Phytochemistry, chelating and reducing potential of *Mesosphaerum suaveolens* (L.) Kuntze (Lamiaceae) essential oil

Fitoquímica, potencial quelante e redutor do óleo essencial de *Mesosphaerum suaveolens* (L.) Kuntze (Lamiaceae)

Fitoquímica, potencial quelante y reductor del aceite esencial de *Mesosphaerum suaveolens* (L.) Kuntze (Lamiaceae)

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Abstract

Products of natural origin with potential chelating or reducing Fe^{+} free become promising in antioxidant research, because they have the ability to soften transition metals that can cause cellular damage by oxidative stress. Thus, the aim of this study is to promote the evaluation of the antioxidant potential of essential oil by the orthophenanthroline method, in addition to promoting phytochemical analysis of the essential oil of *M. suaveolens*. This vegetable, by other antioxidant methods, has promising properties in inhibiting free radicals. To achieve these objectives, a chromatographic analysis was performed, as well as the orthophenanthroline assays to evaluate the formation of the Fe^{2+} 1,10-orthophenanthroline complex. The β-Caryophyllene (20.38%) stands out as the major compound of the species. The essential oil interfered with the formation of the complex, suggesting a moderate chelating activity at the highest concentration tested (480 µg/mL), demonstrating that the oil can chelate Fe^{2+}. The addition of ascorbic acid to the mixture significantly increased the absorbance for all tested concentrations, in the evaluation of the reducing power from Fe^{3+} to Fe^{2+}. Considering a potential source of natural antioxidants, it may be relevant for the management of oxidative stress.

Keywords: Antioxidant; Bamburral; Ferrous; Ferric.

Resumo

Produtos de origem natural com potencial quelante ou redutor o Fe^{+} livre se tornam promissoras em pesquisas antioxidantes, isso porque possuem a capacidade de amenizar metais de transição que podem ocasionar danos celulares pelo estresse oxidativo. Assim, objetiva-se com este estudo promover a avaliação do potencial antioxidante do óleo essencial pelo método de ortofenantrolina, além de promover análise fitoquímica do óleo essencial de *M. suaveolens.* Este vegetal, por outros métodos antioxidantes apresenta propriedades promissoras na inibição de radicais livres. Para alcançar tais objetivos, uma análise cromatográfica foi realizada, assim como os ensaios de ortofenantrolina para avaliação da formação do complexo Fe^{2+}+ 1,10-ortofenantrolina. Destaca-se o β-Caryophyllene (20,38%) como o composto majoritário da espécie. O óleo essencial interferiu com a formação do complexo, sugerindo uma moderada atividade quelante na maior concentração testada (480 µg/mL), demonstrando que o óleo pode quelar Fe^{2+}. A adição de ácido ascórbico para a mistura aumentou significativamente a absorbância para todas as concentrações testadas, na avaliação do poder redutor de Fe^{3+} a Fe^{2+}. Considerando-se uma fonte potencial de antioxidantes naturais, podendo ser relevante para o gerenciamento do estresse oxidativo.

Palavras-chave: Antioxidante; Bamburral; Ferroso; Férrico.

Resumen

Los productos de origen natural con potencial quelante o reductor libres de Fe^{+} se vuelven prometedores en la investigación de antioxidantes, porque tienen la capacidad de suavizar los metales de transición que pueden causar daño celular por estrés oxidativo. Así, el objetivo de este estudio es promover la evaluación del potencial antioxidante del aceite esencial por el método de la ortofenantrolina, además de promover el análisis fitoquímico del aceite esencial de M. suaveolens. Esta verdura, por otros métodos antioxidantes, tiene propiedades prometedoras para inhibir los radicales libres. Para lograr estos objetivos, se realizó un análisis cromatográfico, así como los ensayos de ortofenantrolina para evaluar la formación del complejo Fe^{2+} 1,10-ortofenantrolina. El β-cariofileno (20,38%) se destaca como el principal compuesto de la especie. El aceite esencial interfirió con la formación del complejo, lo que sugiere una actividad quelante moderada a la concentración más alta probada (480 µg/mL), lo que demuestra que el aceite puede quelar el Fe^{2+}. La adición de ácido ascórbico a la mezcla aumentó significativamente la absorbancia para todas las concentraciones probadas, en la evaluación del poder reductor de Fe^{3+} a Fe^{2+}. Considerando una fuente potencial de antioxidantes naturales, puede ser relevante para el manejo del estrés oxidativo.

Palabras clave: Antioxidante; Bamburral; Ferroso; Férrico.

1. Introduction

The oxidative stress is characterized by an excess of reactive species that can cause serious cell damage, even leading to cell death (Barreiros, David, & David, 2006). This stress can be identified by biochemical markers such as excess free Fe^{2+}, which is considered indicative of toxicity induced by free radicals (Engle, Erlandson, & Smith, 1964).

Iron is an essential mineral for normal cell physiology, at low molecular weight, however, excess free iron as a result of oxidative stress can result in molecular damage, since excess iron will react with oxygen generating hydroxyl radicals and superoxide anions (Fenton reaction) (Dunn, Rahmanto, & Richardson, 2007; Wijayanti & Immenschuh, 2004). In this context, the potential for Fe^{2+} chelation to make it unavailable or less available for participation in the Fenton reaction is of particular interest in the treatment of many patients who have iron overload (Mittler et al., 2017).

According to some researchers, a high level of free Fe^{2+} ions has been detected in several diseases such as: cancer, neurodegenerative diseases, aging and cardiovascular disorders (Sang et al., 2019; Dixon, & Stockwell, 2014; Liu et al., 2018). This is because the excessive deposition of iron in different vital organs (such as liver and kidney), which is associated with an overproduction of EROS (reactive oxygen species), can lead to the loss of function of these organs (Engle et al., 1964). Considering that the chelation of free iron can reduce the formation of ROS, as well as prevent damage caused by the normal functions of vital organs where there is an excess formation of free radials, the study of natural products with antioxidant potential are promising.

In this scenario, the species *Mesosphaerum suaveolens* (L) Kuntze, family Lamiaceae, is widely used in folk medicine for the therapeutic treatment of various diseases, among them: carminative, stomach pain and inflammation. A species native to America, commonly known in Brazil as “bamburral” or “lavender-wild” (Costa et al., 2020; Bezerra et al., 2017; Jesus, Falcão, & Lima, 2013). Substantial evidence from the literature reveals that *M. suaveolens* exhibits a variety of pharmacological activities, including antioxidant properties of relevance, by the DPPH method (Bezerra et al., 2018), in addition to being rich in phytochemicals (Bezerra et al., 2017) that show good results in the stability of free radicals (Chanda & Dave, 2009).

In this study, we hypothesized that the essential oil of the species has considerable reducing and chelating potential for free iron. Thus, this work aimed to develop a study on the chromatography of essential oil and promote the evaluation of the potential of essential oil by the orthophenanthroline assay.

2. Materials and Methods

2.1 Botanical material collection and essential oil extraction

The leaves of *M. suaveolens* were collected in the municipality of Quixelô located in the state of Ceará (Brazil) under the coordinates -6°14'22.40" S, -39°16'14.29" W, in March 2015. The species was identified by the botanist José Weverton Almeida-Bezerra, and a voucher was deposited at the Herbário Caririense Dárdano de Andrade-Lima - HCDAL under nº 12.104.

After the collection was carried out, the leaves were put to dry in an oven at 30 °C, then they were placed in a volumetric hydrodistillation flask in a *Clevenger* apparatus with 4 L of distilled water, submitted to constant boiling, under a heating blanket. The temperature was adjusted to the boiling point of the water, after boiling, the time of 2 hours of the extraction cycle was counted. Subsequently, the essential oil was collected and stored in an amber bottle under constant refrigeration until the phytochemistry and evaluation of chelating and iron-reducing power were carried out.

2.2 Phytochemical analysis by gas chromatography

For the phytochemical analysis of the essential oil, gas chromatography (GC) was used, performed with the Agilent Technologies 6890N GC-FID system, equipped with a DB-5 capillary column (30 m × 0.32 mm; 0.50 µm) and connected to an FID detector. The thermal programmer was from 60 ° C (1 min) to 180 °C to 3 ° C/min; injector temperature 220 °C; detector temperature 220 °C; 1:10 division ratio; carrier gas Helium; flow rate: 1 mL/min. The injected volume of *M. suaveolens* essential oil was 1 µL diluted in chloroform (1:10). Two sample replicas were processed in the same way. The relative concentrations of the components were calculated based on the peak areas of the GC without using correction factors.

The identification of the constituents was carried out based on the retention index (RI), determined with reference to the homologous series of n-alkanes, C_7-C_{30}, under identical experimental conditions, in comparison with the research of the mass spectrum library (NIST and Wiley), and the mass spectra literature dates from R Core Team, (2014). The relative quantities of the individual components were calculated based on the peak area of the GC (FID response).

2.3 Chelating activity of Fe^{2+} of the essential oil of *M. suaveolens*

The iron (II) chelation capacity of the essential oil was determined using the method of Minotti and Aust (1987) with some modifications. It was initially preparing the reaction mixture, containing 58 µL of saline solution (0.9%, w / v), 45 µL of Tris-HCl (0.1 M, pH: 7.5), 27 µL of essential oil (60–480 µg / mL) and 36 µL of 110 µM FeSO4 (iron sulfate), distributed on an Elisa plate. Subsequently, 34 µL of 0.10% (w / v) 1.10-phenanthroline were added and the absorbance of the formed orange complex (Fe2 ++ orthophenanthroline) was measured at 0, 10 and 20 min at 510 nm using a spectrophotometer (SpectraMax). The control was carried out without the presence of essential oil, adding a difference of 27 µL of Tris solution.

After the 30 min incubation and readings every 10 min, the reducing agent, ascorbic acid (5 mM) was added to the reaction mixture. Then new readings were performed at an absorbance determined after 5, 10 and 20 minutes after adding ascorbic acid. Where this method was applied to assess whether the *M. suaveolens* essential oil could be oxidizing Fe^{2+} to Fe^{3+} through the formation of a more oxidizable complex, we determined the potential reduction of any Fe^{3+} forming during the 5, 10 and 20 min incubation.

2.4 Test of reducing power of Fe^{3+} of the essential oil of *M. suaveolens*

To further investigate the reducing potential of essential oil, the methodology described above was followed, but using iron chloride ($FeCl_3$ 110 µM) instead of iron sulfate ($FeSO_4$ 110 µM) in the reaction mixture (Kamdem et al. 2013, with adaptations). The increase in absorbance indicates an increase in the reductive capacity (Joshi Verma, & Mathela, 2010). In addition, the white was prepared by the same procedure without essential oil.

2.5 Statistical analysis

The results were expressed as mean ± standard error of the mean (SEM). A statistical analysis was performed using one factor (ANOVA), with one-way analysis. Multiple Bonferroni comparisons were performed to detect significant configurations between controls and treatments. A probability of $p < 0.0001$ was considered statistically significant.

3. Results

3.1 Chemical composition of essential oil

As shown in Table 1, a total of 10 chemical compounds were identified by gas chromatography. The β-Caryophyllene (20.38%) stands out as the major compound in this tested sample. In addition to this, other compounds such as Sabinene (>15%) and Spathulenol (> 11%), stood out with higher percentages of appearance.

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Table 1. Main constituents of *Mesosphaerum suaveolens* essential oil. Relative proportions of essential oil constituents expressed as percentages. a- Experimental retention indices (based on homologous series of n-alkane C_7-C_{30}). b- Literature retention rates.

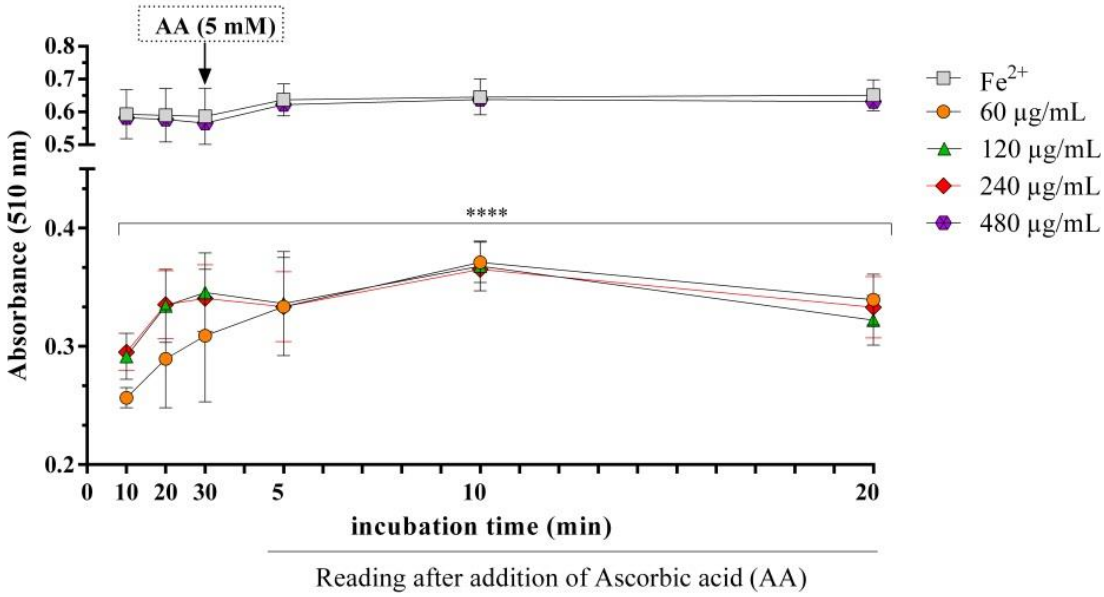
Compounds	**RI**[a]	**RI**[b]	**Oil %**
Sabinene	976	976	15.95
Limonene	1031	1031	5.18
1-8-Cineole	1037	1033	3.03
γ-Terpinene	1060	1061	2.46
4-Tepineol	1178	1177	6.63
β-Caryophyllene	1421	1418	20.38
germacreno D	1481	1480	5.2
Bicyclogermacrene	1501	1488	7.03
Spathulenol	1576	1576	11.08
caryophyllene oxide	1580	1581	3.19

Source: Authors, (2020).

3.2 Chelation of Fe^{2+}

In Fe^{2+} chelation activity (Figure 1), the absorbance rate of the orange complex formed by the interaction of Fe^{2+} and orthophenanthroline allows to estimate a reduction in absorbance only at the highest concentration (480 µg/mL), while the incubation increased significantly the absorbance of the other concentrations tested before the addition of vitamin C, which after addition, significantly increased all concentrations. Incubation of the essential oil of *M. suaveolens* in lower concentrations (60, 120, 240 µg/mL) did not show such activity when compared to the control group, Fe^{2+} isolated.

Figure 1. Oxidation of Fe^{2+} by the essential oil of *Mesosphaerum suaveolens* leaves. The values represent the mean ± SEM of three experiments. AA: Ascorbic acid; ****: $p<0.0001$ statistically significant value.



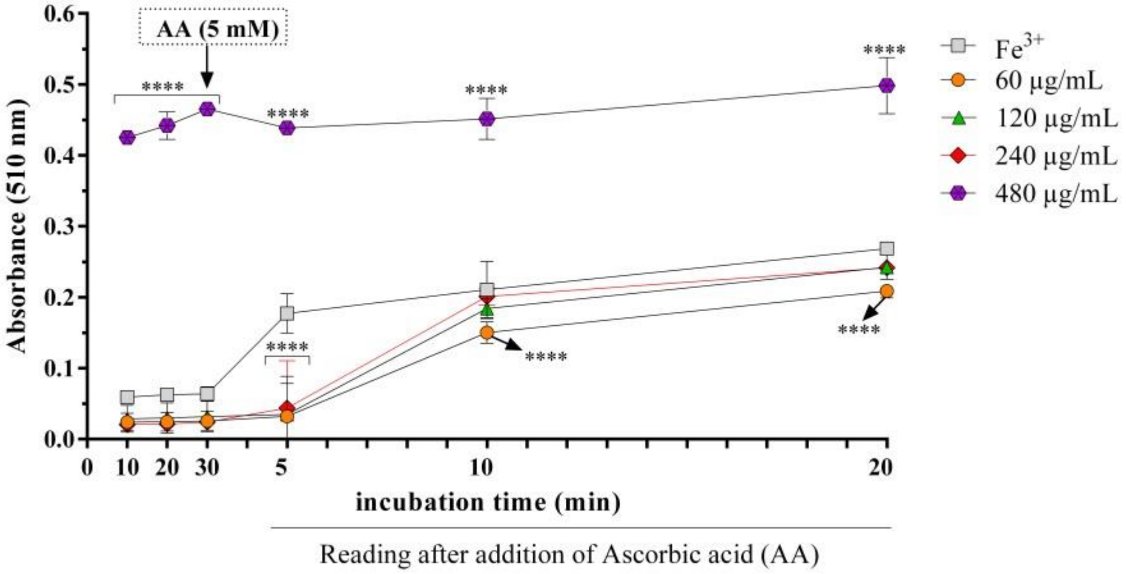
Source: Authors, (2020).

This finding may suggest that essential oil in high concentrations can oxidize free iron. To confirm after a period of 20 min of incubation of the iron with the essential oil, ascorbic acid (AA) was added in the reaction to see if the increase in absorbance in the presence of the oil was attributed to the iron. The absorbance did not change at 10, 20 and 30 min after adding ascorbic acid in the highest concentration (480 µg/mL), however a significant drop was observed in the other concentrations after 10 min. This is because essential oils can oxidize Fe^{2+} to Fe^{3+}, leading to a decrease in absorbance. The most plausible interpretation of these results is that essential oil can chelate Fe^{2+} and accelerate the oxidation of Fe^{2+} to Fe^{3+}.

3.3 Potential for iron reduction Fe^{3+} to Fe^{2+} of essential oil

Figure 2 demonstrates the ability to reduce Fe^{3+}. Similar to that observed in the Fe^{2+} chelation test, the highest concentration (480 µg/mL) was more expressive, showing significant results. Since, the incubation of the essential oil with Fe^{3+} in the presence of orthophenanthroline resulted in a significant increase in absorbance in a dose-dependent manner ($p>$ 0.0001) in comparison with the isolated Fe^{3+} which is the control group. With the addition of ascorbic acid (AA), a significant increase in the absorbance of the sample for the concentrations of 60, 120 and 240 μg/mL and Fe^{3+} isolated, with a moderate reduction in the concentration of 480 μg/mL until the 5 min reading, with subsequent increase in absorbance.

Figure 2. Reduction of Fe3 + to Fe2 + (110 μM) in exposure to *Mesosphaerum suaveolens* essential oil. The values represent the mean ± SEM of three experiments. AA: Ascorbic acid; ****: p> 0.0001 statistically significant value.



Source: Authors, (2020).

4. Discussion

Substances capable of chelating or reducing free iron become promising in antioxidant research, due to the ability to soften transition metals that can cause cellular damage by oxidative stress, as is the case of iron (Fe^{2+}) to ferric (Fe^{3+}) iron chelation (Oboh, Puntel, & Rocha, 2007). This promising antioxidant activity is exerted by phytochemicals, which are electron donors, so that they can react with free radicals to stabilize and/or block radical chain reactions (Chanda & Dave, 2009). Thus, the objective is to evaluate the promising potential of the essential oil of *M. suaveolens*, as well as to determine the terpenes present in the sample under study.

The sesquitepene β-Caryophyllene, which is represented in this study as the major compound of the species (>20%), when tested alone, has promising biological and pharmacological activities (Dahham et al., 2015), among them relevant antioxidant potential, such as example, the study by Calleja et al. (2013), which demonstrates by the DPPH test an

IC_{50} of 9.9 μg/mL, as well as 27.9% inhibition of lipid peroxidation (TBARS). Data that corroborate those of Dahham et al. (2015), in which it presents 1.25 ± 0.06 (DPPH) and 3.23 ± 0.07 (FRAP), results of great significance when compared to vitamin C (control group).

However, complex mixtures such as essential oils, extracts and teas have interactions between compounds, which can often enhance or delay their activity, because there may be possibilities for changes in their structural conformation, significantly altering their bioactivity (Galvão et al., 2017). Thus, knowing that the isolated compounds can be promising as antioxidant studies aimed at combining these compounds in the case of our study, which aims to evaluate the essential oil of the species, can demonstrate satisfactory data.

To date, there are few reports regarding this activity of the essential oil of M. suaveolens, as a chelator and/or free iron reducer. Nevertheless, studies with other antioxidant methods highlight the significant potential of this species in the sequestration of free radicals (Costa et al., 2020; Bezerra et al., 2018).

In the study by Nantitanon, Chowwanapoonpohn and Okonogi (2007), using the in vitro DPPH method, reported significant results in the inhibition of the 2,2-diphenyl-1-picryl-hydrazil radical, presenting an IC_{50} of 3.72 μg/mL. Results from natural products with IC_{50} values below 500 μg/mL are relevant in the scope of scientific research (Razo-Hernández et al. 2014). These data are in accordance with our results.

In this investigation it was possible to demonstrate that after the addition of ascorbic acid to the reaction mixture, a partial increase in absorbance is noticeable after 5, 10 and 20 min of incubation, indicating an oxidoreduction of Fe^{2+} and Fe^{3+} by the essential oil in 60, 120 and 240 μg/mL, except for the concentration of 480 μg/mL in which it had a moderate reduction, after the addition of vitamin C up to 10 min.

These data are in line with studies involving species of this botanical family, such as in the work of Duarte et al., (2015), investigating the potential of *Rhaphiodon echinus* Schauer, reports that the inhibition of lipid peroxidation induced by free radicals may be related to the reduction of Fe^{3+} to Fe^{2+} in exposure to the extracts of the plant under study. As in the study by Carvalho (2018), which reports that *Hyptis lacustris* A.St.-Hil. ex Benth., presents promising results, demonstrating IC_{50} values of 44.75 μg/mL for iron chelation and 5.35 μg/mL for reducing power, when compared to control groups, in addition to presenting 16.73 μg/mL in the DPPH assay and 5.18 μg/mL for the ABTS assay.

In recent years, considerable growth in scientific research in the search to find natural antioxidants that can have a positive impact on clinical conditions and health maintenance has been observed, especially as these compounds intervene (Costa et al., 2020; Chen & Ho, 1995). And one of the well-reported mechanisms of action in which antioxidants exert is the elimination of free radicals, in the process of electron donation to stabilize them, inhibiting their toxic potential (Bezerra et al., 2018; Nantitanon et al., 2007 ; Chen & Ho, 1995), due to the possible ability to cause some harmful effects associated with free radicals., Thus preventing them from binding to molecular structures such as the plasma membrane, proteins, fatty acids and DNA (Luz et al., 2018 ; Kamdem et al., 2013) which can exert serious damage at the molecular level, thus, antioxidants can prevent or repair some harmful effects associated with free radicals.

5. Conclusion

The results obtained in the present study clearly demonstrate that *M. suaveolens* has moderate antioxidant activity by the method of chelation and reduction of free iron, demonstrating significant data, contributing to the pharmacological investigation of natural products for the treatment of diseases caused by oxidative stress. However, further studies must be carried out to ensure the safe use of this vegetable oil.

Declaration of Interests

There are no conflicts of interest in this study.

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