flach, & de Oliveira, 2013; Zorn et al., 2001). The hydroethanolic extract of *A. chica* leaves with high contents of phenolic compounds (16%) demonstrated low oral, acute and subchronic toxicities and lack of cytotoxicity in Chinese hamster ovary cells (CHO-K1) (Mafioleti et al., 2013). Furthermore, *A. chica* presents high potential for the prospection of bioactive compounds (Aro et al., 2013). For example, the presence of flavonoids, anthocyanins, tannins and phytosterols were already reported in *A. chica* leaf extracts cultivated in other localities, distinct from the Amazonia region (Devia et al., 2002; Siraichi et al., 2013). However, no information was found in the literature regarding the carotenoid profile and ascorbic acid contents in this species. Furthermore, the leaves of *A. chica* were reported to possess anti-inflammatory, antibacterial, antifungal,

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antiparasitic, antitumor, antiulcerogenic and antioxidant activities (Mafioleti et al., 2013; Ribeiro et al., 2012; Siraichi et al., 2013).

Therefore, the objective of this study was to identify and quantify the bioactive compounds, by high performance liquid chromatography coupled to diode array and mass spectrometer detectors (HPLC-DAD-MS), of *A. chica* leaves cultivated in the Amazonian region of Brazil. In addition, the *in vitro* antioxidant capacity of the *A. chica* hydrophilic extract to scavenge ABTS radical (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and to quench singlet oxygen ($^1O_2$) was assessed.

## 2. Materials and methods

### 2.1. Chemicals

Scutellarin, apigenin, quercetin, all-*E*-β-carotene, all-*E*-lutein, ascorbic acid, trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid), potassium persulphate, methylene blue (MB), methanol (MeOH), methyl-*tert*-butyl ether (MTBE), acetonitrile, sulfuric acid, formic acid and all other analytical grade salts were purchased from Sigma-Aldrich (St. Louis, USA). L-Tryptophan was obtained from Fisher Scientific (Pittsburgh, PA, USA). Ultrapure water was obtained from the Milli-Q System system (Millipore Corp., Milford, MA, USA). All phenolic compounds and carotenoids standards showed at least 95% of purity degree, as determined by HPLC-DAD.

### 2.2. Samples

The *A. chica* leaves were collected from five plants in May 2017 from the Active Germplasm Bank of EMBRAPA (Brazilian Agricultural Research Corporation) Eastern Amazon Research Center, located in Belém, Pará State, Brazil (01°26′14.7″ S and 48°26′52.2″ W). For each plant, 500 g of leaves from the lower, middle and upper parts were collected to obtain a representative sample. The collected leaves were divided into two portions: one was used for proximate composition analysis and the other was freeze-dried using a benchtop lyophilizer (Liotop, L101, São Paulo, Brazil) and used in the bioactive compounds determination and antioxidant capacity assays. The freeze-dried leaves were milled in a knife mill, vacuum packed in plastic bags and stored at −18 °C until analysis.

### 2.3. Proximate composition

The contents of ashes, total lipids, moisture and total proteins (conversion factor of 6.25 from total nitrogen to total protein) were determined according to AOAC (AOAC (Association of Analytical Chemists), 2000) and expressed as g/100 g (%). Total carbohydrates were calculated by difference [Total carbohydrates = 100–(% moisture + % ashes + % total proteins + % total lipids)]. The total energetic value (kcal/100 g) was obtained according to the Atwater conversion factor for vegetables, as follows: total energetic value (kcal/100 g) = (protein × 2.44 kcal/g) + (lipid × 8.37 kcal/g) + (total carbohydrate × 3.57 kcal/g) (FAO/WHO, 2003). All the experiments were carried out in triplicate ($n = 3$).

### 2.4. Determination of bioactive compounds

#### 2.4.1. Equipments

The identification of phenolic compounds and carotenoids in *A. chica* leaf extracts was carried out by a Shimadzu HPLC (Prominence UFLC model, Kyoto, Japan) consisting of a binary pump (LC-20AD), a degasser unit (DGU-20A3R), an automatic injector (SIL-20AHT), and an oven (CTO-20A), connected in series to a DAD detector (SPD-M20A) and a mass spectrometer with an ion-trap as the *m/z* analyzer from Bruker Daltonics (AmaZon speed ETD, Bremen, Germany), equipped with electrospray (ESI) and atmospheric pressure chemical ionization (APCI) as the ionization sources for phenolic compounds and carotenoids identification, respectively.

The quantification of phenolic compounds, carotenoids and ascorbic acid was carried out by an Agilent HPLC (Agilent 1260 Infinity model, Santa Clara, CA, USA) equipped with a quaternary pump (G1311C), an automatic injector (G7129), an oven (G1316A) and a DAD detector (G1328C). For all the chromatographic analysis, samples and solvents were filtered using membranes of 0.22 and 0.45 µm, respectively, both from Millipore (Billerica, MA, USA).

#### 2.4.2. HPLC-DAD-ESI-$MS^n$ analysis of phenolic compounds

The phenolic compounds of *A. chica* leaves were exhaustively extracted from about 0.5 g of the freeze-dried leaves with MeOH/water (8:2, *v*/v) in a mortar with a pestle (5 min), followed by vacuum filtration and the residue was subjected to the same extraction procedure for 3 times. The filtered extract was transferred to a 50 mL-flask and filled up with the same extraction solution.

The phenolic compounds were separated on a $C_{18}$ Synergi Hydro column (Phenomenex, 4 µm, 250 × 4.6 mm), set at 29 °C. A linear gradient of water with 0.5% formic acid (solvent A) and acetonitrile with 0.5% formic acid (solvent B) was used from A:B 99:1 to 50:50 in 50 min followed to 1:99 in 5 min, keeping the last ratio for another additional 5 min at 0.9 mL/min (Chisté & Mercadante, 2012). The UV-visible (UV-vis) spectra were obtained between 200 and 600 nm, and the chromatograms were monitored at 270, 320, 360 and 480 nm. The column eluate was split to allow only 0.15 mL/min to enter the ESI source. The mass spectra (MS) were acquired after ionization in negative ion mode, with a scanning range of *m/z* from 100 to 1000, and the MS parameters set as described by Chisté and Mercadante (2012). The phenolic compounds were tentatively identified by combining the following information: elution order, retention time on the $C_{18}$ column, UV-vis and mass spectra compared to data available in the literature (Chandrasekara & Shahidi, 2011; El-hela, Al-amier, & Ibrahim, 2010; Ribeiro, Chisté, Lima, & Fernandes, 2016; Siraichi et al., 2013; Surowiec, Szostek, & Trojanowicz, 2007) and authentic standards. The quantification was carried out by comparison with external analytical curves of six points (3.12 to 100 µg/mL, in duplicate) for apigenin [$R^2 = 0.99$, limit of detection (LOD) = 0.17 µg/mL and limit of quantification (LOQ) = 0.51 µg/mL], scutellarin [$R^2 = 0.99$, LOD = 0.14 µg/mL and LOQ = 0.41 µg/mL] and scutellarein [$R^2 = 0.98$, LOD = 0.23 µg/mL and LOQ = 0.69 µg/mL]. LOD and LOQ values were calculated using the parameters (standard deviation and slope) of the analytical curves (ICH, 2005). The phenolic compound contents were expressed as µg/g of freeze-dried leaves (dry basis, d.b.), considering three independent extraction procedures (n = 3).

#### 2.4.3. HPLC-DAD-APCI-MS/MS analysis of carotenoids

The carotenoids of freeze-dried *A. chica* leaves (0.5 g) were exhaustively extracted with acetone, followed by liquid-liquid partition to petroleum ether/diethyl ether (1:1, *v*/v) by washing with distilled water, saponified overnight with 10% KOH in methanol (1:1, v/v), re-partitioned and evaporated under vacuum (T < 38 °C). The dried carotenoid extract was solubilized in MTBE and filtered prior to HPLC injection, according to the procedures described by Chisté and Mercadante (2012).

The carotenoids were separated on a $C_{30}$ YMC (5 µm, 250 mm × 4.6 mm) column set at 29 °C, using as a mobile phase a linear gradient of MeOH (solvent A) and MTBE (solvent B), from A:B 95:5 to 70:30 in 30 min, followed by 50:50 in 20 min at 0.9 mL/min. The UV-vis spectra were recorded between 200 and 600 nm and the chromatograms were processed at 450 nm. The column eluate was directed to the APCI interface, and the mass spectra were obtained after APCI in positive ion mode, with a scan interval at *m/z* from 100 to 800, with the parameters reported by Chisté and Mercadante (2012). The carotenoids were tentatively identified based on the following

combined information: elution order on the $C_{30}$ column, UV-vis [$\lambda_{max}$, spectral fine structure (%III/II), *cis*-peak intensity (%$A_B/A_{II}$)] and mass spectra compared to data available in the literature (De Rosso & Mercadante, 2007; De Faria, De Rosso, & Mercadante, 2009; Chisté & Mercadante, 2012; Berto, Ribeiro, de Souza, Fernandes, & Chisté, 2015;). The assignment of *Z*-isomer of carotenoids was based on the observation of %III/II decrease and %$A_B/A_{II}$ increase (≈7–11% = 9*Z*; ≈45% = 13*Z* and ≈ 56% = 15*Z* carotenoid) as reported by De Rosso and Mercadante (2007). The carotenoids were quantified by HPLC-DAD by external five-point analytical curves (3.12–100 μg/mL, in duplicate) of (all-*E*)-β-Carotene ($R^2$ = 0.99, LOD = 0.98 μg/mL and LOQ = 2.96 μg/mL) and (all-*E*)-Lutein ($R^2$ = 0.98, LOD = 0.20 μg/mL and LOQ = 0.62 μg/mL). The NAS-IOM conversion factor was used to calculate the vitamin A value, considering 12 μg of (all-*E*)-β-carotene and 24 μg of (all-*E*)-α-carotene, (9*Z*)-β-carotene and (13*Z*)-β-carotene corresponding to 1 μg of retinol activity equivalent (RAE). The activity used was 100% for (all-*E*)-β-carotene and 50% for (all-*E*)-α-carotene, (9*Z*)-β-carotene and (13*Z*)-β-carotene (NAS-IOM, 2001). The carotenoid contents were expressed as μg/g of freeze-dried leaves (d.b.), considering three independent extraction procedures (n = 3).

#### 2.4.4. HPLC-DAD analysis of ascorbic acid

The ascorbic acid content was determined in both the freeze-dried leaves of *A. chica* and the hydromethanolic extract used to assess the antioxidant capacity. For the *A. chica* leaves, the ascorbic acid was extracted from 1 g of the freeze-dried leaves with an aqueous solution of 1% oxalic acid (3 times), followed by centrifugation (3000 ×*g* at 4 °C). The supernatant was transferred to a 50 mL flask; the volume was filled up with the same solution. Both the extracts were filtered prior injection into the Agilent HPLC-DAD system. The ascorbic acid was separated on a $C_{18}$ Synergi Hydro column (Phenomenex, 4 μm, 250 × 4.6 mm) set at 25 °C, using an aqueous solution of sulfuric acid (0.001 M, pH 2.5) as the mobile phase, in isocratic mode, at 0.7 mL/min. The chromatogram was processed at 244 nm (Ribeiro et al., 2016). The identification of ascorbic acid was based on the retention time (7 min), co-elution and UV-vis characteristics compared to the authentic standard (data not shown) analyzed under the same conditions. The ascorbic acid was quantified using six-point external analytical curve (1.6–100 μg/mL, in duplicate, $R^2$ = 0.99, LOD = 0.05 μg/mL and LOQ = 0.16 μg/mL) and the results were expressed as μg/g of fresh weight leaves (FW), considering three independent extraction procedures (n = 3).

### 2.5. In vitro antioxidant capacity of A. chica extract

The extract used for the antioxidant capacity assays was obtained according to the same procedure described to the analysis of phenolic compounds.

#### 2.5.1. Scavenging capacity of ABTS radical

The ABTS radical scavenging assay was carried out according to the method described by Re et al. (1999), with modifications. The radical cation ($ABTS^{\cdot+}$) was generated by the chemical reaction of ABTS (7 mM) with potassium persulfate (145 mM) held in the dark during 12–16 h before use. After formation of the $ABTS^{\cdot+}$, 3 mL of this solution (diluted with methanol to ~0.70 absorbance at 734 nm) was mixed with an aliquot (30 μL) of the *A. chica* extract at concentrations ranging from 0.5 to 10 mg/mL or Trolox standard and the absorbance was recorded after 6 min at 734 nm. The analytical curve of trolox (102 to 2048 μM) was constructed and the results (n = 3) were expressed in μM of Trolox equivalent (TE)/g of FW leaves.

#### 2.5.2. Quenching of singlet oxygen ($^1O_2$)

The ability of *A. chica* extract to quench $^1O_2$ was determined according to the method described by Chisté, Benassi, and Mercadante (2011), with adaptations. The experiment was conducted by taking an aliquot (100 μL) of the *A. chica* extract (50 to 375 μg/mL) or quercetin standard (positive control, 0.78 to 4.75 μg/mL), which was added to a solution containing 950 μL of L-tryptophan (TRP) (40 μM) and 950 μL of MB (10 μM) as the sensitizer, under atmospheric air and at room temperature (25 °C), both solutions prepared in phosphate-buffered saline (pH 7.2). The blank experiment was performed under the same conditions, replacing 100 μL of the antioxidant by 100 μL of MeOH/water (8:2, *v*/v). The $^1O_2$ was generated by direct sensitization of MB by a 75 W incandescent bulb, which was used as the excitation source, and two filters (red and orange colored filters) was placed between the excitation source and the cuvette containing the reactants to only excite MB. The reaction was monitored by spectrophotometric measurements in the range of 200–800 nm during 20 min and the absorbance of TRP was recorded at 219 nm. The kinetic data obtained from the decay of the absorbance of TRP was fitted to a first-order reaction (Eq. (1)) using Origin Pro 8 software (OriginLab Corporation, Northampton, MA, USA) to calculate the rate constants (Eq. (2)). The percentage of protection of *A. chica* extract or quercetin against the oxidative damage of $^1O_2$ was calculated through Eq. (3).

$$Y = Y_\infty + A.\ exp^{\left(-\frac{x}{t1}\right)} \quad (1)$$

$$k = \frac{\ln 2}{t_1} \quad (2)$$

$$Protection\ against\ 1O2\ (\%) = \frac{K_{obs}^{TRP} - K_{obs}^{TRP+antioxidant}}{K_{obs}^{TRP}} \times 100 \quad (3)$$

where Y is the intensity absorbance of TRP; $Y_\infty$ is the intensity absorbance of TRP at infinite time; A is pre-exponential factor; *k* is the pseudo-first order rate constant; x is the reaction time; $t_1$ is half-life time (min); $K_{obs}^{TRP}$ is the observed pseudo-first order rate constant fitted to the TRP decay curve (obtained in the blank experiment); and $K_{obs}^{TRP+antioxidant}$ is the observed pseudo-first order rate constant fitted to the TRP decay curve in the presence of antioxidant.

The *in vitro* $IC_{50}$ value (inhibitory concentration to decrease by 50% the amount of reactive species in the tested media) was calculated from the curves of percentage of inhibition *versus* antioxidant concentration using OriginPro 8 software.

## 3. Results and discussion

The *A. chica* leaves presented high moisture (69.95 ± 0.18%), carbohydrates (22.84 ± 0.25%) and ashes (4.66 ± 0.17%) contents, while low contents of total protein (1.82 ± 0.05%) and total lipids (0.73 ± 0.04%) with a total energetic value of 92.07 ± 0.80 kcal/100 g(FW). This is the first report concerning the proximate composition of *A. chica* leaves and these results were lower than those reported for the Nigerian medicinal plants *Chromolina oduratum, Ipomoea aserifolia* and *Emilia santifolia* (Ekpa, 1996), and those found in 40 leaf cultivars of sweet potatoes (*Ipomoea batatas* L.) (Sun, Mu, Xi, Zhang, & Chen, 2014).

Regarding the ascorbic acid content, its value (152.7 ± 13.6 μg/g FW), reported for the first time in the present study, is within the range found for spinach (*Spinacia oleracea* L.) (14.0–460 μg/g FW) at different stages of growth and post-harvest storage (Bergquist, Gertsson, & Olsson, 2006), but lower than broccoli (*Brassica oleracea L., Italian var., Marathon cv.*) inflorescences (665–1077 μg/g FW) during harvesting and storage (Vallejo, Tomás-Barberán, & Garcia-Viguera, 2003). Therefore, *A. chica* leaves could be added to food preparations to increase ascorbic acid ingestion in a healthy diet, since ascorbic acid is considered one of the main antioxidant compounds found in vegetables. Such compound is important for the regeneration of vitamin E, inhibition of nitrosamine formation and in the absorption of inorganic iron, which may promote an enhancement in the immune system and protection against diseases related to oxidative stress (Ribeiro et al., 2016; Ribeiro, De Queiroz, De Queiroz, Campos, & Sant'ana, 2007).

**Table 1**
Phenolic compounds profile of *Arrabidaea chica* leaves by HPLC-DAD-ESI-$MS^n$.

| Peak | Phenolic compound[a] | Concentration (μg/g leaves)[b] | $t_R$ (min)[c] | $\lambda_{max}$ (nm)[d] | $[M-H]^-$ (*m/z*) | $MS^2$ (*m/z*)[e] | $MS^3$ (*m/z*)[e] |
|---|---|---|---|---|---|---|---|
| 1 | Feruloyl hexose (isomer 1)[f] | 1088.07 ± 236.98 | 15.7 | 313 | 355 | 337, 212, **193**, 151 | [355➔193]: **151** |
| 2 | Feruloyl hexose (isomer 2)[f] | 915.26 ± 217.24 | 16.7 | 309 | 355 | 337, 212, **193**, 151 | [355➔ 193]: **161** |
| 3 | Feruloyl derivative[f] | 857.53 ± 72.65 | 24.6 | 273, 327 | 449 | 405, **273**, 257 | [449➔273]: **255**, 231 |
| 4 | Scutellarin[f] | 15,147.22 ± 1660.84 | 27.7 | 282, 334 | 461 | **285**, 178 | [461➔285]: 285**, 267,** 243, 192,169 |
| 5 | Apigenin glucuronide[h] | 807.83 ± 221.15 | 30.7 | 267, 337 | 445 | **269**, 177 | [445➔ 269]: **269,** 225 |
| 6 | Flavone-glucuronyl derivative[f] | 1143.23 ± 209.03 | 31.0 | 275, 328 | 493 | 445, 431, **317**, 273 | [493➔ 317]: 299, **273**, 259, 185 |
| 7 | Scutellarein[g] | 1379.17 ± 91.57 | 34.6 | 282, 337 | 285 | **267**, 225, 200, 173 | [285➔ 267]: 241, 227, 214, **198** |
| 8 | Methyl apigenin glucuronide[h] | 132.14 ± 12.95 | 37.6 | 266, 292, 335 | 459 | **283**, 178 | [459➔283]: **268** |
| 9 | Apigenin[h] | 49.79 ± 40,80 | 42.5 | 267,293, 337 | 269 | 251, **225**, 201, 183 | [269➔225]: nd |
| **Total phenolic (μg/g leaves)** | | 21,520.24 ± 2763.21 | | | | | |

[a]Tentative identification based on Uv-visible and mass spectra as well as relative retention time on $C_{18}$ column and published data. [b]Mean ± standard deviation (n = 3, dry basis). [c]Retention time on the $C_{18}$ Synergi Hydro (4 μm) column. [d]Solvent: gradient of 0.5% formic acid in water and acetonitrile with 0.5% formic acid. [e]In the $MS^2$ and $MS^3$, the most abundant ion is shown in boldface. The peaks were quantified as equivalent of scutellarin[f], scutellarein[g] and apigenin[h]. nd = not detected.

### 3.1. Phenolic compounds profile of A. chica leaves

Regarding the profile of phenolic compounds of *A. chica*, as observed in Table 1, the HPLC-DAD-ESI-$MS^n$ allowed the assignment of nine separated phenolic compounds (Fig. 1).

**Peaks 1** and **2** were tentatively identified as feruloyl hexose isomers since both compounds presented deprotonated molecule ($[M-H]^-$) at *m/z* 355 with fragments at *m/z* 337 and an intense fragment at *m/z* 193 (ferulic acid) in the $MS^2$ spectra, corresponding to a neutral loss of a water molecule (18 u) and a hexose moiety (162 u), respectively. Such characteristics were already reported in the literature for this type of compounds (Chandrasekara & Shahidi, 2011; Ribeiro et al., 2016). **Peak 3** was assigned as feruloyl derivative due to the UV-Vis absorption at 327 nm (hydroxycinnamic acids) and the presence of $[M-H]^-$ at *m/z* 449, followed by a neutral loss of a feruloyl moiety (176 u) as the most intense fragment at *m/z* 273 in the $MS^2$ spectra. The ion at *m/z* 273 was further fragmented into the *m/z* 255, showing the presence of a hydroxyl group in the $MS^3$ spectrum. The same fragmentation pattern was previously reported for the feruloyl derivative compounds found in *Solanum diploconos* fruits (Ribeiro et al., 2016). **Peaks 4–9** are phenolic compounds, which belong to the flavone class, as can be observed by their characteristic UV–vis absorption spectra at the wavelength range of 334–337 nm. **Peak 4** was the major phenolic compound in *A. chica* leaves and identified as scutellarin, with $[M-H]^-$ at *m/z* 461 and a neutral loss of a glucuronyl moiety at *m/z* 285 ($[M-H-176]^-$ in the $MS^2$ spectrum, followed by an intense peak at *m/z* 267 in the $MS^3$ spectrum, which corresponds to the elimination of a hydroxyl group. Scutellarin, identified in this leaf for the first time in the literature, was also confirmed based on co-elution and comparison with the same MS fragmentation pattern exhibited by the authentic standard. Considering that **peak 5** showed $[M-H]^-$ at *m/z* 445 and an intense fragment at *m/z* 269 ($MS^2$ spectra), corresponding to the loss of a glucuronyl unit (176 u), this compound was tentatively assigned as apigenin glucuronide, according to previous report (Surowiec et al., 2007). **Peak 6** was tentatively identified as flavone-glucuronyl derivative as showed a $[M-H]^-$ ion at *m/z* 493 with an intense $MS^2$ fragment at *m/z* 317, corresponding to the loss of a glucuronyl unit, followed by a neutral loss of 18 u at *m/z* 299 ($MS^3$ spectrum). **Peak 7** was identified as scutellarein due to the presence of a $[M-H]^-$ at *m/z* 285 and a $MS^2$ fragment at *m/z* 267, which corresponds to a neutral loss of a hydroxyl group. The identification of scutellarein was confirmed by co-elution and the same MS fragmentation characteristics exhibited by the authentic standard and previous data (Siraichi et al., 2013). **Peak 8** exhibited $[M-H]^-$ at *m/z* 459, with consecutive losses of 176 u (glucuronide moiety) at *m/z* 283 ($MS^2$ spectrum), and 15 u (methyl group) at *m/z* 268 ($MS^3$ spectrum), being identified as methyl apigenin glucuronide (Abubakar et al., 2016; Shi et al., 2013). **Peak 9** was identified as apigenin, with $[M-H]^-$ at *m/z* 269 and fragments at *m/z* 251 (18 u, OH group) and *m/z* 225 (44 u, $CO_2$), typical from this type of compound (Mcnab, Ferreira, Hulme, & Quye, 2009; Shi et al., 2013); identity was confirmed by comparison with the authentic standard.

This is the first study in literature reporting the presence of the feruloyl derivative compounds, scutellarin and methyl apigenin glucuronide in *A. chica* leaves, together with the overall quantification of phenolic compounds by HPLC-DAD. Scutellarin was the major phenolic compound and accounted for ≈70% of the sum of the identified

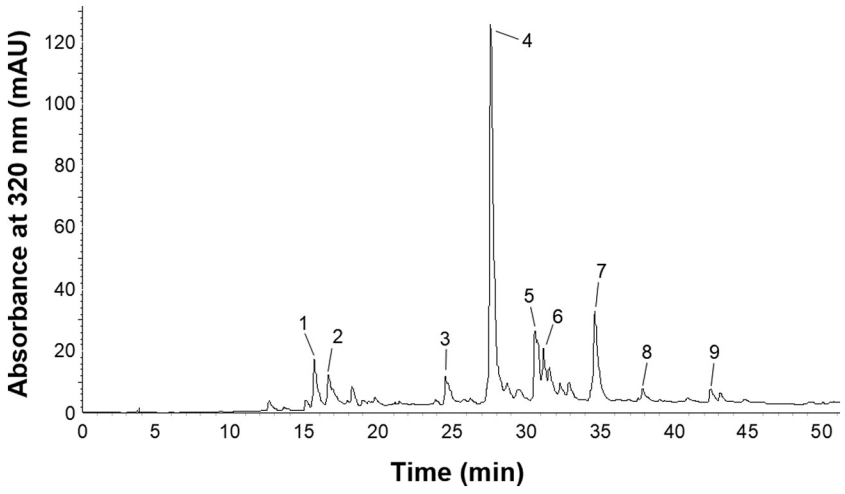


**Fig. 1.** HPLC-DAD chromatogram at 320 nm of phenolic compounds of *Arrabidaea chica* leaf extract. The peak characterization is given in Table 1.

phenolic compounds. Other studies indicated the presence of two desoxyanthocyanidins (carajurin and carajurone) in *A. chica* leaves cultivated in Bogotá (Colombia) (Devia et al., 2002) and Costa Rica (Zorn et al., 2001). In *A. chica* leaves cultivated in Southern Brazil, the presence of isoscutellarein, 6-hydroxyluteolin, hispidulin and luteolin was reported (Siraichi et al., 2013). None of these compounds was detected in the present study. This can be attributed to several factors, such as environmental aspects of plant cultivation, complexity of phenolic compounds and methods of extraction and analysis (Balasundram, Sundram, & Samman, 2006; Tomás-Barberán & Espín, 2001).

The total sum of the identified phenolic compounds of *A. chica* leaves, measured by HPLC-DAD, in this study, was very high (21,520 μg/g, Table 1), which accounts for ≈2% of the composition of the freeze-dried material. According to the literature, *A. chica* leaf extract presented 10,200 μg/g (FW leaves) of total phenolic compounds (expressed as gallic acid equivalent), as spectrophotometrically determined by the Folin-Ciocalteau method (Silva, Souza, Rogez, Rees, & Larondelle, 2007). Such result is higher than that found in our study (≈6456 μg/g, FW) probably due to the fact that many compounds in the extract, *e.g.* ascorbic acid and reducing sugars, also react with the Folin-Ciocalteau reagent.

### 3.2. Carotenoid profile of A. chica leaves

Regarding the profile of carotenoids of *A. chica*, as observed in Table 2, the HPLC-DAD-APCI-MS/MS allowed the assignment of ten separated carotenoids (Fig. 2). The major carotenoids were (all-*E*)-lutein (**peak 5**), followed by (all-*E*)-β-carotene (**peak 9**) and (all-*E*)-α-carotene (**peak 8**). For the identified peaks, the attribution of the protonated molecule $[M + H]^+$ was confirmed by means of the expected MS/MS fragments for both the polyene chain and functional groups of carotenoids, as well as by the UV-vis spectra. (all-*E*)-β-Carotene (**peak 9**) and (all-*E*)-lutein (**peak 5**) were positively confirmed by co-elution and comparison with authentic standards.

All the identified xanthophylls had three or two hydroxyl (OH) groups and, as expected for reverse phase columns, they eluted before the carotenes. **Peaks 1** and **2** were tentatively identified as (13*Z*)-violaxanthin and (all-*E*)-violaxanthin, respectively, because the (*Z*) isomer presented lower values of $\lambda_{max}$, %III/II and high *cis* peak intensity (45%) compared to the corresponding (all-*E*)-isomer. Moreover, both peaks showed the $[M + H]^+$ at *m/z* 601 and fragments at *m/z* 583 $[M + H\text{-}H_2O]^+$ and *m/z* 565 $[M + H\text{-}2H_2O]^+$, due to neutral losses of two hydroxyl groups, at *m/z* 509 $[M + H\text{-}92]^+$ resulting from the loss of toluene and at *m/z* 221, showing the presence of an epoxy group in a hydroxylated β-ring (Chisté & Mercadante, 2012; Crupi et al., 2013). (all-*E*)-luteoxanthin (**peak 3**) presented $\lambda_{max}$ at 398, 421 and 448 nm, high percentage of %III/II (100%), $[M + H]^+$ at *m/z* 601, and two consecutive losses of OH groups were observed in the MS/MS spectrum at *m/z* 583 and *m/z* 565. As also observed for peaks 1 and 2, peak 3 presented the characteristic fragment at *m/z* 221 in the MS/MS spectrum.

**Peak 4** was assigned as a mixture of (*Z*)-lutein + epoxycarotenoids and showed three protonated molecules: *m/z* 601, *m/z* 585 and *m/z* 551 (in-source ionization). The $[M + H]^+$ at *m/z* 601 fragmented at *m/z* 583 $[M + H\text{-}H_2O]^+$, *m/z* 565 $[M + H\text{–}\ 2H_2O]^+$, *m/z* 509 $[M + H\text{-}92]^+$ and at *m/z* 221 in the MS/MS spectrum. The $[M + H]^+$ at *m/z* 585 showed successive OH losses at *m/z* 567 and *m/z* 549, elimination of toluene from the polyene chain at *m/z* 493, loss of toluene combined with an OH (*m/z* 475 $[M + H\text{-}H_2O\text{-}92]^+$) and a fragment at *m/z* 221. The presence of a (*Z*)-carotenoid in peak 4 was noticed by the presence of a *cis*-peak (%$A_B/A_{II}$) at 330 nm in the UV–Visible spectrum, which was confirmed by the observation of an intense in-source fragment at *m/z* 551 in the MS spectrum, which is characteristic of lutein and its (Z)-isomers ($[M + H]^+$ at *m/z* 569), as well as the fragments at *m/z* 533 $[M + H\text{–}\ H_2O]^+$ and at *m/z* 459 $[M + H\text{-}92]^+$.

The (all-*E*)-lutein (**peak 5**) and (all-*E*)-zeaxanthin (**peak 6**) have the

**Table 2**
Carotenoid profile of *Arrabidaea chica* leaves by HPLC-DAD-APCI-MS/MS.

| Peak | Carotenoid[a] | Concentration (μg/g leaves)[b] | $t_R$ (min)[c] | $\lambda_{max}$ (nm)[d] | %III/ II | %$A_B/A_{II}$ | $[M + H]^+$ (*m/z*) | MS/MS (+) (*m/z*) |
|---|---|---|---|---|---|---|---|---|
| 1 | (13*Z*)-Violaxathin[f] | 8.54 ± 0.10 | 6.1 | 313, 405, 427, 455 | 40 | 45 | 601 | 583, 565, 509, 491, 221 |
| 2 | (all-*E*)-Violaxanthin[f] | 39.06 ± 10.31 | 7.7 | 415, 438, 468 | 83 | 0 | 601 | 583, 565, 509, 491, 221 |
| 3 | (all-*E*)-Luteoxanthin[f] | 11.92 ± 0.54 | 8.9 | 398, 421, 448 | 100 | 0 | 601 | 583, 565, 491, 221 |
| 4 | Mix of Z-lutein + epoxycarotenoids[g] | 37.55 ± 0.96 | 10.6 | 330, 419, 440, 467 | 27 | 37 | 601 585 551[e] | 583, 565, 509, 491, 221 567, 549, 493, 475, 221 533, 459 |
| 5 | (all-*E*)-Lutein[g] | 204.28 ± 40.17 | 12.7 | 420, 444, 473 | 60 | 0 | 569 | 551, 533,477, 459 |
| 6 | (all-*E*)-Zeaxanthin[f] | 11.04 ± 1.16 | 15.2 | 420, 450, 476 | 25 | 0 | 569 | 551, 533, 477 |
| 7 | (13*Z*)-β-Carotene[f] | 17.28 ± 1.42 | 26.9 | 338, 415, 444, 469 | 11 | 42 | 537 | 444 |
| 8 | (all-*E*)-α-Carotene[f] | 79.86 ± 5.62 | 29.3 | 420, 445, 473 | 63 | 0 | 537 | 481, 444, 413 |
| 9 | (all-*E*)-β-Carotene[f] | 129.54 ± 8.38 | 33.3 | 420, 450, 477 | 29 | 0 | 537 | 457, 444, 413, 400 |
| 10 | (9*Z*)-β-Carotene[f] | 21.40 ± 0.77 | 35.2 | 340, 420, 446, 472 | 14 | 8 | 537 | 457, 444, 413 |
| Total carotenoids (μg/g leaves) | | 560.46 ± 69.42 | | | | | | |
| Vitamin A value (μg RAE/g leaves) | | 15.73 ± 1.02 | | | | | | |

[a]Tentative identification based on Uv-visible and mass spectra as well as retention times on $C_{30}$ column and published data. [b]Mean ± standard deviation (n = 3, dry basis). [c]Retention time on the $C_{30}$ column. [d]Linear gradient of methanol/MTBE. [e]In-source detected fragment. The peaks were quantified as equivalent of β-carotene[f] and lutein[g]. RAE = retinol activity equivalent.

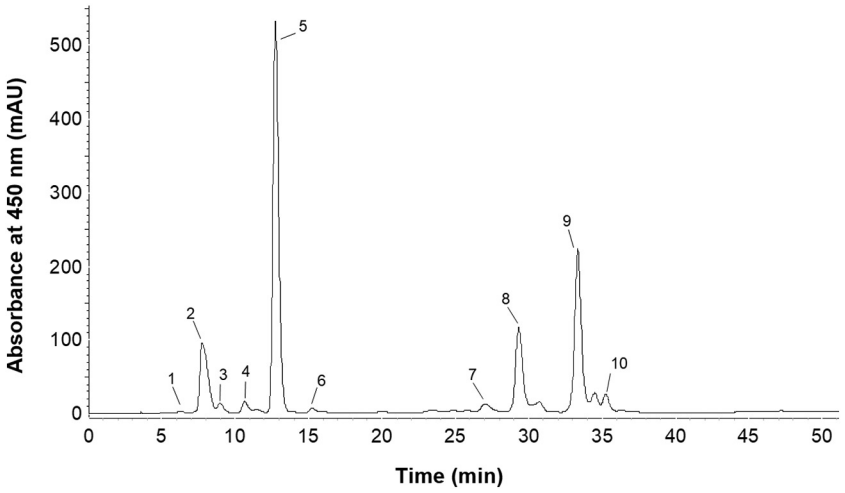


**Fig. 2.** HPLC-DAD chromatogram at 450 nm of carotenoids of *Arrabidaea chica* leaf extract. Peaks: 1) (13*Z*)-violaxathin; 2) (all-*E*)-violaxanthin; 3) (all-*E*)-luteoxanthin;4) Mix of *Z*-lutein + epoxycarotenoids; 5) (all-*E*)-lutein; 6) (all-*E*)-zeaxanthin; 7) (13*Z*)-β-carotene; 8) (all-*E*)-α-carotene; 9) (all-*E*)-*β*-Carotene; 10) (9*Z*)-β-carotene. The peak characterization is given in Table 2.

same chemical formula ($C_{40}H_{56}O_2$) and showed $[M + H]^+$ at *m/z* 569 and MS/MS fragments at *m/z* 551 $[M + H– H_2O]^+$, *m/z* 533 $[M + H-2H_2O]^+$ and at *m/z* 477 $[M + H-92]^+$; however, zeaxanthin has 11 conjugated double bonds (c.d.b.) and two β-rings, while lutein has 10 c.d.b, one β-ring and one ε-ring (De Faria et al., 2009). Both the compounds can be differentiated by the UV-vis spectrum, where lutein presented $\lambda_{max}$ values lower than those of zeaxanthin, and MS spectrum, which showed an intense fragment at *m/z* 551 when compared to the $[M + H]^+$ (*m/z* 569), in contrast to that observed for zeaxanthin (De Rosso & Mercadante, 2007).

The carotenes (13*Z*)-β-carotene (**peak 7**), (all-*E*)-α-carotene (**peak 8**), (all-*E*)-β-carotene (**peak 9**) and (9*Z*)-β-carotene (**peak 10**) showed $[M + H]^+$ at *m/z* 537 and the characteristic fragment at *m/z* 444 $[M-92]^+$ from the polyene chain. The (13*Z*)-β-carotene and (9*Z*)-β-carotene were identified by the presence of the *cis*-peak at the 338–340 nm range in their UV-vis spectra (Table 2). In addition, the assignment of these *Z* isomers assumes that the *cis* peak intensity (%$A_B/A_{II}$) increases and the spectral fine structure (%III/II) decreases as the (*Z*)-double bond approaches the center of the molecule (Britton, Liaaen-Jensen, & Pfander, 2004). The (all-*E*)-α-carotene has the same chromophore as lutein and, therefore, their UV-visible absorption spectra were similar. In addition, (all-*E*)-α-carotene showed the presence of intense fragments at *m/z* 481 and *m/z* 444 in the MS/MS spectrum, corresponding to the neutral losses of the ε-ring and toluene, respectively. In (all-*E*)-β-carotene MS spectrum, the elimination of β-ring with an additional methylene group, $[M + H-137]^+$ resulted in the formation of the fragment at *m/z* 400.

This is the first report concerning the carotenoid profile of *A. chica* leaves. Among the identified carotenoids, only β-carotene (and its *cis* isomer) and α-carotene were the compounds that contributed to vitamin A activity (15.73 μg RAE/g d.b. leaves). The vitamin A activity of *A. chica* leaves was similar to that reported for *Allmania nodiflora* (16.05 μg RAE/g dw), and lower than *Alternanthera pungens* (57.76 μg RAE/g dw), which are other examples of green leafy vegetables, but higher than *Brassica oleracea* (6.41 μg RAE/g dw) and *Mentha spicata* L. (12.46 μg RAE/g dw) (Raju, Varakumar, Lakshminarayana, Parthasarathy, & Baskaran, 2007).

Regarding the lutein content of *A. chica* leaves, it was about thrice higher than the leaves of *Prunus armeniaca*, whose values (56.7–65.7 μg/g) varied during the maturation period (Zeb, Khadim, & Ali, 2017) and about twenty to twenty-seven times higher than the values found for different varieties of *Hippophae rhamnoides* leaves (8–11 μg/g) (Pop et al., 2014). In the same study, the contents of zeaxanthin (5–6 μg/g) were about twice lower that *A. chica* leaves. These data are of paramount importance, since high consumption of dark green leafy vegetables, specifically rich in lutein and zeaxanthin, are associated with a lower risk of cataract and age-related macular degeneration (Moeller, Jacques, & Blumberg, 2000). In addition, according to the classification of good sources of carotenoids (low: 0–1 μg/g, moderate: 1–5 μg/g, high: 5–20 μg/g, very high > 20 μg/g), as suggested by Britton and Khachik (2009), the freeze-dried *A. chica* leaves can be considered as a high source of zeaxanthin and very high of lutein, β-carotene and α-carotene.

### 3.3. In vitro antioxidant capacity of A. chica extract

The MeOH/water extract of *A. chica* leaves was able to scavenge ABTS radical with values (86.81 ± 1.5 μM TE/g, FW) higher than those previously reported for *A. chica* leaves (64 μM TE/g, FW) in an exploratory study with fifteen selected plants from Amazonian region (Silva et al., 2007). Although ABTS radical is a stable and non-biological reactive species, it is a single electron transfer-based assay used by many research laboratories for studying antioxidant capacity due to its operational simplicity. However, when ABTS assay is considered, there are conflicting results on pure antioxidant compounds, associating their structure and ability to donate one or two electrons with their scavenging efficiency (Huang, Ou, & Prior, 2005). As examples, in a study, scutellarin and scutellarein demonstrated $IC_{50}$ values at 3.53 and 3.00 μM, respectively (Qian et al., 2011), and in another study, they exhibited 33.3 μM and 18.3 μM, respectively (Liu, Li, Ouyang, & Chen, 2018).

In this study, the $^1O_2$-quenching ability of *A. chica* leaf extract was assessed since $^1O_2$ is a highly reactive oxygen species (ROS) that can be generated in both physiological and food systems in the presence of light and photosensitizers. The photosensitization system used in this study was efficient in the generation of $^1O_2$ with consequent degradation of tryptophan. The *A. chica* extract, at all the tested concentrations, was able to protect tryptophan against the oxidation damage of $^1O_2$ and this quenching effect was fitted to the first-order reaction with high values of determination coefficient ($R^2 = 0.99$), as shown in Fig. 3a. This is the first report concerning the quenching ability of *A. chica* leaf extract against oxidative damage of $^1O_2$ and a concentration-dependent effect on $^1O_2$ inhibition was observed for the extract (Fig. 3b) with an $IC_{50}$ at 177.2 ± 4.2 μg/mL. The extract of *A. chica* leaves at 375 μg/mL exhibited the highest percentage of protection (90.30%) against $^1O_2$, but lower quenching efficiency than that found for quercetin (positive control) ($IC_{50}$ = 1.88 ± 0.08 μg/mL). However, when compared to the literature, the *A. chica* extract showed higher efficiency as $^1O_2$ quencher than other leaf plant extracts, such as *Terminalia chebula* ($IC_{50}$ = 424.50 μg/mL), *Terminalia belerica* ($IC_{50}$ = 233.12 μg/mL) and *Emblica officinalis* ($IC_{50}$ = 490.42 μg/mL), obtained with MeOH/water (70:30, *v*/v) (Hazra, Sarkar, Biswas, & Mandal, 2010), whereas lower

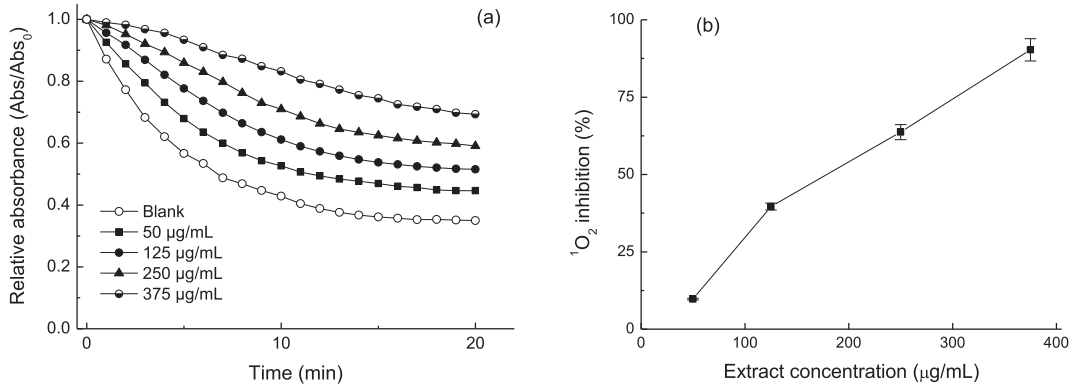


**Fig. 3.** (a) Decay of absorbance intensity of tryptophan in the presence of sensitizer (methylene blue) and *Arrabidaea chica* leaf extracts and (b) percentage of $^1O_2$ inhibition as a function of the *Arrabidaea chica* leaf extract concentration. Each point represents the values obtained in four concentrations, performed in triplicate (Mean ± standard deviation).

than the ethanolic extract of *Vismia cauleflora* ($IC_{50}$ = 27 μg/mL), another Amazonian plant (Ribeiro et al., 2015).

The hydromethanolic extract of *A. chica* leaves exhibited high levels of phenolic compounds, as well as a high antioxidant capacity in both the assays. Since ascorbic acid was not detected in this extract and scutellarin content accounted for ≈70% of the sum of the identified phenolic compounds, it was suggested that this compound might presented high contribution to the assessed antioxidant capacity since a high antioxidant capacity was already reported in the literature for scutellarin (Hong & Liu, 2004; Liu, Yang, Tang, Liu, & Xu, 2005).

## 4. Conclusion

*A. chica* leaves can be seen as a promising source of bioactive compounds with high antioxidant capacity probably due to their high contents of phenolic compounds, which may explain the therapeutic use of these leaves in the folk medicine. For the first time, the presence of feruloyl derivative compounds, scutellarin, methyl apigenin glucuronide and ascorbic acid, and the carotenoid profile of *A. chica* leaves were reported, as well as its protective effect against $^1O_2$, which occurred in a concentration-dependent manner.

## Declaration of Competing Interest

The authors have no conflict of interest.

## Acknowledgements

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## References

Abubakar, M., Abas, F., Wei, S., Shaari, K., Sa, I., Ghazali, H. M., & Lajis, N. H. (2016). Chemical constituents and biological activities of callicarpa maingayi leaves. *South African Journal of Botany, 104*, 98–104. https://doi.org/10.1016/j.sajb.2015.11.010.

AOAC (Association of Analytical Chemists) (2000). *Official methods of analysis* (17th ed.). Gaithersburg, MD, USA: AOAC International.

Aro, A. A., Simões, G. F., Esquisatto, M. A. M., Foglio, M. A., Carvalho, J. E., Oliveira, A. L. R., ... Pimentel, E. R. (2013). Arrabidaea chica extract improves gait recovery and changes collagen content during healing of the achilles tendon. *Injury, 44*(7), 884–892. https://doi.org/10.1016/j.injury.2012.08.055.

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry, 99*, 191–203. https://doi.org/10.1016/j.foodchem.2005.07.042.

Bergquist, S.Å. M., Gertsson, U. E., & Olsson, M. E. (2006). Influence of growth stage and postharvest storage on ascorbic acid and carotenoid content and visual quality of baby spinach (Spinacia oleracea L.). *Journal of the Science of Food and Agriculture, 86*(3), 346–355. https://doi.org/10.1002/jsfa.2373.

Berto, A., Ribeiro, A. B., de Souza, N. E., Fernandes, E., & Chisté, R. C. (2015). Bioactive compounds and scavenging capacity of pulp, peel and seed extracts of the Amazonian fruit quararibea cordata against ROS and RNS. *Food Research International, 77*, 236–243. https://doi.org/10.1016/j.foodres.2015.06.018.

Britton, G., & Khachik, F. (2009). Carotenoids in food. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Vol. Eds.), *Carotenoids: Nutrition and health. Vol. 5. Carotenoids: Nutrition and health* (pp. 45–66). Basel: Birkhäuser.

Britton, G., Liaaen-Jensen, S., & Pfander, H. (2004). *Carotenoids handbook.* Basel: Springer Basel AGhttps://doi.org/10.1007/978-3-0348-7836-4.

Chandrasekara, A., & Shahidi, F. (2011). Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MSn. *Journal of Functional Foods, 3*(3), 144–158. https://doi.org/10.1016/j.jff.2011.03.007.

Chisté, R., Benassi, M. T., & Mercadante, A. Z. (2011). Effect of solvent type on the extractability of bioactive compounds, antioxidant capacity and colour properties of natural annatto extracts. *International Journal of Food Science and Technology, 46*(9), 1863–1870. https://doi.org/10.1111/j.1365-2621.2011.02693.x.

Chisté, R. C., & Mercadante, A. Z. (2012). Identification and quantification, by HPLC-DAD-MS/MS, of carotenoids and phenolic compounds from the Amazonian fruit Caryocar villosum. *Journal of Agricultural and Food Chemistry, 60*(23), 5884–5892. https://doi.org/10.1021/jf301904f.

Choe, E., & Min, D. B. (2015). Chemistry and reactions of reactive oxygen species in foods. *Critical Reviews in Food Science and Nutrition, 46*(1), 1–22. https://doi.org/10.1080/10408390500455474.

Crupi, P., Toci, A. T., Mangini, S., Wrubl, F., Rodolfi, L., Tredici, M. R., ... Antonacci, D. (2013). Determination of fucoxanthin isomers in microalgae (Isochrysis sp.) by high-performance liquid chromatography coupled with diode-array detector multistage mass spectrometry coupled with positive electrospray ionization. *Rapid Communications in Mass Spectrometry,* 1027–1035. https://doi.org/10.1002/rcm.6531 January.

De Faria, A. F., De Rosso, V. V., & Mercadante, A. Z. (2009). Carotenoid composition of jackfruit (Artocarpus heterophyllus), determined by HPLC-PDA-MS/MS. *Plant Foods for Human Nutrition, 64*(2), 108–115. https://doi.org/10.1007/s11130-009-0111-6.

De Rosso, V. V., & Mercadante, A. Z. (2007). Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from Amazonian fruits. *Journal of Agricultural and Food Chemistry, 55*(13), 5062–5072. https://doi.org/10.1021/jf0705421.

Devia, B., Llabres, G., Wouters, J., Dupont, L., Escribano-Bailon, M. T., De Pascual-Teresa, S., ... Tits, M. (2002). New 3-deoxyanthocyanidins from leaves of Arrabidaea chica. *Phytochemical Analysis, 13*(2), 114–120. https://doi.org/10.1002/pca.632.

Dziadek, K., Kopeć, A., & Tabaszewska, M. (2018). Potential of sweet cherry (Prunus avium L.) by-products: Bioactive compounds and antioxidant activity of leaves and petioles. *European Food Research and Technology, 0*(0), 0. https://doi.org/10.1007/s00217-018-3198-x.

Ekpa, O. D. (1996). Nutrient composition of three nigerian medicinal plants. *Food Chemistry, 57*(2), 229–232. https://doi.org/10.1016/0308-8146(95)00206-5.

El-hela, A. A., Al-amier, H. A., & Ibrahim, T. A. (2010). Comparative study of the flavonoids of some Verbena species cultivated in Egypt by using high-performance liquid chromatography coupled with ultraviolet spectroscopy and atmospheric pressure chemical ionization mass spectrometry. *Journal of Chromatography A, 1217*(41), 6388–6393. https://doi.org/10.1016/j.chroma.2010.08.025.

FAO/WHO (2003). *Food energy—methods of analysis and conversion factors. Report of a technical workshop.* FAO Food and Nutrition Paper No. 77Rome: Food and Agriculture Organization of the United Nations. http://www.fao.org/3/Y5022E/y5022e00.htm/Acessed, Accessed date: 20 December 2018.

Halliwell, B. (2012). *Free radicals and antioxidants: Updating a personal view. 70*, 257–265.

https://doi.org/10.1111/j.1753-4887.2012.00476.x 5.
Hazra, B., Sarkar, R., Biswas, S., & Mandal, N. (2010). Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of *Terminalia chebula*, Terminalia belerica and emblica officinalis. *BCM Complementary&Alternative Medicine, 10*, 20. https://doi.org/10.1186/1472-6882-10-20.
Hong, H., & Liu, G. Q. (2004). Scutellarin attenuates oxidative glutamate toxicity in PC12 cells. *Planta Medica, 70*, 427–431. https://doi.org/10.1055/s-2004-818970.
Huang, D., Ou, B., & Prior, R. L. P. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*(6), 1841–1856. https://doi.org/10.1021/jf030723c.
ICH (2005). International conference on harmonization of technical requirements for the registration of pharmaceuticals for human use (ICH). *Guidance for Industry, Q2B Validation of Analytical Procedures: Methodology*ICH Secretariat c/o: Geneva (IFPMA7–100).
Katoch, N., Kaur, P., Kashyap, P., Gupta, S., & Dahiya, R. S. (2013). Role of oxidative stress in cardiovascular diseases. *Research Journal of Pharmaceutical, Biological and Chemical Sciences, 4*(3), 870–881. https://doi.org/10.1097/00004872-200018060-00002.
Liu, H., Yang, X., Tang, R., Liu, J., & Xu, H. (2005). Effect of scutellarin on nitric oxide production in early stages of neuron damage induced by hydrogen peroxide. *Pharmacology Research, 51*, 205–210. https://doi.org/10.1016/j.phrs.2004.09.001.
Liu, Q., Li, X., Ouyang, X., & Chen, D. (2018). Dual effect of Glucuronidation of a Pyrogallol-type phytophenol antioxidant: A comparison between. *Molecules, 23*(12), 3225. https://doi.org/10.3390/molecules23123225.
Mafioleti, L., da Silva Junior, I. F., Colodel, E. M., Flach, A., & de Oliveira, D. T. M. (2013). Evaluation of the toxicity and antimicrobial activity of hydroethanolic extract of Arrabidaea chica (Humb. & Bonpl.) B. Verl. *Journal of Ethnopharmacology, 150*(2), 576–582. https://doi.org/10.1016/j.jep.2013.09.008.
Mcnab, H., Ferreira, E. S. B., Hulme, A. N., & Quye, A. (2009). Negative ion ESI – MS analysis of natural yellow dye flavonoids — An isotopic labelling study. *International Journal of Mass Spectrometry, 284*, 57–65. https://doi.org/10.1016/j.ijms.2008.05.039.
Moeller, S. M., Jacques, P. F., & Blumberg, J. B. (2000). The potential role of dietary Xanthophylls in cataract and age-related macular degeneration. *Journal of the American College of Nutrition, 19*(5), 522S–527S. https://doi.org/10.1080/07315724.2000.10718975.
NAS-IOM (2001). *Dietary reference intakes for vitamin a, vitamin k, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc.* WA: National Academy Press92.
Pop, M. R., Weesepoel, Y., Socaciu, C., Pintea, A., Vincken, J., & Gruppen, H. (2014). Carotenoid composition of berries and leaves from six romanian sea buckthorn (*Hippophae rhamnoides* L.) varieties. *Food Chemistry, 147*, 1–9. https://doi.org/10.1016/j.foodchem.2013.09.083.
Qian, L., Li, N., Tang, Y., Zhang, L., Tang, H., Wang, Z., ... Ding, A.-W. (2011). Synthesis and bio-activity evaluation of Scutellarein as a potent agent for the therapy of ischemic cerebrovascular disease. *International Journal of Molecular Sciences, 12*, 8208–8216. https://doi.org/10.3390/ijms12118208.
Raju, M., Varakumar, S., Lakshminarayana, R., Parthasarathy, T., & Baskaran, V. (2007). Carotenoid composition and vitamin A activity of medicinally important green leafy vegetables. *Food Chemistry, 101*, 1598–1605. https://doi.org/10.1016/j.foodchem.2006.04.015.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Catherine, R.-E. (1999). Antioxidant activity applying an improved abts radical cation decolorization assay. *Free Radical Biology & Medicine, 26*(98), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.
Ribeiro, A. B., Berto, A., Chisté, R. C., Freitas, M., Visentainer, J. V., & Fernandes, E. (2015). Bioactive compounds and scavenging capacity of extracts from different parts of Vismia cauliflora against reactive oxygen and nitrogen species. *Pharmaceutical Biology, 0209*, 1267–1276. https://doi.org/10.3109/13880209.2014.974063.
Ribeiro, A. B., Chisté, R. C., Lima, J. L. F. C., & Fernandes, E. (2016). Solanum diploconos fruits: Profile of bioactive compounds and in vitro antioxidant capacity of different parts of the fruit. *Food & Function, 7*(5), 2249–2257. https://doi.org/10.1039/C6FO00326E.
Ribeiro, A. F. C., Telles, T. C., Ferraz, V. P., Souza-Fagundes, E. M., Cassali, G. D., Carvalho, A. T., & Melo, M. M. (2012). Effect of arrabidaea chica extracts on the ehrlich solid tumor development. *Brazilian Journal of Pharmacognosy, 22*(2), 364–373. https://doi.org/10.1590/S0102-695X2011005000225.
Ribeiro, S. M., De Queiroz, H. J., De Queiroz, M. E. L. R., Campos, F. M., & Sant'ana, H. M. P. (2007). Antioxidant in mango (*Mangifera indica* L.) pulp. *Plant Foods for Human Nutrition, 62*, 13–17. https://doi.org/10.1007/s11130-006-0035-3.
Sharififar, F., Dehghn-nudeh, G., & Mirtajaldini, M. (2009). Major flavonoids with antioxidant activity from Teucrium polium L. *Food Chemistry, 112*, 885–888. https://doi.org/10.1016/j.foodchem.2008.06.064.
Shi, Y., Wu, C., Chen, Y., Liu, W., Feng, F., & Xie, N. (2013). Comparative analysis of three callicarpa herbs using high performance liquid chromatography with diode array detector and electrospray ionization-trap mass spectrometry method. *Journal of Pharmaceutical and Biomedical Analysis, 75*, 239–247. https://doi.org/10.1016/j.jpba.2012.11.038.
Silva, B. A., Ferreres, F., Malva, O., & Dias, A. C. P. (2005). Phytochemical and antioxidant characterization of Hypericum perforatum alcoholic extracts. *Food Chemistry, 90*, 157–167. https://doi.org/10.1016/j.foodchem.2004.03.049.
Silva, E. M., Souza, J. N. S., Rogez, H., Rees, J. F., & Larondelle, Y. (2007). Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chemistry, 101*(3), 1012–1018. https://doi.org/10.1016/j.foodchem.2006.02.055.
Siraichi, J. T. G., Felipe, D. F., Brambilla, L. Z. S., Gatto, M. J., Terra, V. A., Cecchini, A. L., ... Cortez, D. A. G. (2013). Antioxidant capacity of the leaf extract obtained from arrabidaea chica cultivated in Southern Brazil. *PLoS One, 8*(8), 1–10. https://doi.org/10.1371/journal.pone.0072733.
Sun, H., Mu, T., Xi, L., Zhang, M., & Chen, J. (2014). Sweet potato (*Ipomoea batatas* L.) leaves as nutritional and functional foods. *Food Chemistry, 156*, 380–389. https://doi.org/10.1016/j.foodchem.2014.01.079.
Surowiec, I., Szostek, B., & Trojanowicz, M. (2007). HPLC-MS of anthraquinoids, flavonoids, and their degradation products in analysis of natural dyes in archeological objects. *Journal of Separation Science, 30*(13), 2070–2079. https://doi.org/10.1002/jssc.200700041.
Tomás-Barberán, F. A., & Espín, J. C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agricuture, 81*(February 2001), 853–876. https://doi.org/10.1002/jsfa.885.
Vallejo, F., Tomás-Barberán, F., & Garcia-Viguera, C. (2003). Health-promoting compounds in broccoli as influenced by refrigerated transport and retail sale period. *Journal of Agricultural and Food Chemistry, 51*, 3029–3034. https://doi.org/10.1021/jf021065j.
Zeb, A., Khadim, N., & Ali, W. (2017). Changes in the polyphenolic profile, carotenoids and antioxidant potential of apricot (*Prunus armeniaca* L.) leaves during maturation. *Agriculture, 7*(9), https://doi.org/10.3390/agriculture7020009.
Zorn, B., García-Pieres, A. J., Castro, V., Murillo, R., Mora, G., & Merfort, I. (2001). 3-Desoxyanthocyanidins from Arrabidaea chica. *Phytochemistry, 56*(8), 831–835. https://doi.org/10.1016/S0031-9422(01)00038-3.

# ANEXO B

*antioxidants*

MDPI

*Article*

# Scavenging Capacity of Extracts of *Arrabidaea chica* Leaves from the Amazonia against ROS and RNS of Physiological and Food Relevance

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**Abstract:** *Arrabidaea chica*, a medicinal plant found in the Amazon rainforest, is a promising source of bioactive compounds which can be used to inhibit oxidative damage in both food and biological systems. In this study, the in vitro scavenging capacity of characterized extracts of *A. chica* leaves, obtained with green solvents of different polarities [water, ethanol, and ethanol/water (1:1, *v*/*v*)] through ultrasound-assisted extraction, was investigated against reactive oxygen (ROS) and nitrogen (RNS) species, namely superoxide anion radicals ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl), and peroxynitrite anion ($ONOO^-$). The extract obtained with ethanol–water presented about three times more phenolic compound contents (11.8 mg/g) than ethanol and water extracts (3.8 and 3.6 mg/g, respectively), with scutellarein being the major compound (6.76 mg/g). All extracts showed high scavenging efficiency against the tested ROS and RNS, in a concentration-dependent manner with low $IC_{50}$ values, and the ethanol–water extract was the most effective one. In addition, all the extracts were five times more efficient against $ROO^{\bullet}$ than Trolox. Therefore, the extracts from *A. chica* leaves exhibited high promising antioxidant potential to be used against oxidative damage in food and physiological systems.

**Keywords:** antioxidant capacity; Amazonian plant; reactive oxygen species; reactive nitrogen species; green solvents; phenolic compounds



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## 1. Introduction

In biological systems, reactive oxygen (ROS) and nitrogen (RNS) species play an essential role in maintaining the body's normal physiological condition [1,2]. However, the overproduction of ROS/RNS, as a result of physiological disorders, combined with the deficiency of endogenous and exogenous antioxidants in the human body, induces oxidative and nitrosative stress, damaging basic components for function and cell survival, which may be related to the occurrence of several chronic degenerative diseases, such as cardiovascular diseases, diabetes, Alzheimer's, and cancer [3–5].

In addition to the deleterious effect caused to physiological systems, oxidative reactions induced by the presence of ROS and RNS can also be observed in food systems. The oxidative effects of ROS can occur during food harvesting, storage, and processing, producing off-flavors as well as changes in color and texture, which adversely affect the overall quality and food safety due to the degradation of essential fatty acids, amino acids,

and vitamins, and might promote the formation of toxic and carcinogenic compounds [6–9]. Due to these harmful effects, the inhibition of ROS and RNS in food systems is highly desirable and, therefore, the use of antioxidants as food additive (natural or artificial) can be seen as a very efficient strategy to delay oxidative reactions that decrease the shelf life of processed foods.

Scientific evidences suggest that antioxidants obtained from natural sources, such as fruits and other vegetables, are efficient replacements to synthetic antioxidants with the same purpose of preventing the formation of undesirable oxidation products, improving the quality of food products, consequently improving the life quality of consumers [10–14]. Scientific data, combined with the perception of modern consumers on the need for foods with natural and healthier ingredients in the formulation, resulted in a growing trend at the scientific level and in the food industry for the incorporation of natural additives.

In this context, the Amazon is known as one of the richest biomes in the world concerning biodiversity, including several claimed medicinal plants, which present a number of bioactive molecules in their composition, with promising biological activities [15]. Among these plants, *Arrabidaea chica* (Humb. & Bonpl.) B. Verlot (pariri), from the Bignoniaceae family, is potentially rich in phenolic compounds that has been used to treat various diseases [16–18]. Some studies have also reported anti-inflammatory, antimicrobial, and antifungal properties for the leaves of *A. chica* [15,19].

According to a recent study published by our research group, *A. chica* leaves were considered to be promising sources of phenolic compound contents, with feruloyl hexose, scutellarin, apigenin glucuronide, flavone-glucuronyl derivative, scutellarein, methyl apigenin glucuronide, and apigenin being tentatively identified, in addition to exhibiting high quenching abilities against singlet oxygen ($^1O_2$) [20], a highly reactive ROS derived from molecular oxygen.

Other studies also showed that the extract of *A. chica* leaves has higher antioxidant capacity when compared to scutellarein and apigenin isolated from the leaf, as evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl), β-carotene/linoleic acid, total reactive antioxidant potential (TRAP), and peroxyl radical ($ROO^•$) assays [21,22]. Furthermore, in an in vitro cellular study, post-treatment with *A. chica* in L929 fibroblasts decreased oxidative damage by inhibiting intracellular ROS and mitochondrial superoxide anion radicals ($O_2^{•-}$) induced by UV-A and UV-B irradiation [5]. However, to the best of our knowledge, there are no reports in the literature regarding the direct antioxidant effect of *A.chica* extracts against other ROS and RNS commonly found in physiological and food systems, namely $O_2^{•-}$, hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl), and peroxynitrite anion ($ONOO^-$). Such information may stimulate relevant applicability to extracts obtained from *A. chica* leaves to confer antioxidant action in several applications of interest to food, pharmaceutical, and cosmetic industries.

On the other hand, it should be taken into account that several factors can significantly affect the phytochemical content and antioxidant capacity of natural extracts, including the type and polarity of the solvent used [23,24]. In addition, there is a greater appeal for the use of solvents that favor green chemistry, associated with the use of emergent extraction techniques, for example, ultrasound-assisted extraction (UAE), which is safer and less time-consuming, promotes high-efficiency extractions, and can minimize environmental impacts [25,26].

Therefore, our study investigated the in vitro antioxidant capacity of *A. chica* extracts, obtained through UAE technique using three green solvents of different polarities [water, ethanol and ethanol/water mixture (1:1, *v*/*v*)] against the ROS and RNS of food and physiological relevance, namely $O_2^{•-}$, $H_2O_2$, HOCl, $^1O_2$, $ROO^•$, and $ONOO^-$ (in the presence and absence of $NaHCO_3$ to simulate physiological conditions).

## 2. Materials and Methods

### *2.1. Chemicals*

Scutellarin, apigenin, quercetin, L-ascorbic acid, quercetin 6-hydroxy-2,5,7,8 -tetramethylchroman-2-carboxylic acid (trolox), lucigenin, fluorescein Dihydrorodamine (DHR), phenazine methosulfate (PMS), nitroblue tetrazolium chloride (NBT), α,α′-Azodiisobutyramidine dihydrochloride (AAPH), dimethyl sulfoxide (DMSO), sodium hypochlorite solution (NaClO, with 4% available chlorine), β-nicotinamide adenine dinucleotide (NADH), Tris-buffer HCl, methylene blue (MB), hydrogen peroxide (30%), ethanol (EtOH), methanol (MeOH), acetonitrile, formic acid, methylene blue (MB), sulfuric acid, formic acid, sodium chloride, tribasic sodium phosphate dodecahydrate, potassium chloride, sodium nitrite, sodium bicarbonate, monobasic potassium phosphate, and dibasic sodium phosphate were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-tryptophan was obtained from Fisher Scientific (Pittsburgh, PA, USA). For all chromatographic analyses, samples and solvents were filtered using, respectively, 0.22 and 0.45 µm membranes, both from Millipore (Billerica, MA, USA). Ultrapure water was obtained from the Milli-Q system (Millipore Corp., Milford, MA, USA).

### *2.2. Arrabidaea chica Leaves*

The leaves of *A. chica* (≈250 g), collected from five different plants belonging to the Active Germplasm Bank of *EMBRAPA Amazonia Oriental*, located in Belém, Pará, Brazil (01°26′14.7″ S and 48°26′52.2″ W), were freeze-dried (Liotop, L101, São Paulo, Brazil), ground in a knife mill, vacuum packed in plastic bags, and stored at −18 °C until analysis.

Access to the selected leaves was registered in the Brazilian National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen #A89EDD3).

### *2.3. Extracts of Arrabidaea chica Leaves*

Three extracts from the leaves of *A. chica* were prepared, according to the methodology described by Chisté et al. [27] with some modifications, using the following green solvents: water, ethanol, and the ethanol/water mixture (1:1, *v*/*v*). The choice for these solvents considered the permissibility of residues in the extracts after evaporation, in accordance with Directive 95/45/EC of the Commission of European Communities [28].

The freeze-dried leaves of *A. chica* (3 g) were subjected to UAE with each green solvent in an ultrasonic bath (QUIMIS—model 03350, Diadema-SãoPaulo/Brazil) for 5 min at room temperature (25 °C), and fixed ultrasonic frequency at 25 KHz, at a solid/liquid ratio of 1:10 (*w*/*v*). After the UAE procedure, the extracts were centrifuged (Heraeus multifuge x 1R Thermo Electron Led GMBH, Göttingen, Germany) at 11,648× *g* for 5 min. The extraction procedure was repeated seven times for each solvent and the supernatants were combined after vacuum filtration. The extracts containing ethanol in the composition were subjected to evaporation at reduced pressure in a rotary evaporator (T < 38 °C). Water extract and the remaining water in the ethanol/water extract were frozen and freeze-dried. All the dried extracts were sealed under $N_2$ flow and stored at −18 °C under light-free conditions, until analysis. The extractions were carried out in triplicate ($n$ = 3).

### *2.4. HPLC-DAD Determination of Phenolic Compounds in the A. chica Extracts*

The phenolic compound compositions of *A. Chica* extracts were determined by high-performance liquid chromatography (HPLC), coupled with a diode array detector (DAD) on an Agilent HPLC (model Agilent 1260 Infinity, Santa Clara, CA, USA) equipped with a quaternary pump (G1311C), an automatic injector (G7129), an oven (G1316A), and a DAD detector (G1328C).

The *A. chica* extracts were analyzed after solubilizing 10 mg of each dried extract in methanol/water (80:20, *v*/*v*) and the phenolic compounds were separated on a $C_{18}$ Synergi Hydro column (Phenomenex, Torrance, CA, USA, 4 µm, 250 × 4.6 mm), at a temperature of 29 °C at 0.9 mL/min, with a linear gradient consisting of water–formic acid (99.5:0.5,

*v*/*v*) and acetonitrile/formic acid (99.5:0.5, *v*/*v*) [29]. The UV–visible (UV–vis) spectra were recorded from 200 to 600 nm, and chromatograms were processed at 270, 320, and 360 nm. The phenolic compounds were identified by combining the following information: elution order, retention time in the $C_{18}$ column, comparison with authentic standards analyzed under the same conditions, and UV–vis spectra compared to the phenolic compounds previously identified for *A. chica* leaves by LC-MS in our research group [20]. The phenolic compounds were quantified using six-point analytical curves (3 to 100 µg/mL in duplicate) of scutellarin ($r^2$ = 0.99, limit of detection (LOD) = 0.14 µg/mL, and limit of quantification (LOQ) = 0.41 µg/mL), scutellarein ($r^2$ = 0.98, LOD = 0.23 µg/mL, and LOQ = 0.69 µg/mL), and apigenin ($r^2$ = 0.99, LOD = 0.17 µg/mL and LOQ = 0.51 µg/mL). The parameters of the analytical curves (standard deviation and the slope) were used to calculate the LOD and LOQ values [30]. The phenolic compound contents were expressed as mg/g of dried extracts (dry basis, d.b.), considering three independent extraction procedures ($n$ = 3).

### *2.5. In Vitro Scavenging Capacity Determination against ROS and RNS*

The ROS and RNS scavenging assays were carried out at 37 °C, in a microplate reader (Synergy HT, BioTek, Winooski, VT, USA) equipped with a thermostat using fluorescence, absorbance, or chemiluminescence modes of detection. Each antioxidant assay corresponds to, at least, four individual experiments, in triplicate, using five concentrations (0.03 to 500 µg/mL). The dried extracts of *A. chica* were dissolved in ethanol/water (1:1, *v*/*v*) for all the assays, and analyzed immediately to avoid the degradation of bioactive compounds. Quercetin (0.001 to 30 µg/mL) and scutellarein (0.10–500 µg/mL) were used as positive controls in the $O_2^{\bullet-}$, HOCl, $^1O_2$ and $ONOO^-$ scavenging assays, while scutellarein and ascorbic acid (15 to 500 µg/mL) were used for $H_2O_2$. $IC_{50}$ values (µg/mL) were calculated from curves of antioxidant concentrations versus the inhibition percentage using Origin Pro 8 software (OriginLab Corporation, Northampton, MA, USA). Before carrying out each assay, additional tests to check interference effects among the *A. chica* extracts and the solvents with the used probes or selected wavelengths were carried out and no interference was observed for the assay conditions.

#### 2.5.1. Superoxide Anion Radical ($O_2^{\bullet-}$) Scavenging Assay

*A. chica* extracts were evaluated in relation to their capacity to scavenge $O_2^{\bullet-}$, using a non-enzymatic system containing NADH/PMS/$O_2$ [31]. This system is able to produce $O_2^{\bullet-}$, which reduces NBT to a purple diformazan. The reaction mixtures in the microplate wells contained the following reagents at final concentrations (in a final volume of 300 µL): NADH (166 µM), NBT (43.3 µM), PMS (2.7 µM), the extracts dissolved in EtOH/$H_2O$ solution (1:1, *v*/*v*), and quercetin dissolved in DMSO. NADH, NBT, and PMS were dissolved in 19 mM phosphate buffer, pH 7.4. The inhibition percentage of NBT reduction to diformazan, by the extract/standards, was monitored by spectrophotometry at 560 nm after 10 min of plate introduction.

#### 2.5.2. Hydrogen Peroxide ($H_2O_2$) Scavenging Assay

The scavenging capacity of *A.chica* extracts against $H_2O_2$ was determined by monitoring the inhibition of chemiluminescence resulting from the $H_2O_2$-induced oxidation of lucigenin [31]. The reaction mixtures in the wells contained the following reagents at final concentrations (in a final volume of 250 µL): 50 mM Tris-HCl buffer (PH 7.4), lucigenin (0.8 mM) dissolved in Tris-HCl buffer, extracts of *A. chica*, and 1% (*w*/*w*) $H_2O_2$. The chemiluminescence signal was detected in the microplate reader after 5 min of plate introduction. Results were expressed as an inhibition percentage of the $H_2O_2$-induced oxidation of lucigenin.

#### 2.5.3. Hypochlorous Acid (HOCl) Scavenging Assay

The scavenging capacity of *A. chica* extracts against HOCl was determined by monitoring the inhibition of HOCl-induced oxidation of DHR to rhodamine 123 [31]. HOCl was

prepared by adjusting a 1% (*v*/*v*) NaClO solution to pH 6.2 with $H_2SO_4$ 10% (*v*/*v*, followed by HOCl quantification by spectrophotometry at 235 nm, using the molar absorption coefficient of 100 $M^{-1} \cdot cm^{-1}$ [32]. The reaction mixture was composed of the following reagents at the indicated final concentrations (final volume of 300 µL): DHR (5 µM), HOCl (5 µM), and *A. chica* extracts. The fluorescence signal, at an emission wavelength at 528 ± 20 nm and excitation at 485 ± 20 nm, was detected in the microplate reader immediately after plate insertion. Results were expressed as an inhibition percentage of the HOCl-induced oxidation of DHR.

#### 2.5.4. Peroxynitrite Anion ($ONOO^-$) Scavenging Assay

The scavenging capacity of the *A. chica* extracts against $ONOO^-$ was determined by monitoring the inhibition of the $ONOO^-$-induced oxidation of DHR to fluorescent rhodamine 123 [32]. $ONOO^-$ was synthesized as described by [32]. The reaction mixture was composed of the following reagents at the indicated final concentrations (final volume of 300 µL): DHR (5 µM), extract or standard, and $ONOO^-$ (600 nM). The fluorescence signal, at an emission wavelength at 528 ± 20 nm and excitation at 485 ± 20 nm, was detected in the microplate reader after a 2 min incubation period. In a set of parallel experiments, assays were conducted in the presence of 25 mM $NaHCO_3$ to simulate physiological conditions of $CO_2$ concentration. The results were expressed as an inhibition percentage of the $ONOO^-$-induced oxidation of DHR.

#### 2.5.5. Peroxyl Radical Scavenging Assay ($ROO^•$) (ORAC)

$ROO^•$ was generated by the thermodecomposition of AAPH at 37 °C, and the $ROO^•$ scavenging capacity was measured by monitoring the effects of *A. chica* extract on the inhibition of fluorescence decay, due to fluorescein oxidation, induced by $ROO^•$ [33,34]. The reaction mixtures consisted of the following reagents at the final concentrations (final volume of 200 µL): fluorescein (61.2 nM), AAPH solution (19.1 mM), and different concentrations of *A. chica* extracts dissolved in 75 mM phosphate buffer (pH 7.4). Trolox was used as a positive control. The fluorescence signal, at the emission wavelength of 528 nm with excitation at 485 nm, was monitored every minute until the total decay of fluorescence. The relative ability to capture $ROO^•$ was expressed as the ratio between the slope of the curve of each extract or positive control and the slope obtained for trolox, as proposed by [34].

#### 2.5.6. Singlet Oxygen ($^1O_2$) Quenching Assay

The scavenging capacity of *A. chica* extracts and positive controls (quercetin and scutellarein) to inhibit $^1O_2$ was evaluated according to the method described by De Siqueira et al. [20]. The $^1O_2$ was generated, at room temperature (25 °C) and under atmospheric air, via the direct sensitization of methylene blue (MB) using a 75W incandescent lamp, used as an excitation source, and two filters (red and orange) were placed between the excitation source and the cuvette containing the reactants (*A. chica* extratcs, L-tryptophan, and MB), to excite MB only. The reaction was monitored by spectrophotometry, in the range of 200–800 nm, for 20 min, and the absorbance of tryptophan was recorded at 219 nm. The kinetic data obtained from the tryptophan absorbance decay were fitted to a first-order reaction to calculate the rate constants. The protection percentage of the *A. chica* extract, or the positive controls, against the oxidative damage of $^1O_2$ was calculated through Equation (1).

$$Protection\ (\%) = \frac{K_{obs}^{TRP} - K_{obs}^{TRP+antioxidant}}{K_{obs}^{TRP}} \times 100 \quad (1)$$

where $K_{obs}^{TRP}$ is the rate constant for the observed pseudo-first-order reaction fitted to the TRP decay curve (obtained in the blank experiment) and $K_{obs}^{TRP+antioxidant}$ is the rate constant for the observed pseudo-first-order reaction fitted to the decay curve of TRP in the presence of the antioxidant compound.

### 2.6. Statistical Analysis

The $IC_{50}$ values (mean ± standard deviation) were subjected to ANOVA analysis of variance and the means were classified by Tukey's test at the 95% significance level using Statistica 7.0 software (Statsoft Inc., Tulsa, OK, USA). Analytical curves were plotted by linear regression ($p < 0.05$) using Origin 8 Software (OriginLab Corporation, Northampton, MA, USA).

## 3. Results and Discussion

### 3.1. Phenolic Compounds Composition of Arrabidaea chica Extracts

Any modification in the composition of a selected solvent alters its polarity, and consequently promotes changes in the phenolic compounds' composition during extraction procedures [31]. Considering the low toxicity, good extraction yield, safety for human consumption, and its application in the food industry, ethanol, ethanol/water mixture (1:1, *v*/*v*), and water were used as green solvents. Thus, in addition to the cavitation promoted by the UAE procedure that facilitates bioactive compound extraction from plant tissues, the composition of phenolic compounds identified in the extracts of *A. chica* leaves was the result of their solubility in each solvent (Table 1).

**Table 1.** Profile of phenolic compounds from extracts of *A. chica* determined by HPLC-DAD.

| Peak | Phenolic Compound * | $t_R$ (min) [a] | $\lambda_{max}$ (nm) [b] | Concentration (mg/g Extract) [c] | | |
|---|---|---|---|---|---|---|
| | | | | EtOH/$H_2O$ | $H_2O$ | EtOH |
| 1 | Feruloyl hexose (isomer 1) [d] | 15–16.3 | 313 | 0.85 ± 0.03 | 1.71 ± 0.06 | nd |
| 2 | Feruloyl hexose (isomer 2) [d] | 16.6–17.5 | 309 | 0.49 ± 0.05 | 1.28 ± 0.03 | nd |
| 3 | Feruloyl derivative [d] | 24.7 | 273, 327 | nd | nd | 0.45 ± 0.01 |
| 4 | Scutellarin [e] | 27.7 | 282, 334 | nd | nd | 0.67 ± 0.09 |
| 5 | Flavone glucuronyl derivative [d] | 31.6–32.6 | 275, 328 | 2.18 ± 0.36 | 0.82 ± 0.05 | 0.88 ± 0.07 |
| 6 | Scutellarein [d] | 34.6–35.5 | 282, 337 | 6.79 ± 0.59 | nd | 1.51 ± 0.13 |
| 7 | Apigenin [f] | 42.3–43.3 | 267,293, 337 | 1.49 ± 0.09 | nd | 0.09 ± 0.02 |
| | **Total sum (mg/g)** | | | **11.80 ± 1.13** | **3.81 ± 0.15** | **3.62 ± 0.25** |

* Tentative identification based on the retention time on $C_{18}$ column, UV–visible spectra and comparison with the identification previously carried out by our research group for *A. chica* leaves (De Siqueira et al. 2019 [20]). [a] Retention time ($t_R$) on $C_{18}$ column. [b] Solvent: gradient of water with 0.5% formic acid and acetonitrile with 0.5% formic acid. [c] Mean ± standard deviation ($n = 3$, dry basis). The peaks were quantified as equivalent of [d] scutellarein, [e] scutellarin, and [f] apigenin. Abbreviations: nd, not detected; EtOH/$H_2O$, ethanol/water (1:1, *v*/*v*); $H_2O$, water; EtOH, ethanol.

HPLC analysis of the freeze-dried extracts of *A. chica* leaves allowed the separation and quantification of seven phenolic compounds (Figure 1). However, variations were observed among the extracts regarding the individual compounds. The highest levels of phenolic compounds were found in the ethanol/water (1:1, *v*/*v*) extract (Table 1), about three times higher than the extracts obtained with ethanol and water separately.

The mixture of solvents of different polarities, such as ethanol (which has medium polarity) and water (which is a strong polar solvent), promotes high efficiency in the extraction of a range of phenolic compounds of different degrees of polarity [35–37].

In addition, mixing water with organic solvents was reported to increase the efficiency of phenolic compound extraction from dry samples, since it allows the hydration of dry particles and the swelling of plant tissues, thus favoring the penetration of the organic solvent into the plant matrix [38] and consequently increasing mass transfer by molecular diffusion [39]. Furthermore, ethanol breaks the bonds between the sample matrix and phenolic compounds, which increases the recovery of these compounds [39].

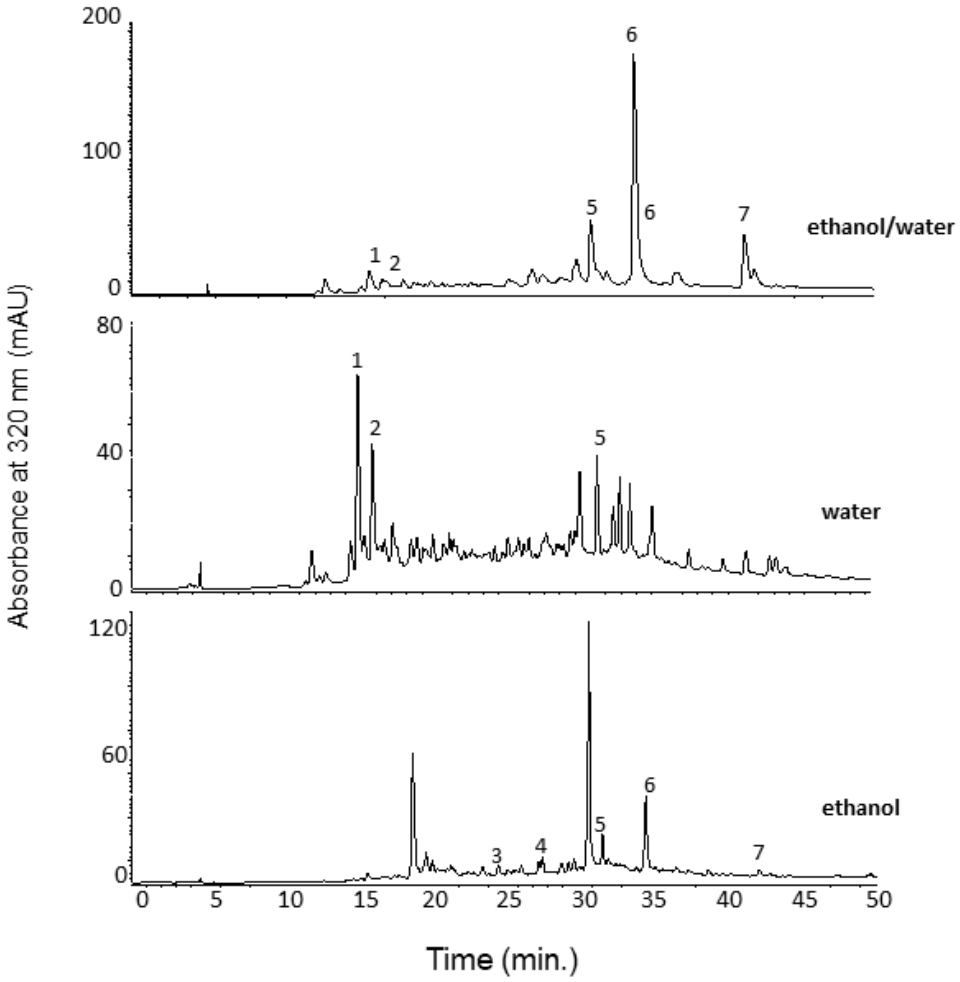


**Figure 1.** HPLC-DAD chromatogram at 320 nm of phenolic compounds of the extracts of *Arrabidaea chica* leaves. Peak characterization is given in Table 1.

Unlike the extract of *A. chica* leaves using methanol/water (8:2, *v*/*v*), as previously published by our research group [20], which showed scutellarin as the major compound, in the ethanol/water extract (1:1, *v*/*v*) (Figure 1), only its aglycone form (scutellarein) was identified, comprising about 57% of the total area of the phenolic compounds (Table 1). Both the compounds were found in the extract obtained with ethanol, with scutellarein found at the highest concentration (41%).

On the other hand, in the extract obtained with water, the flavonoid concentrations were lower (glucuronyl flavone derivative) or not detected (scutellarin, scutellarein, and apigenin). However, water extract presented the highest levels of phenolic acids (isomers of ferulic acid glycosides), confirming the selectivity of water for compounds of high polarity in the composition of *A. chica* leaves.

The ethanol–water extract of *A. chica* leaves showed that the total phenolic compound contents were ≈10 times lower than that found in the hydromethanolic extract of the same plant (21.5 mg/g) [20]. By comparing extracts obtained from other vegetables, it showed lower values than the hydroalcoholic extract of artichoke leaves (*Cynara cardunculus*) (73 mg/g), similar to *Eryngium foetidum* (9.99 mg/g) [40] and higher than extracts obtained from the peel (5.4 mg/g) and pulp (1.8 mg/g) of *Antrocaryon amazonicum* fruits [41].

### 3.2. ROS- and RNS-Scavenging Capacity of the Arrabidaea chica Extracts

*A. chica* extracts and scutellarein (authentic standard) could scavenge all the ROS and RNS tested, with $IC_{50}$ values at low μg/mL ranges (Table 2) and in a concentration-dependent manner (Figure 2).

**Table 2.** Scavenging capacity of extracts of *A. chica* leaves and standard compounds against reactive oxygen species (ROS) and reactive nitrogen species (RNS).

| Extract/ Compound | ROS | | | | | RNS | |
|---|---|---|---|---|---|---|---|
| | $IC_{50}$ (μg·mL$^{-1}$) * | | | | $S_{sample}/S_{trolox}$ | $IC_{50}$ (μg·mL$^{-1}$) | |
| | $O_2^{\bullet-}$ | $H_2O_2$ | HOCl | $^1O_2$ | ROO• | $ONOO^-$ | |
| | | | | | | Absence of $NaHCO_3$ | Presence of $NaHCO_3$ |
| $H_2O$ | 204 ± 13 $^a$ | 198 ± 4 $^a$ | 127 ± 9 $^b$ | 271 ± 1 $^a$ | 5.00 ± <0.01 $^a$ | 16.5 ± 0.2 $^b$ | 21 ± 1 $^b$ |
| EtOH/$H_2O$ | 10 ± 1 $^c$ | 4.3 ± 0.5 $^d$ | 2.9 ± 0.3 $^c$ | 35 ± 6 $^c$ | 5.00 ± <0.01 $^a$ | 0.34 ± 0.07 $^d$ | 11.1 ± 0.7 $^c$ |
| EtOH | 196 ± 2 $^a$ | 210 ± 9 $^a$ | 166 ± 14 $^a$ | 143 ± 8 $^b$ | 5.00 ± <0.01 $^a$ | 40.3 ± 0.6$^a$ | 28.3 ± 0.9 $^a$ |
| **Positive control** | | | | | | | |
| Scutellarein | 107 ± 9 $^b$ | 151 ± 5 $^b$ | 4.40 ± 0.10 $^c$ | 7.8 ± 0.2 $^d$ | 0.15 ± <0.01$^b$ | 7 ± 0.4$^c$ | 4.7 ± 0.4 $^d$ |
| Quercetin | 12.9 ± 0.5 $^c$ | - | 13 ± 1 $^c$ | 1.9 ± 0.1 $^d$ | - | 0.01± < 0.01$^d$ | 0.01 ± <0.01 $^e$ |
| Ascorbic acid | - | 41 ± 7 $^c$ | - | - | - | - | - |
| Trolox | | | | | 1.00 | | |

* $IC_{50}$, inhibitory concentration in vitro to decrease the oxidizing effect of each reactive species by 50% (mean ± standard deviation) ($n$ = 3, dry basis). Sample = slope for the curve of *A. chica*. $S_{Trolox}$ = slope for the trolox curve. $H_2O$ = water; EtOH/$H_2O$ = ethanol–water; EtOH = ethanol. Means at the same column with the same lowercase superscript letters are statistically equal at 95% significance (Tukey's test).

In general, the extract obtained with ethanol/water (1:1, *v*/*v*), which presented the highest contents of phenolic compounds, exhibited higher antioxidant efficiency against all the ROS and RNS tested, with lower $IC_{50}$ values (Table 2). Interestingly, all the extracts showed the same scavenging capacity against ROO•, with five times higher values than trolox (positive control).

In relation to $O_2^{\bullet-}$, the extract of *A. chica* obtained with ethanol/water (1:1, *v*/*v*) was significantly more effective than those obtained with ethanol and water, and it showed higher scavenging capacity than quercetin (positive control) (Table 2), as well as other medicinal plants, such as *Castanea sativa* (13.60 μg/mL) and *Quercus robur* (11.00 μg/mL) [42], and was about five times more efficient than the leaves of *Vismia cauliflora* (medicinal plant from the Amazon biome) (54.00 μg/mL) [12]. The high efficiency of the ethanol/water extract (1:1, *v*/*v*) may be associated, in addition to the presence of scutellarein (major compound), to the synergy with other phenolic compounds, given the fact that this extract was considered a more effective scavenger of $O_2^{\bullet-}$ when compared with scutellarein (about 10 times).

$O_2^{\bullet-}$ can be formed enzymatically and chemically from triplet oxygen (molecular oxygen, $^3O_2$). This ROS is very important in the reduction of oxygen to generate other reactive species, such as $H_2O_2$, hydroxyl radicals (HO•), and $^1O_2$. In food systems, it can be generated, in addition to the action of enzymes, such as xanthine oxidase, by ohmic food processing, gamma irradiation, microwaves, and pulsed electric field, and via the reaction of $^3O_2$ with the decomposition products of some azo compounds, such as azo dyes [7,43].

In most organisms, $O_2^{\bullet-}$ is converted into $H_2O_2$ with the enzyme superoxide dismutase (SOD). Although $H_2O_2$ is not a free radical, it has a reactive potential, and in the presence of metal ions, it produces HO•, which is an oxidizing species with very high reactivity [44,45]. Likewise, in foods, $H_2O_2$ can indirectly impart the loss of quality, since HO• can act as an initiator of lipid peroxidation [7]. The ethanol/water extract (1:1, *v*/*v*) of *A. chica* was the most efficient in scavenging $H_2O_2$ among the tested extracts, with higher efficiency than scutellarein and ascorbic acid, both used as positive controls (Table 2). On the other hand, all the tested extracts also showed high efficiency when compared to other plant extracts, with $IC_{50}$ values about 1 to 66 times lower than those of *Vismia cauliflora* leaves (289.00 μg/mL) [12] and 2 to 88 times of extracts of *Juglans regia* (383 μg/mL) [42].

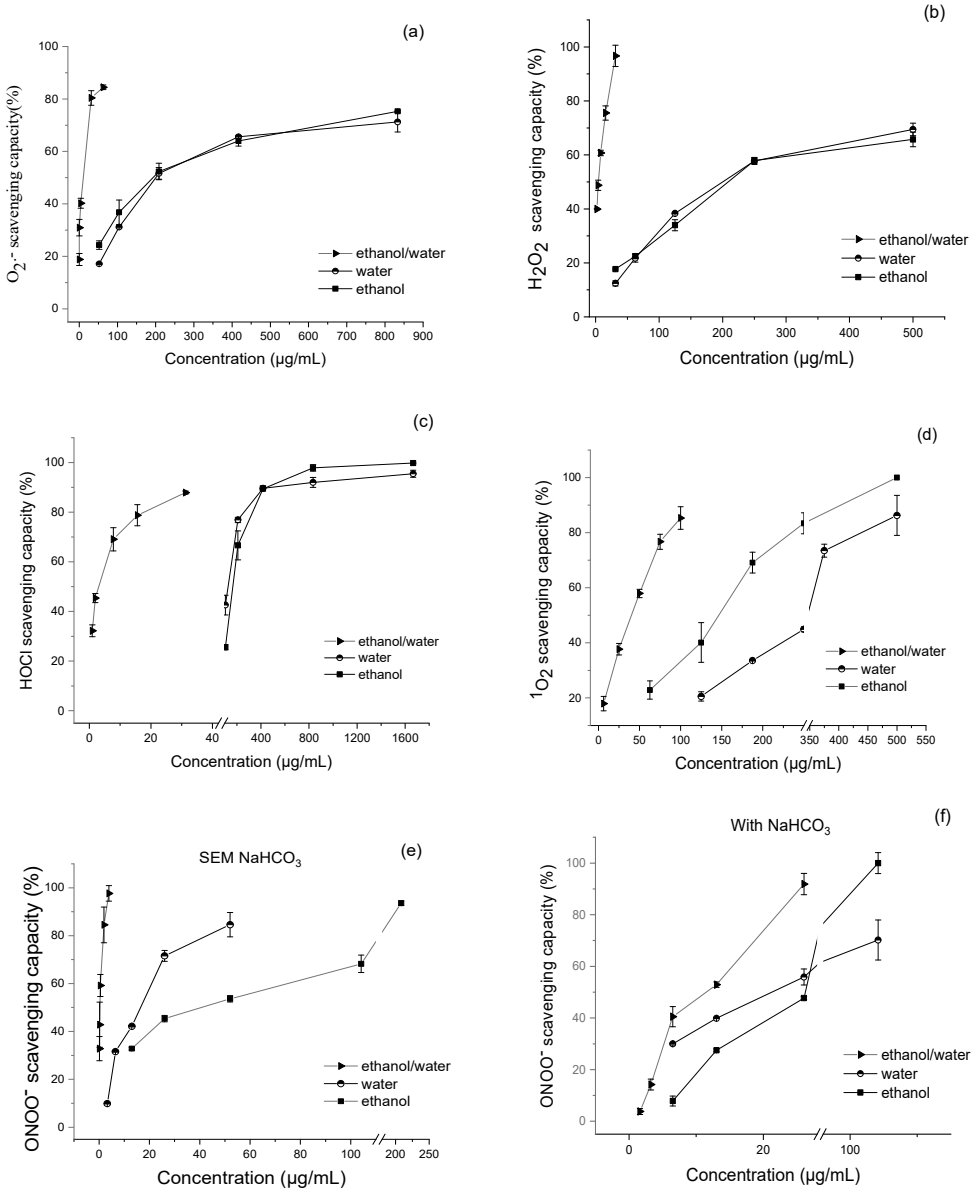


**Figure 2.** Concentration-dependent behavior of extracts of *Arrabidaea chica* leaves against (**a**) superoxide anion radicals ($O_2^{\bullet-}$), (**b**) hydrogen peroxide ($H_2O_2$), (**c**) hypochlorous acid (HOCl), (**d**) singlet oxygen ($^1O_2$), and (**e**) peroxynitrite anion ($ONOO^-$) in the absence and (**f**) presence of $NaHCO_3$. Each point represents the values obtained by four experiments, at five concentrations, carried out in triplicate (mean ± standard deviation).

Another important ROS, HOCl, can be generated in the presence of $H_2O_2$, produced by activated neutrophils and monocytes, where the enzyme myeloperoxidase (MPO) catalyzes the oxidation of chloride ion ($Cl^-$). HOCl is highly harmful and causes oxidation and chlorination reactions in biological systems [46]. It is considered a potent pro-inflammatory agent and consequently associated with a number of diseases resulting from chronic and degenerative inflammation and various types of cancer [47,48]. In this study, the ethanol/water extract (1:1, *v*/*v*) was also the most potent HOCl scavenger, followed by the water and ethanol extracts, which is even more efficient than the quercetin and scutellarein (Table 2). Likewise, it showed higher scavenging efficiency than other hydrophilic extracts of Amazonian fruits, such as *Byrsonima crassifolia* (10 µg/mL) [49] and *Solanum sessiliflorum* (13 µg/mL) [50], and the $IC_{50}$ value was close to the values reported for extracts of artichoke (*Cynara cardunculus*) leaves (3.7 to 4.7 µg/mL) [51].

Regarding the ability to quench $^1O_2$, a highly reactive ROS that is frequently generated in both physiological and food systems, the ethanol/water extract (1:1, *v*/*v*) of *A. chica*, also showed the highest antioxidant capacity, yet was less effective than quercetin (1.88 μg/mL) and scutellarein (7.84 μg/mL). The ethanol–water extract presented an $IC_{50}$ value close to the values reported for extracts of *Vismia cauliflora* leaves (27.00 μg/mL) [12] and *Cynara cardunculus* (29.00 μg/mL) [51], and was about seven times more efficient than *Solanum diploconos* (269 μg/mL), a native Brazilian fruit [4]. Natural extracts that exhibit high $^1O_2$-quenching ability are highly desirable, since this ROS, induced by light and in the presence of photosensitizers such as chlorophyll, phaeophytins, riboflavin, myoglobin and heavy metals, may react with unsaturated fatty acids via non-radical pathways, as in photo-oxidation processes, to speed up lipid peroxidation [7,9]. As an example, the incidence of light on foods, such as beer, milk, and cheese, in the presence of riboflavin and other photosensitizers, can promote the formation of off-flavors due to the degradation of lipid and protein molecules [52]. Likewise, in biological organisms, photo-oxidation also contributes to oxidative damage, such as in skin diseases such as photoaging and photocarcinogenesis [9]. Thus, the incorporation of extracts with high scavenging capacity against ROS/RNS in food systems or even in cosmetic formulations is frequently seen as an effective strategy to delay direct oxidative reactions.

As another very important ROS highly associated with oxidative damages in both food and biological systems, $ROO^{\bullet}$ are originated in the propagation step during the chain reactions of lipid peroxidation. The presence of double bonds in unsaturated and polyunsaturated fatty acids of edible oils and other animal derived products makes them highly susceptible to oxidative damage, leading to the degradation of essential fatty acids and fat-soluble vitamins, promoting sensory changes and formation of chemical compounds with harmful effects on health, which compromises the nutritional quality and safety of foods [9,53]. Lipid autoxidation is observed in the presence of molecular oxygen and reactive species, and it involves a chain reaction that includes initiation, propagation, and termination steps. After initiation, where lipid radicals are formed from lipid molecules, propagation reactions take place, where the lipid-free radicals quickly react with molecular oxygen-generating $ROO^{\bullet}$, which produce hydroperoxides (ROOH) after abstracting hydrogen from another intact molecule of unsaturated fatty acid [54]. Furthermore, in the presence of transition metals, such as Fe and Cu, or in light or high temperatures, ROOH can be decomposed into alkoxyl radicals ($RO^{\bullet}$), and then form by-products of lipid oxidation, such as ketones, aldehydes, acids, esters, and other derived compounds (such as malonaldehyde) [7].

A common practice in the oilseed industries is the addition of synthetic antioxidant compounds, such as butylhydroxytoluene (BHT), butylhydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ), and propyl galatte (PG), to delay or inhibit the oxidative processes of edible oils [55,56]. However, there is an increasing demand in the food industry to incorporate antioxidants of natural origin in the processing of food products, as replacements to the artificial ones, to accomplish the increased world tendency for the development of healthier products as part of healthy habits. In our study, all the *A. chica* extracts presented high potential to be used as a natural alternative to increase the stability of food systems. All the tested extracts exhibited the same scavenging capacity against $ROO^{\bullet}$ (Table 2), and they were 5 times more efficient than trolox (positive control) and about 33 times more efficient than scutellarein, which can raise the hypothesis of synergy between the other phenolic compounds in the extracts. When compared to other plant species, the *A. chica* extracts were considered effective scavengers of $ROO^{\bullet}$, about 26 times more effective than the extracts of pulp + skin (0.19, $S_{sample}/S_{trolox}$) and seeds (0.16, $S_{sample}/S_{trolox}$) of *Citharexylum solanaceum* [57], and were 16 times more efficient than the hydroalcoholic extract of artichoke leaves (*Cynara cardunculus*) (0.31, $S_{sample}/S_{trolox}$) [51].

Regarding RNS, $ONOO^-$ is a strong oxidizing and nitrosative agent, which can affect the quality of foods, with $O_2^{\bullet -}$ and nitric oxide ($^{\bullet}NO$) being precursors. $ONOO^-$ can initiate lipid oxidation in foods, with the formation of free radicals that can lead to the loss

of essential fatty acids, vitamins, and proteins [58,59]. In biological systems, $ONOO^-$ can also cause nitrosative damage to biomolecules, including DNA, proteins and lipids, and enzymatic inactivation, among other reactions [45,52]. In the scavenging capacity assays of $ONOO^-$, it is also important to determine the ability to scavenge $ONOO^-$ in the presence of $NaHCO_3$ as a function of the predominance of the reaction between $ONOO^-$ and $CO_2$ under physiological conditions [60].

Regarding the scavenging capacity of *A. chica* extracts against $ONOO^-$, the ethanol–water extract also showed higher efficiency than the other extracts, in the presence and absence of $NaHCO_3$, followed by the water extract (Table 2). The ethanol–water extract was less efficient than quercetin (positive control), but was more efficient than other plant extracts, such as the extracts from the whole fruit of *Solanum diploconos* [4], presenting $IC_{50}$ about eighty times lower values in the absence of $NaHCO_3$ (27.8 μg/mL) and twice in the presence of $NaHCO_3$ (27.3 μg/mL). Additionally, the scavenging capacity of the ethanol–water extract was about seventeen times higher than that of the extracts of *Vismia cauliflora* leaves (5.8 μg/mL) in the absence of $NaHCO_3$ [12].

In this study, the *A. chica* extracts showed high levels of phenolic compounds, and one of the advantages of using plant extracts as natural antioxidants might be related to the synergistic effects between the constituents of the extracts. The fact that the ethanol/water extract (1:1, *v*/*v*) of *A. chica* was as effective as the positive controls against ROS and RNS highlights the interest in its use as alternative components in food formulations. In addition, another advantage that lies with the use of natural antioxidants is the replacement of synthetic ones, which express safety concerns due to their suspected carcinogenic effects [61].

## 4. Conclusions

This is the first report concerning the ability of *A. chica* leaf extracts to scavenge physiologically relevant ROS and RNS, namely HOCl, $H_2O_2$, $O_2^{\bullet-}$, and $ONOO^-$. All the *A. chica* extracts and scutellarein were able to scavenge all the reactive species tested in a concentration-dependent manner. Among the extracts, the one produced with ethanol–water as green solvent presented the highest content of phenolic compounds, where scutellarein was the major compound, and exhibited the highest scavenging capacity against all the ROS and RNS tested.

The antioxidant potential herein investigated may be useful to delay or minimize the oxidative damages induced by the overproduction of ROS/RNS in both physiological and food systems.

**Author Contributions:** F.C.d.S.: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing—original draft, and writing—review and editing. A.P.P.B.-C., D.d.S.T.C.L., G.C.A.C.-J. and K.F.F.: formal analysis, investigation, and writing—original draft. A.S.L.: funding acquisition, resources, writing—original draft, and writing—review & editing. R.C.C.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, visualization, writing—original draft, and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

## References


1. Öztaskın, N.; Taslimi, P.; Maraş, A.; Gülcin, İ.; Göksu, S. Novel Antioxidant Bromophenols with Acetylcholinesterase, Butyrylcholinesterase and Carbonic Anhydrase Inhibitory Actions. *Bioorg. Chem.* **2017**, *74*, 104–114. [CrossRef] [PubMed]
2. Huyut, Z.; Beydemir, Ş.; Gülçin, I. Antioxidant and antiradical properties of selected flavonoids and phenolic compounds. *Biochem Res Int.* **2017**, *2017*, 1–10. [CrossRef] [PubMed]
3. Anraku, M.; Gebicki, J.M.; Iohara, D.; Tomida, H.; Uekama, K.; Maruyama, T.; Hirayama, F.; Otagiri, M. Antioxidant activities of chitosans and its derivatives in in vitro and in vivo studies. *Carbohydr. Polym.* **2018**, *199*, 141–149. [CrossRef] [PubMed]
4. Ribeiro, A.B.; Chisté, R.C.; Lima, J.L.F.C.; Fernandes, E. *Solanum Diploconos* Fruits: Profile of bioactive compounds and in vitro antioxidant capacity of different parts of the fruit. *Food Funct.* **2016**, *7*, 2249–2257. [CrossRef] [PubMed]
5. Ribeiro, F.M.; Volpato, H.; Lazarin-Bidóia, D.; Desoti, V.C.; de Souza, R.O.; Fonseca, M.J.V.; Ueda-Nakamura, T.; Nakamura, C.V.; de Oliveira Silva, S. The extended production of UV-Induced Reactive Oxygen Species in L929 Fibroblasts Is Attenuated by Posttreatment with *Arrabidaea Chica* through Scavenging Mechanisms. *J. Photochem. Photobiol. B Biol* **2018**, *178*, 175–181. [CrossRef]
6. Shahidi, F.; Zhong, Y. Measurement of Antioxidant Activity. *J. Funct. Foods* **2015**, *18*, 757–781. [CrossRef]
7. Choe, E.; Min, D.B. Chemistry and Reactions of Reactive Oxygen Species in Foods. *Crit. Rev. Food Sci. Nutr.* **2006**, *46*, 1–22. [CrossRef] [PubMed]
8. Pateiro, M.; Gómez-Salazar, J.A.; Jaime-Patlán, M.; Sosa-Morales, M.E.; Lorenzo, J.M. Plant Extracts Obtained with Green Solvents as Natural Antioxidants in Fresh Meat Products. *Antioxidants* **2021**, *10*, 181. [CrossRef] [PubMed]
9. Shahidi, F.; Zhong, Y. Lipid Oxidation and Improving the Oxidative Stability. *Chem. Soc. Rev.* **2010**, *39*, 4067–4079. [CrossRef]
10. Chong, Y.M.; Chang, S.K.; Sia, W.C.M.; Yim, H.S. Antioxidant Efficacy of Mangosteen (*Garcinia Mangostana Linn.*) Peel Extracts in Sunflower Oil during Accelerated Storage. *Food Biosci.* **2015**, *12*, 18–25. [CrossRef]
11. Mezza, G.N.; Borgarello, A.V.; Grosso, N.R.; Fernandez, H.; Pramparo, M.C.; Gayol, M.F. Antioxidant Activity of Rosemary Essential Oil Fractions Obtained by Molecular Distillation and Their Effect on Oxidative Stability of Sunflower Oil. *Food Chem.* **2018**, *242*, 9–15. [CrossRef] [PubMed]
12. Ribeiro, A.B.; Berto, A.; Chisté, R.C.; Freitas, M.; Visentainer, J.V.; Fernandes, E. Bioactive compounds and scavenging capacity of extracts from different parts of *Vismia cauliflora* against reactive oxygen and nitrogen species. *Pharm. Biol.* **2015**, *53*, 1267–1276. [CrossRef] [PubMed]
13. Silva Faria, W.C.; de Oliveira, M.G.; Cardoso da Conceição, E.; Silva, V.B.; Veggi, N.; Converti, A.; Miguel de Barros, W.; Fernandes da Silva, M.; Bragagnolo, N. Antioxidant Efficacy and in Silico Toxicity Prediction of Free and Spray-Dried Extracts of Green Arabica and Robusta Coffee Fruits and Their Application in Edible Oil. *Food Hydrocoll.* **2020**, *108*, 106004. [CrossRef]
14. Wang, Y.Z.; Fu, S.G.; Wang, S.Y.; Yang, D.J.; Wu, Y.H.S.; Chen, Y.C. Effects of a Natural Antioxidant, Polyphenol-Rich Rosemary (Rosmarinus Officinalis L.) Extract, on Lipid Stability of Plant-Derived Omega-3 Fatty-Acid Rich Oil. *LWT Food Sci. Technol.* **2018**, *89*, 210–216. [CrossRef]
15. Oliveira, A.A.; Segovia, J.F.; Sousa, V.Y.; Mata, E.C.; Gonçalves, M.C.; Bezerra, R.M.; Junior, P.O.; Kanzaki, L.I. Antimicrobial activity of amazonian medicinal plants. *Springerplus* **2013**, *2*, 371–376. [CrossRef] [PubMed]
16. Mafioleti, L.; da Silva Junior, I.F.; Colodel, E.M.; Flach, A.; de Oliveira, D.T.M. Evaluation of the Toxicity and Antimicrobial Activity of Hydroethanolic Extract of *Arrabidaea Chica* (Humb. & Bonpl.) B. Verl. *J. Ethnopharmacol.* **2013**, *150*, 576–582. [CrossRef]
17. Rodrigues, I.A.; Azevedo, M.M.B.; Chaves, F.C.M.; Alviano, C.S.; Alviano, D.S.; Vermelho, A.B. *Arrabidaea Chica* Hexanic Extract Induces Mitochondrion Damage and Peptidase Inhibition on Leishmania Spp. *BioMed Res.* **2014**, *2014*, 1–7. [CrossRef]
18. Zorn, B.; García-Pieres, A.J.; Castro, V.; Murillo, R.; Mora, G.; Merfort, I. 3-Desoxyanthocyanidins from Arrabidaea Chica. *Phytochemistry* **2001**, *56*, 831–835. [CrossRef]
19. Barbosa, W.L.R.; Pinto, L.D.N.; Quignard, E.; Vieira, J.M.D.S.; Silva, J.O.C.; Albuquerque, S. *Arrabidaea Chica* (HBK) Verlot: Phytochemical Approach, Antifungal and Trypanocidal Activities. *Braz. J. Pharmacog.* **2008**, *18*, 544–548. [CrossRef]
20. De Siqueira, F.C.; Leitão, D.S.T.C.; Mercadante, A.Z.; Chisté, R.C.; Lopes, A.S. Profile of Phenolic Compounds and Carotenoids of *Arrabidaea Chica* Leaves and the in Vitro Singlet Oxygen Quenching Capacity of Their Hydrophilic Extract. *Food Res. Int.* **2019**, *126*, 108597. [CrossRef]
21. Silva, E.M.; Souza, J.N.S.; Rogez, H.; Rees, J.F.; Larondelle, Y. Antioxidant Activities and Polyphenolic Contents of Fifteen Selected Plant Species from the Amazonian Region. *Food Chem.* **2007**, *101*, 1012–1018. [CrossRef]
22. Siraichi, J.T.G.; Felipe, D.F.; Brambilla, L.Z.S.; Gatto, M.J.; Terra, V.A.; Cecchini, A.L.; Cortez, L.E.R.; Rodrigues-Filho, E.; Cortez, D.A.G. Antioxidant Capacity of the Leaf Extract Obtained from Arrabidaea Chica Cultivated in Southern Brazil. *PLoS ONE* **2013**, *8*, 1–10. [CrossRef]
23. Chisté, R.C.; de Toledo Benassi, M.; Mercadante, A.Z. Efficiency of Different Solvents on the Extraction of Bioactive Compounds from the Amazonian Fruit *Caryocar Villosum* and the Effect on Its Antioxidant and Colour Properties. *Phytochem. Anal.* **2014**, *25*, 364–372. [CrossRef]
24. Lou, S.N.; Hsu, Y.S.; Ho, C.T. Flavonoid Compositions and Antioxidant Activity of Calamondin Extracts Prepared Using Different Solvents. *J. Food Drug Anal.* **2014**, *22*, 290–295. [CrossRef] [PubMed]
25. Cvjetko Bubalo, M.; Vidović, S.; Radojčić Redovniković, I.; Jokić, S. New Perspective in Extraction of Plant Biologically Active Compounds by Green Solvents. *Food Bioprod. Process.* **2018**, *109*, 52–73. [CrossRef]

26. Filippi, D.; Bilibio, D.; Bender, J.P.; Carniel, N.; Priamo, W.L. Kinetic Extraction of Total Polyphenols from Pitanga (*Eugenia Uniflora* L.): Effect of Ultrasonic Treatment, Modeling and Antioxidant Potential. *J. Food Process. Eng.* **2015**, *38*, 320–328. [CrossRef]
27. Chisté, R.C.; Benassi, M.T.; Mercadante, A.Z. Effect of Solvent Type on the Extractability of Bioactive Compounds, Antioxidant Capacity and Colour Properties of Natural Annatto Extracts. *Int. J. Food Sci.* **2011**, *46*, 1863–1870. [CrossRef]
28. Commission Directive 95/45/EC. Laying down specific purity criteria concerning colours for use in foodstuffs. *Off. J. Eur. Community* **1995**, *226*, 1–45.
29. Chisté, R.C.; Mercadante, A.Z. Identification and Quantification, by HPLC-DAD-MS/MS, of Carotenoids and Phenolic Compounds from the Amazonian Fruit Caryocar Villosum. *J. Agric. Food Chem.* **2012**, *60*, 5884–5892. [CrossRef]
30. ICH. *International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use. Guidance for Industry, Q2B Validation of Analytical Procedures: Methodology*; International Conference on Harmonization Secretariat, c/o International Federation of Pharmaceutical Manufacturers and Associations: Geneva, Switzerland, 2005; pp. 7–10.
31. Chisté, R.C.; Mercadante, A.Z.; Gomes, A.; Fernandes, E.; Lima, J.L.F.D.C.; Bragagnolo, N. In Vitro Scavenging Capacity of Annatto Seed Extracts against Reactive Oxygen and Nitrogen Species. *Food Chem.* **2011**, *127*, 419–426. [CrossRef]
32. Gomes, A.; Fernandes, E.; Silva, A.M.S.; Santos, C.M.M.; Pinto, D.C.G.A.; Cavaleiro, J.A.S.; Lima, J.L.F.C. 2-Styrylchromones: Novel Strong Scavengers of Reactive Oxygen and Nitrogen Species. *Bioorg. Med. Chem.* **2007**, *15*, 6027–6036. [CrossRef] [PubMed]
33. Ou, B.; Hampsch-Woodill, M.; Prior, R.L. Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the Fluorescent Probe. *J. Agric. Food Chem.* **2001**, *49*, 4619–4626. [CrossRef] [PubMed]
34. Rodrigues, E.; Mariutti, L.R.B.; Faria, A.F.; Mercadante, A.Z. Microcapsules Containing Antioxidant Molecules as Scavengers of Reactive Oxygen and Nitrogen Species. *Food Chem.* **2012**, *134*, 704–711. [CrossRef]
35. Alcântara, M.A.; de Lima Brito Polari, I.; de Albuquerque Meireles, B.R.L.; de Lima, A.E.A.; da Silva Junior, J.C.; de Andrade Vieira, É.; dos Santos, N.A.; de Magalhães Cordeiro, A.M.T. Effect of the Solvent Composition on the Profile of Phenolic Compounds Extracted from Chia Seeds. *Food Chem.* **2019**, *275*, 489–496. [CrossRef] [PubMed]
36. Ilaiyaraja, N.; Likhith, K.R.; Sharath Babu, G.R.; Khanum, F. Optimisation of Extraction of Bioactive Compounds from Feronia Limonia (Wood Apple) Fruit Using Response Surface Methodology (RSM). *Food Chem.* **2015**, *173*, 348–354. [CrossRef] [PubMed]
37. Pereira, G.A.; Molina, G.; Arruda, H.S.; Pastore, G.M. Optimizing the homogenizer-assisted extraction (HAE) of total phenolic compounds from banana peel. *J. Food Process Eng.* **2017**, *40*, 12438. [CrossRef]
38. Shi, D.; Ding, H.; Xu, S. Optimization of Microwave-Assisted Extraction of Wedelolactone from Eclipta Alba Using Response Surface Methodology. *Front. Chem. Sci. Eng.* **2014**, *8*, 34–42. [CrossRef]
39. Ghitescu, R.E.; Volf, I.; Carausu, C.; Bühlmann, A.M.; Gilca, I.A.; Popa, V.I. Optimization of Ultrasound-Assisted Extraction of Polyphenols from Spruce Wood Bark. *Ultrason. Sonochem.* **2015**, *22*, 535–541. [CrossRef]
40. do Leitão, D.S.T.C.; Siqueira, F.C.; de Sousa, S.H.B.; Mercadante, A.Z.; Chisté, R.C.; Lopes, A.S. Amazonian *Eryngium Foetidum* Leaves Exhibited Very High Contents of Bioactive Compounds and High Singlet Oxygen Quenching Capacity. *Int. J. Food Prop.* **2020**, *23*, 1452–1464. [CrossRef]
41. Pereira Barbosa-Carvalho, A.P.; do Texeira Costa Leitão, D.S.; Campos de Siqueira, F.; Zerlotti Mercadante, A.; Campos Chisté, R. Antrocaryon Amazonicum: An Unexploited Amazonian Fruit with High Potential of Scavenging Reactive Oxygen and Nitrogen Species. *J. Food Sci.* **2021**, *86*, 4045–4059. [CrossRef]
42. Almeida, I.F.; Fernandes, E. Protective Effect of Castanea Sativa and Quercus Robur Leaf Extracts against Oxygen and Nitrogen Reactive Species. *J. Photochem. Photobiol. B Biol.* **2008**, *91*, 87–95. [CrossRef] [PubMed]
43. Pastor, I.; Esquembre, R.; Micol, V.; Mallavia, R.; Mateo, C.R. A Ready-to-Use Fluorimetric Biosensor for Superoxide Radical Using Superoxide Dismutase and Peroxidase Immobilized in Sol-Gel Glasses. *Anal. Biochem.* **2004**, *334*, 335–343. [CrossRef] [PubMed]
44. Berto, A.; Ribeiro, A.B.; de Souza, N.E.; Fernandes, E.; Chisté, R.C. Bioactive Compounds and Scavenging Capacity of Pulp, Peel and Seed Extracts of the Amazonian Fruit Quararibea Cordata against ROS and RNS. *Food Res. Int.* **2015**, *77*, 236–243. [CrossRef]
45. Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M.T.D.; Mazur, M.; Telser, J. Free Radicals and Antioxidants in Normal Physiological Functions and Human Disease. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 44–84. [CrossRef]
46. Freitas, M.; Lima, J.L.F.C.; Fernandes, E. Optical Probes for Detection and Quantification of Neutrophils' Oxidative Burst. A Review. *Anal. Chim. Acta* **2009**, *649*, 8–23. [CrossRef] [PubMed]
47. Malle, E.; Marsche, G.; Arnhold, J.; Davies, M.J. Modification of Low-Density Lipoprotein by Myeloperoxidase-Derived Oxidants and Reagent Hypochlorous Acid. *Biochim. Biophys. Acta Mol. Cell. Biol. Lipids* **2006**, *1761*, 392–415. [CrossRef] [PubMed]
48. Ho, E.; Karimi Galougahi, K.; Liu, C.C.; Bhindi, R.; Figtree, G.A. Biological Markers of Oxidative Stress: Applications to Cardiovascular Research and Practice. *Redox Biol.* **2013**, *1*, 483–491. [CrossRef]
49. Mariutti, L.R.B.; Rodrigues, E.; Chisté, R.C.; Fernandes, E.; Mercadante, A.Z. The Amazonian Fruit Byrsonima Crassifolia Effectively Scavenges Reactive Oxygen and Nitrogen Species and Protects Human Erythrocytes against Oxidative Damage. *Food Res. Int.* **2014**, *64*, 618–625. [CrossRef]
50. Rodrigues, E.; Mariutti, L.R.B.; Mercadante, A.Z. Carotenoids and Phenolic Compounds from Solanum Sessiliflorum, an Unexploited Amazonian Fruit, and Their Scavenging Capacities against Reactive Oxygen and Nitrogen Species. *J. Agric. Food Chem.* **2013**, *61*, 3022–3029. [CrossRef]

51. Pistón, M.; Machado, I.; Branco, C.S.; Cesio, V.; Heinzen, H.; Ribeiro, D.; Fernandes, E.; Chisté, R.C.; Freitas, M. Infusion, Decoction and Hydroalcoholic Extracts of Leaves from Artichoke (*Cynara Cardunculus* L. Subsp. Cardunculus) Are Effective Scavengers of Physiologically Relevant ROS and RNS. *Food Res. Int.* **2014**, *64*, 150–156. [CrossRef]
52. Halliwell, B.; Gutteridge, J.M.C. *Free Radicals in Biology and Medicine*, 5th ed.; OUP Oxford: Oxford, UK, 2015.
53. Zhang, Y.; Yang, L.; Zu, Y.; Chen, X.; Wang, F.; Liu, F. Oxidative Stability of Sunflower Oil Supplemented with Carnosic Acid Compared with Synthetic Antioxidants during Accelerated Storage. *Food Chem.* **2010**, *118*, 656–662. [CrossRef]
54. Apak, R.; Özyürek, M.; Güçlü, K.; Çapanoğlu, E. Antioxidant Activity/Capacity Measurement. 3. Reactive Oxygen and Nitrogen Species (ROS/RNS) Scavenging Assays, Oxidative Stress Biomarkers, and Chromatographic/Chemometric Assays. *J. Agric. Food Chem.* **2016**, *64*, 1046–1070. [CrossRef] [PubMed]
55. Castro-Vargas, H.I.; Baumann, W.; Parada-Alfonso, F. Valorization of Agroindustrial Wastes: Identification by LC-MS and NMR of Benzylglucosinolate from Papaya (*Carica Papaya* L.) Seeds, a Protective Agent against Lipid Oxidation in Edible Oils. *Electrophoresis* **2016**, *37*, 1930–1937. [CrossRef] [PubMed]
56. Souza, A.L.; Martínez, F.P.; Ferreira, S.B.; Kaiser, C.R. A Complete Evaluation of Thermal and Oxidative Stability of Chia Oil: The Richest Natural Source of α-Linolenic Acid. *J. Therm. Anal. Calorim.* **2017**, *130*, 1307–1315. [CrossRef]
57. Barizão, É.O.; Visentainer, J.V.; de Cinque Almeida, V.; Ribeiro, D.; Chisté, R.C.; Fernandes, E. Citharexylum Solanaceum Fruit Extracts: Profiles of Phenolic Compounds and Carotenoids and Their Relation with ROS and RNS Scavenging Capacities. *Food Res. Int.* **2016**, *86*, 24–33. [CrossRef]
58. Brannan, R.G.; Connolly, B.J.; Decker, E.A. Peroxynitrite: A potential initiator of lipid oxidation in food. *Trends Food Sci. Technol.* **2001**, *12*, 164–173. [CrossRef]
59. Skibsted, L.H. Nitric Oxide and Quality and Safety of Muscle Based Foods. *Nitric Oxide* **2011**, *24*, 176–183. [CrossRef]
60. Gomes, A.; Fernandes, E.; Lima, J.L.F.C. Use of fluorescence probes for detection of reactive nitrogen species: A review. *J. Fluoresc.* **2006**, *16*, 119–139. [CrossRef]
61. Ramful, D.; Aumjaud, B.; Neergheen, V.S.; Soobrattee, M.A.; Googoolye, K.; Aruoma, O.I.; Bahorun, T. Polyphenolic Content and Antioxidant Activity of Eugenia Pollicina Leaf Extract in Vitro and in Model Emulsion Systems. *Food Res. Int.* **2011**, *44*, 1190–1196. [CrossRef]