Antioxidant activity and flavonoid content of Lippia origanoides Kunth.

Actividad antioxidante y contenido de flavonoides de Lippia origanoides Kunth.

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ABSTRACT

Introduction Lippia origanoides Kunth. is an aromatic shrub, it is used as a condiment and for medicinal purposes. Besides the presence of the essential oil, studies have reported the presence of flavonoids, which have the ability to act as free radical scavenger antioxidants.
Objective: To determine the antioxidant capacity and the total flavonoid content in leaf extract of L. origanoides accessions in the in vivo germplasm bank of the Institute of Agrarian Sciences of the Federal University of Minas Gerais.
Methods: Evaluation of antioxidant activity was carried out by the DPPH radical sequestration method with extraction from leaves. The effective concentration (EC50) antioxidant activity index (AAI) was calculated. The total flavonoid content was determined by the aluminum chloride method, the results were submitted to analysis of variance, and means were compared by Tukey test at 5% probability.
Results: The effective concentration (EC50) presented mean values from 27.09 µg.mL-1 (ICA 1) to 44.38 µg.mL-1 (ICA 6), accession ICA 1 has more effective antioxidant action, as it presented the lowest EC50. The leaf ethanolic extracts of L. origanoides showed moderate antioxidant activity. The average total flavonoid content of accession ICA 6 (417.04 mg RE g-1) was statistically higher than the others, while accession ICA 2 total flavonoid content (114.69 mg RE g-1) was lower than the others. The extract total flavonoid content had a significant positive correlation (r = 0.83) with antioxidant activity.
Conclusions: Ethanolic extracts from the studied accessions present variation in antioxidant and flavonoid content and a promising potential for application as a natural antioxidant.

Keywords: carvacrol, thymol, DPPH, flavonoid, germplasm in vivo.

RESUMEN

Introducción: Lippia origanoides Kunth. es un arbusto aromático, se utiliza como condimento y para propósitos medicinales. En el aceite esencial, los estudios han reportado la presencia de flavonoides, que tienen la capacidad de actuar como barredores de radicales libres antioxidantes.
Objetivos: Determinar la capacidad antioxidante y el contenido de flavonoides totales en extractos hojas de L. origanoides de accesos del banco de germoplasma in vivo del Instituto de Ciencias Agrícolas de la Universidad Federal de Minas Gerais.
Métodos: Accesos de L. origanoides son de la colección del banco de germoplasma in vivo del Instituto de Ciencias Agrícolas de la Universidad Federal de Minas Gerais. La evaluación de la capacidad antioxidante se llevó a cabo por DPPH método secuestro radical con los extractos de las hojas. Se calculó la concentración eficaz (EC50) y el índice de actividad antioxidante (IAA). El contenido total de flavonoides se determinó por el método de cloruro de aluminio y los resultados fueron sometidos a análisis de varianza y las medias se compararon mediante la prueba de Tukey al 5% de probabilidad.
Resultados: La concentración eficaz (EC50) mostraron valores medios de 27,09 µg ml-1 (ICA1) a 44,38 µg ml-1 (ICA 6) a los diferentes accesos. ICA 1 acceso era más eficaz acción antioxidante, ya que se presenta la CE50 más baja. Los extractos etanolicos de hojas de L. origanoides mostraron capacidad antioxidante moderada. El contenido promedio de flavonoide del acceso total a ICA 6 (417,04 mg RE g-1) fue significativamente mayor que el otro, mientras que el acceso ICA 2 (114,69 mg RE g-1) fue menor que los otros accesos. El contenido de extractos de flavonoides totales tenían una correlación positiva significativa (r = 0,83) con la actividad antioxidante.
Conclusión: Los extratos etanólicos de los accesos estudiados presentan variación en la actividad antioxidante y no teor de flavonoides con un potencial promisorio para una aplicación como antioxidante natural.

Palabras claves: carvacrol, timol, DPPH, flavonoides, germoplasma in vivo.

INTRODUCTION

Lippia origanoides Kunth. is a shrub native to northeastern South America and some countries in the Caribbean and Central America[1]. Popularly known as "salva-de-marajó" or "alecrim-pimenta", is used as a spice and for medicinal purposes [2].

The L. origanoides medicinal action is usually associated with the essential oil obtained from the leaves that have, as main constituents, two isomers and phenolic monoterpenes, thymol and carvacrol2. In addition to the essential oil, other metabolites such as flavonoids were detected in leaf ethanolic extracts [3-4]. Flavonoids are natural substances with variable phenolic structures and have the ability to act as radical sequestrant antioxidants[5].

In recent years, research on natural antioxidants has increased due to their importance in fighting free radicals and other oxygen reactive forms, which are responsible for diseases such as cancer and age-related diseases[6].

The objective of this study was to determine the total flavonoid content and antioxidant activity in hydroalcoholic extracts of L. origanoides leaves maintained in an in vivo germplasm bank.

MATERIAL AND METHODS

Leaf samples from 8 accessions of L. origanoides from the in vivo germplasm bank collection of the Institute of Agrarian Sciences of the Federal University of Minas Gerais (ICA-UFMG) were collected. The accessions belong to 6 regions of Minas Gerais, accession ICA 01 being from Salinas, MG; ICA 2 and 3 from Turmalina, MG; ICA 06 from Cristália, MG; ICA 07 from Montes Claros, MG; ICA 09 from Glaucilândia, MG; and ICA 11 and ICA 12 from Buenópolis, MG. Exsiccates of the species were deposited in the Agricultural Company of Minas Gerais (EPAMIG) herbarium under No 56524 and No 56526 and identified by Fatima Salimena.

The leaves from each of the eight accessions were collected in February 2014 in the morning, taken to the medicinal plants laboratory and dried in a forced air circulation oven (Nova Ética) at 40°C until constant weight. The plant material was ground in an analytical mill (QUIMIS, No. 289) and stored (-20°C) in plastic capped tubes until the time of analysis. The extracts were prepared at the concentration of 50 mg mL-1 with 60% ethanol. The extract was maintained on an orbital shaker (Shaker, SK-180-Pro) for 24 h at room temperature, and subsequently filtered on paper filter whatman No 01. The resulting fraction was stored in an amber bottle at -4°C until analysis[7].

Evaluation of antioxidant activity was carried out by the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical sequestration method according to Kondo8. For the analysis, ethanolic extracts (60%) from L. origanoides leaves were used in a concentration of 1 mg ml-1, in triplicate, and DPPH at the concentration of 60 µM. Five concentrations of each accession extract were used and added to the DPPH (3 ml), then left to react in the dark.

The absorbance readings were performed after 30 min of reaction in spectrophotometer UV-Vis (Cary60, Agilent). Rutin, at a concentration of 1 mg mL^{-1} and BHT (Butylated Hydroxytoluene) at a concentration of 0.5 mg mL^{-1} were used as analytical standards. Antioxidant activity percentage (%A.A) was determined according to Maisuthisakul et al. (2007) using the following equation[9]:

$$\%AA = \left(\frac{Control\ \ absorbance - Sample\ \ absorbance}{Control\ \ absorbance} \right) \times 100$$

Where: Control Abs. = Control absorbance (DPPH solution without antioxidant) and Sample Abs. = absorbance of the sample (extract) being tested.

The effective concentration (EC_{50}) was calculated from antioxidant activity quadratic regression equations as a function of sample concentration (µg.mL^{-1}) in the reaction. The antioxidant activity index (AAI) was calculated according to Scherer and Godoy[10]:

$$IAA = \left(\frac{C_{DPPH}}{CE_{50}} \right)$$

Where: C_{DPPH} is the DPPH concentration (µg ml^{-1}).

The total flavonoid content was determined by the aluminum chloride method according to Liu[7], modified. The plant extract was diluted (2 mL) in a test tube and a 2% aluminum chloride solution (2 ml) was added. The mixture was subjected to vortexing and allowed to rest (30 min) in the dark. Rutin (Sigma-Aldrich, USA) was used as standard for the calibration curve, set up with five different concentrations (0.01; 0.02; 0.04; 0.06; 0.08 mg mL^{-1}).

The analysis and calibration curves were performed in the UV-Vis spectrophotometer (Cary60, Agilent), together with the complex (rutin-$AlCl_3$) maximum absorption peak determination. Readings were taken at 405 nm and the total flavonoids

were expressed in mg rutin equivalent (mg RE g^{-1}).

The results were submitted to analysis of variance and means were compared by Tukey test at 5% probability. The Pearson correlation coefficient was used to assess the relationship between flavonoid and antioxidant activity. The interaction between flavonoid content and antioxidant activity was performed.

RESULTS

The amount of extract required to decrease the DPPH initial concentration by 50% (effective concentration - EC_{50}) presented mean values from 27.09 $\mu g.mL^{-1}$ (ICA 1) to 44.38 $\mu g.mL^{-1}$ (ICA 6). The higher the DPPH consumption by a sample, the lower its EC_{50} and greater its antioxidant activity[11]. Thus, accession ICA 1 (27.09 $\mu g.mL^{-1}$) has more effective antioxidant action, as it presented the lowest EC_{50}, followed by accessions ICA 11 (31.28 $\mu g.mL^{-1}$) and ICA 7 (32.47 $\mu g.mL^{-1}$) (**Table 1**).

Table 1. Effective concentration (EC_{50}), antioxidant activity index (AAI) and average total flavonoid content of *Lippia origanoides* accession leaf extracts from the germplasm collection at the Institute of Agricultural Sciences, Federal University of Minas Gerais (ICA-UFMG).

Sample	EC_{50} ±SD (µg/mL)	AAI	* Average total flavonoid content mg RE g^{-1}
ICA6	44.38 ab	0.54±0.02b	417.04 a
ICA1	27.09 c	0.9±0.16 a	284.04 ab
ICA7	32.47 c	0.75±0.17 ab	280.7 ab
ICA9	38.36 ab	0.63±0.03 b	280.7 ab
ICA12	34.23 abc	0.70±0.01 ab	230.4 bc
ICA3	41.94ab	0.57±0.02 b	223.22 bc
ICA11	31.28 c	0.77±0.03 a	205.58 bc
ICA2	40.92ab	0.61±0.04 b	114.69 c
Rutin	2.2	10.83±0.52	-
BHT	48.35	0.5±0.07	-

Note: Means followed by the same letter in the column do not differ significantly by Tukey's test at 5% probability.

The L. origanoides leaf ethanolic extracts had higher antioxidant capacity compared to the BHT synthetic antioxidant, however, both the extracts and the BHT had lower antioxidant capacity compared to rutin.

Regarding total flavonoids content from the eight accessions, three (ICA 1, ICA 7 and ICA 9) were not statistically different from each other (284.04 mg RE g^{-1}, 280.7 mg RE g^{-1} and 280.7 RE mg g^{-1}). The same occurred with accessions ICA 12 (230.4 mg RE g^{-1}), ICA 3 (223.22 mg RE g^{-1}) and ICA 11 (205.58 mg RE g^{-1}). The average total flavonoid content of accession ICA 6 (417.04 mg RE g^{-1}) was statistically higher than the others, while accession ICA 2 total flavonoid content (114.69 mg RE g-1) was lower than the others.

The extract total flavonoid content had a significant positive correlation (r = 0.83) with antioxidant activity.

DISCUSSION

In a study by Silva[12], with Lippia thymoides (Verbenaceae) species, EC50 was 15.4 ± 1.6 $\mu g.mL^{-1}$, in a methanolic extract. In another study by Almeida[4], with an ethanolic extract of Lippia sidoides leaves, EC_{50} was 16.3 $\mu g.mL^{-1}$.

According to the index defined by Scherer and Godoy[10], samples are classified as being of low antioxidant activity when the AAI < 0.5; moderate antioxidant activity when 0.5 < AAI < 1.0; strong antioxidant activity when 1.0 < AAI < 2.0; and very strong antioxidant activity when AAI > 2.0. The BHT and the extracts showed moderate antioxidant activity, while rutin antioxidant activity was very strong. BHT (butylated hydroxytoluene) is a synthetic antioxidant commonly used in food containing lipids, but it presents safety and toxicity problems[13].

The difference in the average total flavonoid content among the accessions may be related to the genetic factors, as the plants are from different places, but are kept in the same environmental conditions and have the same age.

The extract total flavonoid content had a significant positive correlation antioxidant activity. This indicates that the extract antioxidant activity is strongly related to flavonoids. Flavonoid antioxidant activity is assigned to the reducing power of phenolic groups, which reduce free radicals and produces phenoxyl radical which, in turn, is stabilized by resonance[5].

Ethanolic extracts from the studied accessions present variation in antioxidant and flavonoid content, while being kept in the same soil and climatic conditions, indicating a genetic variation among accessions and a promising potential for application as a natural antioxidant.

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