Antioxidant potential of *Pfaffia glomerata* and *Arrabidaea chica* extracts combination

Potencial antioxidante da combinação do extrato de *Pfaffia glomerata* com extrato de *Arrabidaea chica*

Potencial antioxidante de la combinación de extractos de *Pfaffia glomerata* y *Arrabidaea chica*

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Abstract

Several therapeutic benefits of medicinal plants are attributed to antioxidant properties, since antioxidant compounds play an important role in the prevention of various diseases and aging. Many products have synergistic action from combinations of plant extracts that contain natural antioxidants. The present study aimed to evaluate the synergistic effect of *Pfaffia glomerata* extract with *Arrabidaea chica* extract for antioxidant action. In the study the extract of the inflorescences of *P. glomerata* and the extract of the leaves of *A. chica* were used. The synergism between *P. glomerata* extract and *A. chica* extract was evaluated using the ratio between experimental and theoretical antioxidant activity, which was determined using the DPPH method and the potential total reactive antioxidant method (TRAP). The combination of *P. glomerata* extract and *A. chica* extract showed a synergistic effect, in which the antioxidant property of this combination was substantially superior to the sum of the individual antioxidant effects. This demonstrates that the combined extracts can be important for the development of products that have a high antioxidant potential.

**Keywords:** Brazilian ginseng; Pariri; Antioxidant activity; Synergism.

Resumo

Vários benefícios terapêuticos das plantas medicinais são atribuídos às propriedades antioxidantes, uma vez que compostos antioxidantes apresentam um papel importante na prevenção de várias doenças e do envelhecimento. Muitos produtos têm ação sinérgica de combinações de extratos vegetais que contêm antioxidantes naturais. O presente estudo teve como objetivo avaliar o efeito sinergista da combinação do extrato de *Pfaffia glomerata* com extrato de *Arrabidaea chica* quanto à ação antioxidante. No estudo foram utilizados o extrato das inflorescências da *P. glomerata* e o extrato das folhas de *A. chica*. O sinergismo entre o extrato de *P. glomerata* e extrato de *A. chica* foi avaliado usando a proporção entre atividade antioxidante experimental e teórica, a qual foi determinada usando o método DPPH e o método potencial antioxidante reativo total (TRAP). A combinação do extrato de *P. glomerata* e extrato de *A. chica* mostrou um efeito sinérgico, em que a propriedade antioxidante desta combinação foi substancialmente superior à soma dos efeitos antioxidantes individuais. Isto demonstra que os extratos combinados podem ser importantes para o desenvolvimento de produtos que tenham alto potencial antioxidante.

Palavras-chave: Ginseng brasileiro; Pariri; Atividade antioxidante; Sinergismo.

Resumen

Varios beneficios terapéuticos de las plantas medicinales se atribuyen a las propiedades antioxidantes, ya que los compuestos antioxidantes juegan un papel importante en la prevención de diversas enfermedades y el envejecimiento. Muchos productos tienen una acción sinérgica a partir de combinaciones de extractos de plantas que contienen antioxidantes naturales. El presente estudio tuvo como objetivo evaluar el efecto sinérgico del extracto de *Pfaffia glomerata* con el extracto de *Arrabidaea chica* con respecto a su acción antioxidante. En el estudio se utilizó el extracto de las inflorescencias de *P. glomerata* y el extracto de las hojas de *A. chica*. El sinergismo entre el extracto de *P. glomerata* y el extracto de *A. chica* se evaluó mediante la relación entre la actividad antioxidante experimental y teórica, que se determinó mediante el método DPPH y el método antioxidante reactivo total potencial (TRAP). La
La combinación de extracto de *P. glomerata* y extracto de *A. chica* mostró un efecto sinérgico, en el que la propiedad antioxidante de esta combinación fue sustancialmente superior a la suma de los efectos antioxidantes individuales. Esto demuestra que los extractos combinados pueden ser importantes para el desarrollo de productos que tienen un alto potencial antioxidante.

**Palabras clave:** Ginseng brasileiro; Pariri; Actividad antioxidante; Sinergismo.

1. **Introducción**

Reactivos de especies oxigenadas son radicales libres comunes que son conocidos por causar daño a lipidos, proteínas, carbohidratos, y nucleídos, causando daño cellular o tisular que se manifiesta en varios trastornos, incluyendo cáncer, enfermedad de Parkinson, aterosclerosis, enfermedad cardiovascular, enfermedad inflamatoria, y envejecimiento (Radi, 2018; Vellosa et al., 2021). Reacciones de oxidación pueden producir radicales libres que inician reacciones de cadena que pueden dañar células de piel, dando lugar a la aparición de arrugas, envejecimiento por exposición solar, elastosis, sequedad y pigmentación cutánea (Kusumawati y Indrayanto, 2013).

A través de la acción de antioxidantes es posible neutralizar los radicales libres responsables de la oxidación de células, lo cual conduce a envejecimiento más precoz. El antioxidante puede retardar o prevenir la oxidación, promoviendo la protección de las células del cuerpo contra la oxidación de radicales libres (Lima, Santana y Moreira, 2018). El organismo humano tiene un mecanismo endógeno antioxidante que incluye varias enzimas y antioxidantes no enzimáticos (Kaim y Backes, 2019). Además del producido naturalmente de antioxidantes endógenos, existen fuentes exógenas que se pueden adquirir a través de la dieta o mediante el uso tópico (de Freitas et al., 2020).

Las plantas tienen algunas de las antioxidantes naturales más potentes y son ampliamente utilizadas por su efecto antioxidante y anti-envejecimiento (Kalim, Bhattacharyya, Banerjee, y Chattopadhyay, 2010). Además, el consumo de antioxidantes naturales ha sido asociado con una incidencia más baja de trastornos relacionados con estrés oxidativo (de Azevedo et al., 2020). Una de las principales metas de la investigación industrial es el uso de antioxidantes naturales para alimentos, cosméticos y medicamentos (Alves y Souza, 2020). Estas tres áreas promueven productos similares, que se refieren a como alimentos funcionales, suplementos alimenticios, nutracéuticos y cosmeceuticos (Peschel et al., 2006). Muchas patentes y productos cosméticos comerciales incluyen combinaciones de extractos de plantas con antioxidantes naturales (Kusumawati y Indrayanto, 2013).

*Pfaffia glomerata* (Spreng.) Pedersen (Amaranthaceae), conocido como ginseng brasileiro, es ampliamente utilizado en Brasil para tratar varias condiciones patológicas (Franco et al., 2021). Se estudian que el extracto de *P. glomerata* es ampliamente utilizado en la industria farmacéutica, debido a sus propiedades antimicrobianas, anti-rheumáticas, antitumorales, anti-diabéticas, entre otras propiedades, en adición a acciones como anti-inflamatorias, curativas y antioxidantes (Silva et al., 2010; Oliveira y Sakai, 2020; Souza, Fonseca, Ferrari, y Felipe, 2021).

Una empresa coreana ha registrado dos patentes para cosméticos que contienen extracto de *P. glomerata* junto con otros ingredientes activos. La patente KR1116447 reportó que un cosmético que contiene extracto de las raíces de *P. glomerata*, sales de arginina de L-pirrolidone carboxylic acid (PCA arginina), extracto de Melia azedarach y extracto de levadura como componentes activos, puede aumentar la dinámica celular de la piel, prevenir la aparición de arrugas, iluminar y hidratar la piel, además de aliviar problemas de piel (Lee et al., 2012). La patente KR1155180 presentó un cosmético anti-envejecimiento que contiene extracto de las raíces de *P. glomerata* y PCA arginina, que inhibe el daño a los neurocitos de la piel, además de inhibir daño a los fibroblastos y queratinocitos causados por radiación ultravioleta (Gang et al., 2012).

El componente principal de *Pfaffia glomerata* es la 3-ecdisona, un fetoecdisteroides, encontrado en las raíces, tallos, inflorescencias y hojas del plant (Oliveira y Sakai, 2020; Serra, Felipe, y Cortez, 2012; Souza, Fonseca, Ferrari, y Felipe, 2021). Muchos efectos farmacológicos se atribuyen a la 3-ecdisona, una de las cuales es una acción antioxidante (LaFont; Dinan, 2003; Daniel et al., 2005). Investigaciones por Nsimba, Kikuzaki, y Konishi (2008), mostraron que fetoecdisteroides, incluyendo 3-ecdisona, pueden ser considerados compuestos eficaces para prevenir o retrasar daño de la piel relacionado con colagenasa y estrés oxidativo. En adición a
these actions, Dinan and Lafont (2006) reported that \( \beta \)-ecdysone has wound healing and skin regenerating activity. The US5609873 patent determines the use of ecdysteroids, mainly \( \beta \)-ecdysone, for dermatological and cosmetic preparations, because \( \beta \)-ecdysone strengthens the water barrier function of the skin, increases keratinocyte differentiation, and gives the skin a smoother and softer appearance (Meybeck, Bonte, and Redziniak, 1997).

In addition to ecdysteroids, which have been considered natural antioxidants, many investigations have revealed that phenolic compounds and flavonoids also contribute to the antioxidant activity of plants (Cai, Luo, Sun, and Corke, 2004; Zhang et al., 2011). The flavonoids are widely used in the cosmetic, medicine and food industries, due to their antioxidant properties, with application in anti-aging products (Henrique and Lopes, 2017). These compounds were identified in the inflorescences of *P. glomerata* in a study carried out by Felipe et al., (2014), which showed that the main compounds identified, in addition to \( \beta \)-ecdysone, were glycosylated flavonoids, such as quercetin-3-O-glycoside, kaempferol-3-O-glycoside and kaempferol-3-O-(6-p-cumaryl)-glycoside, which are important bioactive compounds for use in phytotherapy and in the cosmetics industry.

A study carried out by Henrique and Lopes (2017), showed the increasing use of biodiversity, for the development of new cosmetic assets that can act effectively against skin aging, highlighting the importance of flavonoids. These compounds are used clinically in cosmetic formulations for oral or topical use, since they provide protection to the skin against harmful endogenous and exogenous agents, and enable the prevention and treatment of skin disorders, in addition to preventing aging.

Studies demonstrate that there is a positive correlation between antioxidant activity and flavonoid content, indicating that these types of polyphenols could be the main responsible for the antioxidant activity observed in various plant extracts (Jiménez-Estrada et al., 2013). For example, studies have shown that the extract of *Arrabidaea chica* Verlot. (Bignoniaceae) has significant antioxidant activity, which could be attributed to the presence of the mixture of flavonoids in the plant extract, mainly scutellarin and apigenin (Siraichi et al., 2013). Other studies have reported that *A. chica* extract has healing and anti-inflammatory properties, antifungal activity and antitumor activity (Jorge et al., 2008; Barbosa et al., 2008; Ribeiro et al., 2012). The patent PI0703095 reported a pharmaceutical formulation containing *A. chica* for photoprotection and pigmentation (Dias Filho et al., 2009).

The *A. chica* plant, popularly known as “Pariri”, is a shrub that is present in tropical America, particularly in the Amazon Basin, and has been grown in Southern Brazil (Barbosa et al., 2008; Siraichi et al., 2013). Several studies have determined the content of phenolic compounds and flavonoids in the leaves of *A. chica*, which is the part used in folk medicine (Silva et al., 2007; Barbosa et al., 2008; Siraichi et al., 2013).

In phytotherapy, greater efficacy can be achieved with the application of a combination of plant extracts than with generally high doses of an individual extract. This association at the same time has the advantage of reducing or eliminating side effects due to the lower doses of the compounds present in the mixture of extracts (Wagner, 2006). Some combinations of compounds exhibit synergistic interactions, while others show additive interactions, and still others show antagonistic interactions (Kirakosyan et al., 2010).

Therapeutic synergism has been described for several plant extracts (Williamson, 2001). For example, a combination of kava (*Piper methysticum*) and valerian (*Valeriana officinalis*) appears to be better for the treatment of stress-induced insomnia than the use of these plants individually (Wheatley, 2001). Studies have reported synergistic interactions between natural antioxidants (Liu et al., 2008). Fuhrman, Volkova, Rosenblat, and Aviram, (2000) found that a combination of different antioxidants is superior to the action of an individual antioxidant in protecting low-density lipoprotein (LDL) against oxidation.

The synergistic action of combinations of antioxidant extracts in lower concentrations can be a strategy for the cosmetic and food industries (Beddows, Jagait, and Kelly, 2001; Peschel et al., 2006). The study of natural substances with
strong antioxidant potential that can act alone or synergistically with other additives has emerged as an alternative to the use of synthetics due to sustainability, ease of obtaining and economic viability (Massarolo et al., 2017; Wróblewska, Baby, Guaratini, and Moreno, 2019).

The choice of appropriate active plant extracts, confirmation of their activity, and synergistic effects are important factors when developing effective products (Kusumawati and Indrayanto, 2013). Thus, the present study aimed to evaluate the synergistic effect of the extract of *P. glomerata* in combination with the extract of *A. chica*, regarding the antioxidant action.

### 2. Methodology

#### 2.1 Plant material

In the present research, the extract of the inflorescences of *P. glomerata* and the extract of the leaves of *A. chica* were used, which were acquired from the Laboratory in Natural Products and Biotechnology, located at the State University of Maringá, Maringá, Paraná. The extract of the inflorescences of *P. glomerata* obtained was standardized in the study carried out by Souza, Fonseca, Ferrari, and Felipe, (2021), which was prepared from the plant collected in Querência do Norte, Paraná, Brazil (in the month of April, at 9:00 a.m. approximately, transported in a closed package), and presented β-ecdysone (91.35 µg / mL) as a major compound.

The *A. chica* leaf extract was standardized in the study by Siraichi et al., (2013). The standardized extract was obtained from the plant cultivated in Southern Brazil, collected in the month of February, at 9:00 a.m. approximately, and it was transported in a closed package to protect from moisture. The plant contained a mixture of flavonoids, mainly scutellarin (818.6 µg/ml) and apigenin (112.04 µg/ml), in addition to other minor flavonoids, such as isoscutellarein, 6-hydroxyluteolin, hispidulin, and luteolin.

#### 2.2 Antioxidant assays for calculation of synergism

The antioxidant activity of the *P. glomerata* inflorescence extract alone was compared with the combination with the *A. chica* leaf extract to evaluate the synergistic effect of this combination. The antioxidant activity of the *P. glomerata* extract, *A. chica* extract, and a combination of their extracts were determined using the DPPH radical scavenging assay and total reactive antioxidant potential (TRAP) method. The experiments were performed in triplicate.

##### 2.2.1 DPPH radical scavenging assay

The DPPH radical scavenging capacity was determined according to the procedure of Brand-Williams, Cuvelier, and Berset (1995). From stock solutions prepared in methanol at a concentration of 100 µg/ml, the *P. glomerata* inflorescence extract and *A. chica* leaf extract were diluted at concentrations of 75 and 7.5 µg/ml, respectively, which are the concentrations of the two extracts in combination that produce a 50% antioxidant effect (IC₅₀), calculated as described below.

An aliquot (3 ml) of sample solution was mixed thoroughly with 300 µl of DPPH solution and then left for 30 min at room temperature in the dark. The absorbance of the solutions was measured at 515 nm against a blank (3 ml sample solution and 300 µl methanol) using a spectrophotometer. Three replicates were made for each test sample. A methanolic solution that contained 3 ml methanol and 300 µl of the DPPH solution was used as a negative control. The DPPH radical scavenging activity (%) was calculated using the following equation described by Scherer and Godoy (2009):

\[
\text{Scavenging activity (\%)} = \left[ \frac{(\text{Abs}_0 - \text{Abs}_1)}{\text{Abs}_0} \right] \times 100
\]
where Abs₀ is the absorbance of the negative control, and Abs₁ is the absorbance in the presence of the extract. The scavenging ability of the extracts is expressed as the IC₅₀ value, which is the concentration of the sample that is required to scavenge 50% of DPPH free radicals. The IC₅₀ values were calculated using linear regression plots, in which the ordinate represents the scavenging activity (%), and the abscissa represents the concentration of the tested extracts.

2.2.2 TRAP method
The TRAP method was performed as described in vivo by Repetto et al., (1996) and reproduced in vitro by Ghiselli, Serafini, Natella, & Scaccini (2000) e Pellegrini et al., (2003). This technique evaluates the levels of total antioxidants. In this method, ABAP was used as a generator system for peroxyl radicals by thermal decomposition, producing photons that were amplified and measured by a TD 20/20 luminometer via a chemiluminescence reaction. The reaction was inhibited by analogs of vitamin E (Trolox), which was used as an antioxidant standard.

The antioxidant activity of the individual extracts and their combination was analyzed. The P. glomerata inflorescence extract at a concentration of 0.33 µg/ml, A. chica leaf extract at the same concentration, and these extracts combined at a dose ratio of 1:1 were mixed with 200 mM luminol and 200 mM ABAP to a final volume of 1 ml. The TRAP of these samples was calculated using the following equation:

\[
\text{TRAP (µM Trolox)} = \frac{(\text{Tind sample} \times \text{µM Trolox})}{\text{Tind Trolox}}
\]

where Tind represents the induction time for which the antioxidant produces peroxyl formation/number of photons readings in 1 min. The TD20/20 luminometer detects chemiluminescence at a wavelength of 300-650 nm, has 50.1% sensitivity, and can produce 450-12,500 readings. The luminometer was connected to a computer through Spreadsheet Interface v. 1.0 software to record the chemiluminescence emission. Origin v. 8.0 software was used to plot chemiluminescence curves.

2.3 Calculation of synergism between Pfaffia glomerata extract and Arrabidaea chica extract
The synergistic effects of combinations of the P. glomerata extract with the A. chica extract were based on the ratio between experimental antioxidant activity (EAA) and theoretical antioxidant activity (TAA). The EAA of the extracts in combination was calculated using the equations previously described for the DPPH method and TRAP method. Theoretical antioxidant activity is the sum of the antioxidant activity of each extract. For the DPPH method, TAA was calculated using the following equation described by Fuhrman, Volkova, Rosenblat, and Aviram (2000):

\[
\%\text{TAA} = (A + B) - (A \times B / 100)
\]

where A is the percent EAA of the individual P. glomerata extract, and B is the percent EAA of the individual A. chica extract. Synergistic effects were calculated using the following equation described by Fuhrman, Volkova, Rosenblat, and Aviram (2000):

\[
\text{Synergistic effect} = \frac{\text{EAA}}{\text{TAA}}
\]

where synergism is shown when the synergistic effect is > 1.
2.4 Statistical analysis

All of the experiments were performed at least in triplicate, and the results are expressed as mean ± standard deviation (SD). In the antioxidant assays, the data were compared using analysis of variance (ANOVA) followed by Tukey’s test. For the synergism analysis, Student’s t-test was performed to compare the two means (EAA and TAA). The level of significance was set at 5%, and differences were considered statistically significant at $p < 0.05$. The statistical analysis was performed using GraphPad Prism v. 5.0 software (GraphPad, San Diego, CA, USA).

3. Results and Discussion

To investigate the synergism between the *P. glomerata* inflorescence extract and *A. chica* leaf extract, the antioxidant activity of the single extracts and their combination was examined. We focused primarily on these extracts because of their relatively high antioxidant capacities, which was reported in the research by Souza, Fonseca, Ferrari, and Felipe, (2021) for *P. glomerata* and in the study by Siraichi et al., (2013) for *A. chica*.

The antioxidant activity of single extracts, determined by the DPPH method and TRAP method, is presented in Tables 1 and 2. The TRAP method, similar to the DPPH method, indicates the free radical scavenging ability of antioxidants via the hydrogen atom transfer pathway, but against peroxyl radicals (Ghiselli, Serafini, Natella, and Scaccini, 2000). The antioxidant capacity of the *A. chica* extract was higher than the *P. glomerata* extract using both methods. This may be explained by the fact that the *A. chica* extract presents a high content of flavonoids, which are the major constituents that contribute to antioxidant activity in most plants and act as an effective scavenger of free radicals (Siraichi et al., 2013; Jiménez-Estra et al., 2013).

Based on the data obtained from the single extracts, the TAA of the extracts in combination was calculated, in addition to EAA, to examine synergistic effects (Tables 1 and 2). If the EAA is greater than the TAA, then an interaction is evident among the antioxidants, thus displaying synergism. Mathematically, a ratio of EAA/TAA > 1 would indicate a synergistic effect (Liu et al., 2008). Considering the antioxidant activity determined by the DPPH method (Table 1), a significant ($p < 0.05$) synergistic effect (1.17) was produced by the combination of the *P. glomerata* extract and *A. chica* extract. The EAA and TAA values were $51.7 \pm 1.72\%$ and $44.2 \pm 1.60\%$, respectively, whereas the antioxidant activity of the *P. glomerata* extract alone was $20.9 \pm 0.66\%$, indicating that the EAA of the extracts in combination exceeded the TAA by 17%.

Table 1. Synergistic effect (SE) of the *P. glomerata* extract with *A. chica* extract, considering the antioxidant activity determined by the DPPH method.

<table>
<thead>
<tr>
<th>Extract concentration</th>
<th>% Antioxidant activity*</th>
<th>%TAA*</th>
<th>% EAA*</th>
<th>SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. glomerata</em> 75 µg/ml</td>
<td>20.9 ± 0.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. chica</em> 7.5 µg/ml</td>
<td>29.4 ± 0.82</td>
<td>44.2 ± 1.60</td>
<td>51.7 ± 1.72</td>
<td>1.17*</td>
</tr>
</tbody>
</table>

Theoretical antioxidant activity (TAA) was calculated from data for the extracts alone. The asterisk denote a significant difference compared with the respective experimental antioxidant activity (EAA) value ($^* p < 0.05$, ANOVA). *The data are expressed as the mean ± standard deviation of triplicate measurements. *SE > 1: synergistic effect shown.
Source: Authors (2021).
Based on the antioxidant activity evaluated by the TRAP method (Table 2), the combined extracts also exhibited a significant ($p < 0.05$) synergistic effect (1.25), with an EAA value of $2.44 \pm 0.28 \ \mu M$ Trolox and TAA value of $1.95 \pm 0.14 \ \mu M$ Trolox. The *P. glomerata* extract alone had antioxidant activity of $0.65 \pm 0.15 \ \mu M$ Trolox, indicating that the EAA of the combination of the extracts exceeded the TAA by 25.1%.

**Table 2.** Synergistic effect (SE) of the *P. glomerata* extract with *A. chica* extract, considering the antioxidant activity determined by the TRAP method.

<table>
<thead>
<tr>
<th>Extract concentration</th>
<th>Extract alone</th>
<th>Extracts in combination (<em>P. glomerata + A. chica</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antioxidant activity (µM Trolox)</td>
<td>TAA(µM Trolox)</td>
</tr>
<tr>
<td><em>P. glomerata</em> 0.33 µg/ml</td>
<td>$0.65 \pm 0.15$</td>
<td></td>
</tr>
<tr>
<td><em>A. chica</em> 0.33 µg/ml</td>
<td>$1.30 \pm 0.13$</td>
<td>$1.95 \pm 0.14$</td>
</tr>
</tbody>
</table>

Theoretical antioxidant activity (TAA) was calculated from data for the extracts alone. The asterisk denote a significant difference compared with the respective experimental antioxidant activity (EAA) value (*$p < 0.05$, ANOVA). aThe data are expressed as the mean ± standard deviation of triplicate measurements. bSE > 1: synergistic effect shown. Source: Authors (2021).

Fuhrman, Volkova, Rosenblat, and Aviram (2000) reported that lycopene acts synergistically with several natural antioxidants as an effective antioxidant against LDL oxidation. They showed that when the lycopene was combined with rosmarinic acid or vitamin E, the inhibition of LDL oxidation, measured as TBARS formation, also exceeded the expected additive inhibition of the individual antioxidants by 17% and 25%, respectively. A study carried out by de Souza, Correa, Silva & Conceição (2021), showed that plant extracts containing natural antioxidants combined with synthetic antioxidants showed synergism, in other words, the mixture of each tested extract with the synthetic antioxidant presented a better performance than used individually.

Several plant extracts have been shown to exhibit synergistic interactions (Williamson, 2001). Scholey and Kennedy (2002) studied the synergism between different extracts in a product that contained *Panax ginseng* extract with *Ginkgo biloba* extract, which was demonstrated to be more effective in improving cognitive function than the extracts alone. A study by Capasso and Sorrentino [69] used a standardized extract combination of *Kava-Kava* and *Passiflora*, and the superiority of the combination over the individual extracts in a sedative and hypnotic test was found. They observed an approximately 50% higher efficacy of the extract combination compared with the individual extracts. In the present study, the results showed 59.6% higher antioxidant activity of the combination of *P. glomerata* and *A. chica* compared with the *P. glomerata* extract alone using the DPPH method, and 73.4% higher antioxidant activity was found using the TRAP method.

The present study found evidence that the *P. glomerata* extract acts synergistically with the *A. chica* extract, in which the combined extracts had effective DPPH and peroxy radicals scavenging activity. Previous studies reported that only compounds with strong antioxidant capacities exhibit synergistic interactions (Kirakosyan et al., 2010). Liu et al., (2008) showed that specific concentrations and combinations of antioxidants were more effective than the corresponding individual antioxidants in scavenging DPPH. They reported that the synergistic effect appeared to be partially based on the reducing potential of the various antioxidants and their ability to convert antioxidant free radicals to their native form.
Flavonoids may play a role in increasing the biological activity of other compounds through synergistic mechanisms (Williamson, 2001). Thus, these compounds that according Siraichi et al., (2013), are present at high concentrations in A. chica leaves, could have contributed to the synergistic effects observed between the combined extracts, once that interaction may have occurred between flavonoids and β-ecdysone, which is the major compound in P. glomerata inflorescences. Additionally, synergistic interactions may be explained by enhanced absorption, metabolism and reduced excretion or by enhanced effectiveness at the target site(s) of action (Kirakosyan et al., 2010). In the case of flavonoids, they likely affect membrane lipid viscosity or protein movement (Tarahovsky, Muzafarov, & Kim, 2008).

The general understanding of synergism is that combinations of substances have greater effects than the substances alone. This can apply to an increased therapeutic effect, reduced side-effect profile at low doses, or both. Synergistic interactions are important in phytomedicine for constituents within a total extract of a single herb and between different herbs in a formulation (Williamson, 2001). The present work demonstrated that the combined extracts can be an alternative for the development of products that have high antioxidant potential. O uso de tais produtos é uma estratégia interessante para combater o estresse oxidativo que frequentemente é associado ao envelhecimento precoce e ao surgimento de diversas doenças crônicas (de Moraes, Ferreira, Souza, and Moreira, 2020). In addition to antioxidant substances, there is a great trend in the cosmetic industry in the development of multifunctional cosmetics that also combine moisturizing and photoprotective substances, and synergistic effects can be obtained from many combinations (Gaspar, Camargo, Gianeti, and Campos, 2008).

4. Conclusion

The present study demonstrates that the P. glomerata inflorescence extract acted synergistically with the A. chica leaf extract, with effective antioxidant capacity, suggesting that these extracts in combination offer great potential for the prevention of photoaging and diseases associated with oxidative stress. Additionally, the effects of the extracts combined may be important for the development of multifunctional cosmetics, in which the mixture of the extracts could combine the antioxidant ability with other beneficial effects on the skin, such as the moisturizing property of the P. glomerata extract and photoprotective activity of the A. chica extract. These effects could improve skin conditions and promote healthier skin.

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