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Antioxidant capacity and total phenol content of *Hyptis* spp., *P. heptaphyllum*, *T. panamensis*, *T. rhoifolia* and *Ocotea* sp.

Abstract

In this work, the possible correlation between the antioxidant activities and the Total Phenolic Content (TPC) and chemical composition of Lamiaceae (*H. conferta, H. dilatata, H. mutabilis, H. suaveolens*), Burseraceae (*P. heptaphyllum, T. rhoifoila, T. panamensis*), and Lauraceae (*Ocotea* sp.) were evaluated. The Trolox Equivalent Antioxidant Capacity or the Total Antioxidant Activity (TAA) was determined by using a colorimetric assay with the ABTS radical cation, Effective Concentration (EC_{50}) was evaluated with the DPPH radical, and the TPC was established by the Folin-Ciocalteu method, for ethanolic extracts obtained by cold maceration and evaporation to dryness. Both the TAA and the EC_{50} were highly correlated with the TPC. The barks of *T. rhoifolia* and *T. panamensis* demonstrated the highest antioxidant capacities. The Burseraceae spp. exhibited the highest TPC, and the Lamiaceae (*Hyptis* spp.) demonstrated the lowest TPC.

Keywords: ABTS, Folin-Ciocalteu, Lamiaceae, Burseraceae, Lauraceae.

Capacidad antioxidante y contenido de fenoles totales de *Hyptis* spp., *P.* Heptaphyllum*, T. Panamensis, T. Rhoifolia*, y *Ocotea* sp.

Resumen

En este trabajo se evaluó la posible correlación entre las actividades antioxidantes, el contenido de fenoles totales (CFT) y la composición química de Lamiaceae (*H. conferta, H. dilatata, H. mutabilis, H. suaveolens*), Burseraceae (*P. heptaphyllum, T. rhoifoila, T. panamensis*) y Lauraceae (*Ocotea* sp.). Para los extractos etanólicos obtenidos por maceración en frio y evaporación a sequedad, la Capacidad Antioxidante Equivalente al Trolox o la Actividad Antioxidante Total (AAT), fueron determinadas mediante un ensayo colorimétrico con el catión radical ABTS, la Concentración Efectiva (EC_{50}) fue evaluada con el radical DPPH, y el Contenido de Fenoles Totales (CFT), fue establecido mediante el método de Folin-Ciocalteu. Tanto la AAT como la EC_{50} estuvieron altamente correlacionados con el CFT. Las cortezas de *T. rhoifolia* y *T. panamensis* mostraron las capacidades antioxidantes más altas. Las Burseraceae spp. mostraron los TPC más altos y las Lamiaceae (*Hyptis* spp.) mostraron los TPC más bajos.

Palabras clave: ABTS, Folin-Ciocalteu, Lamiaceae, Burseraceae, Lauraceae.

Capacidade antioxidante e conteúdo de fenóis totais de *Hyptis* spp., *P. Heptaphyllum, T. Panamensis, T. Rhoifolia,* e *Ocotea* sp.

Resumo

Neste trabalho foi avaliada a possível correlação entre as atividades antioxidantes, o conteúdo de fenóis totais e a composição química de Lamiaceae (*H. conferta, H. dilatata, H. mutabilis, H. suaveolens*), Burseraceae (*P. heptaphyllum, T. rhoifoila, T. panamensis*), e Lauraceae (*Ocotea* sp.). Para os extratos de etanol obtidos por maceração em frio e evaporação até a secura, a Capacidade Antioxidante Equivalente ao Trolox ou à Atividade Antioxidante Total (AAT), foi determinada por meio de um ensaio colorimétrico com o cátion radical ABTS, a concentração eficaz (EC_{50}) foi avaliada com o radical DPPH, e o Conteúdo de Fenóis Totais (CFT) foi estabelecido pelo método do Folin-Ciocalteu. Tanto a AAT quanto a EC_{50} estiveram altamente relacionadas com a CFT. A casca de *T. rhoifolia* e *T. panamensis* apresentaram as maiores capacidades antioxidantes. As Burseraceae spp. apresentaram o CFT mais alto, e as Lamiaceae (*Hyptis* spp.) apresentaram o CFT mais baixo.

Palavras chave: ABTS, Folin-Ciocalteu, Lamiaceae, Burseraceae, Lauraceae.

Introduction

Lamiaceae are the most widely distributed angiosperms in the world. They comprise approximately 221 genera and 6000 species. In Colombia, 23 genera and over 190 species of Labiatae have been identified *(1)*. Studies of plants in this family indicate that Lamiaceae have traditionally been used as condiments or drugs because of their antioxidant, insecticidal, antimicrobial (antibacterial, antiherpes), antiinflammatory, antitumor, antihypertensive, and gastroprotective properties *(2,3)*. Additionally, they are used in the perfume, cosmetic, food, and pharmaceutical industries because of the diversity of flavors present in the essential oils of several species *(1)*.

The content and type of phenolic compounds found in the essential oils and extracts of these species are among the factors that determine their biological activity *(4)*. The species of the *Hyptis* genera (Lamiaceae) are used in applications such as repellents and insecticides as well as for antinociceptive, antihyperglycemic, antifungal, antibacterial, antiinflammatory, antimalarial, and gastrointestinal purposes *(5,6)*.

Burseraceae are a source of exudate and resins with increased aromatic compounds that are used in traditional medicine and perfumery. The Online Collection of the Instituto de Ciencias Naturales of the Universidad Nacional de Colombia (ICN-UN) reported eight genera of Burseraceae: *Bursera, Canarium, Crepidospermum, Dacryodes, Hemicrepidospermum, Protium, Tetragastris*, and *Trattinnickia.* The *Protium* genus contains the largest number of species, followed by *Bursera* and *Trattinickia (7)*.

The resins, essential oils of resins and leaves, and extracts of leaves, barks and stems of the *Protium* genus have all been evaluated for potential applications. Species of this genus are antiinflammatory, antinociceptive, analgesic, expectorant, antimalarial, repellent, possess antitumor and acaricide activities, and are gastric and liver protectors *(8)*.

The *Tetragastris* and *Trattinnickia* species have been less thoroughly studied. The bark of *Tetragastris panamensis* has been used for antihemorrhagic, antiviral, leishmanicidal, and antimalarial purposes *(9)*.

Lauraceae has 55 genera and approximately 3000 species. It is composed of a wide variety of trees and shrubs that grow in moist tropical dry forests. It is distributed throughout America and Asia, with a considerable number of species in Australia and Madagascar and a small number in Africa (*10*). Genus *Ocotea* is the most diverse and abundant of the Lauraceae, with approximately 350 species mainly distributed in the Neotropics. They are found from Mexico to Argentina, in Africa, Madagascar, and one species in the Canary Islands (*10*). Many species of *Ocotea* have antirheumatic, analgesic, purgative, and tonic properties (*11*), they have shown analgesic, antiinflammatory, antithrombotic, antiplaquetales, antioxidant, and antimicrobial properties as well *(12)*. For example *Ocotea paulii* (*O. atirrensis*) has antifungal properties *(13),* and *O. bullata* has shown antiinflammatory activity *(14)*. Also several species of this genus are important in the field of perfumery such as *O. pretiosa, O. sassafras, O. caudate*, and *O. cymbarum* (*15)*.

The species of Lamiaceae, Lauraceae, and Burseraceae are culturally important and have the potential to treat different diseases. Studies of antioxidant activities and Total Phenol Content (TPC) can be used to explain the biological activities that have been reported. In this work, the antioxidant capacities of the ethanolic extracts of *H. conferta, H. dilatata, H. mutabilis, H. suaveolens, P. heptaphyllum, T. panamensis, T. rhoifolia,* and *Ocotea* sp., which were collected from areas near Arauca (Orinoco, Colombia) were evaluated. Assays with the DPPH and ABTS radicals were used to evaluate the Total Antioxidant Capacity (TAA), and the Folin-Ciocalteu reagent was used to determine the TPC.

Materials and methods

Reagents and materials

Vitamin E (97%), DPPH (90%), gallic acid (98%), Folin-Ciocalteu reagent ® (2 N), and ethanol (99.8%) were purchased from Sigma-Aldrich® (Saint Louis, MO, USA). Spectrophotometric data were obtained on a Shimadzu model 2401PC® (Columbia, Maryland, USA). Quartz cuvettes (1 cm x 1 cm x 4 cm) were used to determine the absorbance in the visible range.

Plant material

Images of the samples collected for this study are shown in Figure 1.

Hyptis conferta was collected at La Saya village (Arauca, Colombia); coordinates: 7°04'N–70°45'W, 206 meters above sea level (m.a.s.l.). *Hyptis dilatata* was collected at Mata Corozo farm, La Comarca village of Cravo Norte (Arauca, Colombia); coordinates: 6°21›2.75"N–70°14'27.14"W, 102 m.a.s.l. *Tetragastris panamensis* and *Trattinnickia rhoifolia* were collected at La Reforma farm, Rincón Hondo village of Tame (Arauca, Colombia); coordinates: 6°28'50.03"N–71°41'15.09"W, 445 m.a.s.l. *Hyptis mutabilis* and *Hyptis suaveolens* were collected at Las Mercedes farm, in Mata de Gallina village of Arauca (Arauca, Colombia); coordinates: 6°58'25,45"N–70°42'24.69"W, 127 m.a.s.l. *Protium heptaphyllum* was collected in La Mancha farm, on Puerto San Salvador, in Tame (Arauca, Colombia); coordinates: 6°27'N–71°44'W, 240 m.a.s.l.

Taxonomic identification of the following species was performed in the Herbario Nacional Colombiano of the ICN-UN, Bogotá: *H. conferta* (COL 563485, 2012), *H. dilatata* (COL 563486, 2012), *T. panamensis* (2012), *T. rhoifolia* (COL 566451, 2012), *H. mutabilis* (COL 553356, 2011), *H. suaveolens* (COL 553357, 2011) and *P. heptaphyllum* (COL 557313).

Ocotea sp., was collected on El Porvenir village of Toledo, Norte de Santander (Colombia). Preliminary identification was performed by Venezuelan Forest.

Preparation of ethanolic extract

Extracts were obtained from dried plant material, crushed, and homogenized by exhaustive extraction with ethanol as the solvent (leaves or bark). The extracts were dried by vacuum distillation. Inflorescences were used for the extractions of *H. conferta,* including leaves of *H. dilatata, H. mutabilis, H. suaveolens*, *P. heptaphyllum*, and *Ocotea* sp., and the stem barks of *T. panamensis* and *T. rhoifolia*.

Reactivity to ABTS and DPPH radicals

The assay for ABTS radical was performed according to the method reported by Re *et al. (16*). TEAC or TAA values (Trolox Equivalent Antioxidant Capacity or Total Antioxidant Activity) were obtained in this test. ABTS (38.5 mg) was dissolved in distilled water (10 mL) and potassium persulfate was added to the mixture (6.9 mg). The final solution was allowed to stand for 20 h in the dark at room temperature to obtain the ABTS cation radical. An aliquot of the ABTS solution was diluted in ethanol to achieve an absorbance of 0.70 at 734 nm. A calibration curve was performed with the reference antioxidant (trolox). The decrease in absorbance (dA) of the solution of ABTS (3 mL) was plotted for 6 min following the addition of 30 µl trolox standard mixtures (0.2-1.9 mM). Five mixtures of ethanolic extracts were prepared to assess the decrease

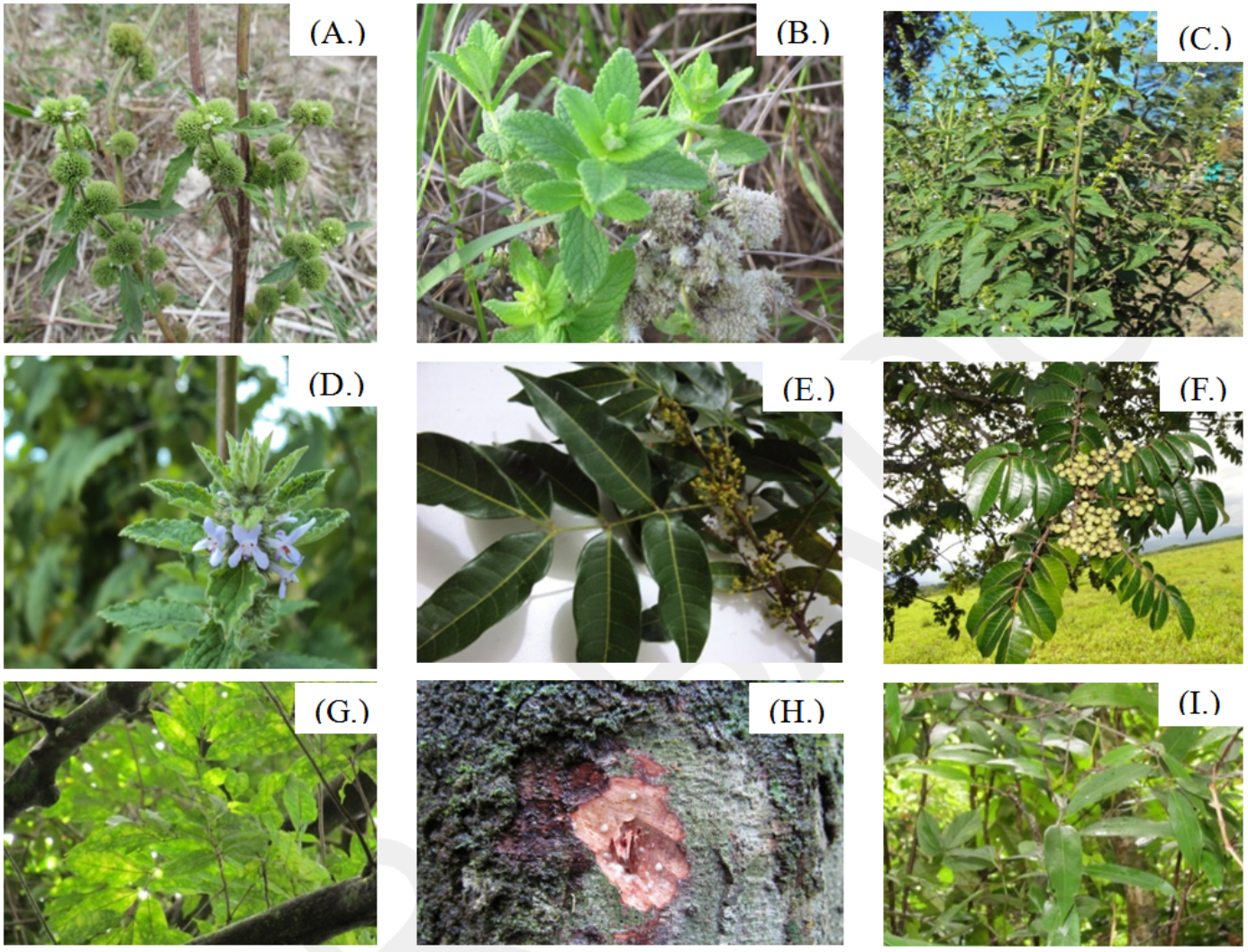

Figure 1. Photographs of *H. conferta* (A.), *H. dilatata* (B.), *H. mutabilis* (C.), *H. suaveolens* (D.), *P. heptaphyllum* (E.), *T. rhoifolia* (F.), *T. panamensis* (G. y H.), and *Ocotea* sp. (I.).

in absorbance of the ABTS solution with the reference antioxidant (trolox, vitamin E). The TAA of ethanolic extracts was estimated with reference to trolox (mmol trolox/kg extract). The ratio between the slopes of the curves evaluated for the ethanolic extract (dA vs kg/L), and trolox (dA vs mM) were used to estimate TEAC or TAA.

The assay for DPPH radical was performed according to the procedure described by Brand-Williams *et al. (17)*. EC_{50} values (Equivalent Concentration of antioxidant that reduces the concentration of the DPPH radical by 50%) were obtained using this test. A calibration curve was prepared with DPPH standard solutions in ethanol (514 nm). The steady state (time when the DPPH concentration or the absorbance stops decreasing) was measured after the addition of 0.5 mL of ethanolic extract to 2.5 mL of the DPPH standard solution. The steady state of the DPPH standard solution was evaluated for five solutions of ethanolic extracts of each plant material. Graphs of DPPH remaining (%) vs EC (Effective Concentration: kg extract/mol initial DPPH) were constructed based on the data obtained for each ethanolic extract (DPPH absorbance vs time), and plant material. The EC_{50} values were interpolated from these graphs.

Total Phenolic Content (TPC)

The TPC was calculated as gallic acid equivalents (g GA/g extract) according to the procedure described by Dastmalchi *et al.* (*18*). An aliquot of ethanolic extract (1 mL) was transferred to a test tube containing distilled water (6 mL). The Folin-Ciocalteu reagent (500 µL) was added to the test tube. After 5 min, Na_2CO_3 (1.5 mL, 200 g/L) and water were added to a final volume of 10 mL. When the reaction was complete (2 h at room temperature), the absorbance at 760 nm was determined and compared to a GA calibration curve.

Results and discussion

The yields, antioxidant capacities (EC_{50} and TAA), and TPC evaluated for the ethanolic extracts of Lamiaceae, Burseraceae, and Lauraceae are shown in Table 1. Higher yields were obtained for Lamiaceae and Burseraceae than those for Lauraceae. For Lamiaceae, *H. conferta*, and *H. dilatata*, the yields were higher than those obtained for *H. mutabilis* and *H. suaveolens*.

The results shown in Table 1 indicate that the barks of *T. rhoifolia* and *T. panamensis* (Burseraceae), had the highest antioxidant capacities (TAA), followed by the *Hyptis* spp., *H. dilatata* and *H. conferta*. *P. heptaphyllum*, *Ocotea* sp., *H. mutabilis*, and *H. suaveolens* exhibited the lowest values of TAA. The Burseraceae spp. also had the highest TPC, whereas the Lamiaceae had the lowest. Burseraceae exhibited the lowest EC_{50} values and Lamiaceae exhibited the highest.

For the ethanolic extracts of the *Hyptis* spp., the *H. dilatata* and *H. conferta* demonstrated the best antioxidant capacities. Their TAA values were the highest, which is related to the largest TPC and the lowest EC_{50} values. Regarding the Burseraceae, the barks of *T. rhoifolia* and *T. panamensis* exhibited the best antioxidant capacities compared to the leaves of *P. heptaphyllum*. The bark of *T. panamensis* possessed the highest antioxidant capacity and had the highest values for TAA and TPC, and the lowest EC_{50} value.

The highest antioxidant activities and TPC belonged to the barks of *T, rhoifolia* and *T. panamensis*. These values can be attributed to the large number of tannins (hydrolyzable and condensates) present in wood tissues *(19,20)*. Tannins have demonstrated considerable antimicrobial and antioxidant capacities (*19,20)*. The leaves of *P. heptaphyllum* contained a moderate level of antioxidant activity and TPC, lower than *T, rhoifolia* and *T. panamensis*. The promising antioxidant activities of *T, rhoifolia*, *T. panamensis*, and *P. heptaphyllum* may be attributed to the high content of triterpenoids in the Burseraceae spp. *(21-23)*. The pentacyclic triterpenes have demonstrated antitumor, antiinflammatory and antioxidant potential *(24)*.

Resins, essential oils (EO), and extracts of leaves, barks and stems of the *Protium* species have been evaluated to assess their pharmacological potential *(25,26)*. They have shown antiinflammatory, antinociceptive, analgesic, expectorant, antitumor, repellent, acaricide, and antimalarial activities, and may be gastric and hepatic protectors *(25,26)*. Biological activities for *T. panamensis* and *T. rhoifolia* were not previously reported. For Burseraceae, this study suggests that the barks of *T rhoifolia* and *T. panamensis* possess promising bio-activity compared to the leaves of *P. heptaphyllum*.

The antioxidant activities of the *Hyptis* spp., *H. dilatata* and *H. conferta*, may be attributed to the content of sesquiterpenoids and/or diterpenoids *(27,28)*. Tricyclic diterpenes have been reported in *H. dilatata (29)*. Less antioxidant activity was found for *H. mutabilis* and *H. suaveolens.* The EO of *H. mutabilis* may consist of a majority of sesquiterpenes *(27)*. Triterpenoids have also been detected for this species *(30)*. Volatile oils, starches, proteins, tannins, saponins, fats, alkaloids and glycosides have been reported for *H. suaveolens (31)*. Additionally, abietane type endoperoxides, diterpenes, and pentacyclic triterpenes have been detected *(32,33)*.

Cytostatic and cytotoxic activities against tumor cell lines have been reported for *H. dilatata (34)*. No biological activity was found for *H. conferta*. Gastrointestinal, antiparasitic (malaria), and repellent activities have been reported for *H. mutabilis (5,30)*. Antihyperglycemic, insecticidal, antifungal, antiinflammatory, antibacterial, antinociceptive, antiplasmodial and antidermatitis activities have been reported for *H. suaveolens* (*6,32,35*).

The results obtained in this study suggest that *H. conferta* and *H. dilatata* could have promising biological activities.

Monoterpenes, sesquiterpenes, phenylpropanoids, flavonoids, lignans, and alkaloids have been detected in the *Ocotea* spp. (Lauraceae) *(12,36-41)*. Analgesic, antiinflammatory, antithrombotic, antiplaquetal, antioxidant and antimicrobial activities are reported for the *Ocotea* genus *(13-15,38,41)*.

The low values for TAA and TPC from the extract of *Ocotea* sp., indicate that the leaves of this species contain a low number of biologically active compounds such as antioxidants. They are less active than the Burseraceae and Lamiaceae that were analyzed.

Conclusions

This work determined the possible relation between antioxidant activities and the TPC, and the chemical composition of *H. conferta, H. dilatata, H. mutabilis, H. suaveolens*, *P. heptaphyllum, T. rhoifoila, T. panamensis* and *Ocotea* sp.

The barks of *T. rhoifolia* and *T. panamensis* showed the highest antioxidant capacities (high TAA and low EC_{50}), followed by *H. dilatata* and *H. conferta*.

P. heptaphyllum, *Ocotea* sp. *H. mutabilis* and *H. suaveolens*, exhibited the lowest antioxidant activities.

Burseraceae spp. also demonstrated the highest TPC, whereas the Lamiaceae (*Hyptis* spp.) exhibited the lowest TPC.

Table 1. Antioxidant capacities (TAA and EC_{50}), TPC and yields (%, g of extract/100 g of dry plant material) of the etanolic extracts of Lamiaceae, Burseraceae and Lauraceae.

Species	TAA (mmol trolox/kg extract)		TPC (g GA/g extract)		EC_{50} (kg extract/µmol DPPH)		Yield (%)
	X*	VC**	X*	VC**	X*	VC**	
H. conferta	721 ± 27	4	0.107 ± 0.009	8	$4.50 \times 10^{-7} \pm 4 \times 10^{-8}$	10	5.3
H. dilatata	903 ± 64	7	0.071 ± 0.002	3	$4.60 \times 10^{-7} \pm 3 \times 10^{-8}$	7	9.6
H. mutabilis	227 ± 11	5	0.012 ± 0.0007	6	$5.70 \times 10^{-7} \pm 2 \times 10^{-8}$	8	1.8
H. suaveolens	76 ± 2	2	0.020 ± 0.002	10	$1.70 \times 10^{-6} \pm 2 \times 10^{-7}$	10	4.5
P. heptaphyllum	581 ± 69	12	0.220 ± 0.03	12	$1.80 \times 10^{-7} \pm 2 \times 10^{-9}$	1	ND
T. rhoifolia	1980 ± 369	6.1	0.270 ± 0.03	10	ND	ND	3.4
T. panamensis	2687 ± 156	2.6	0.320 ± 0.02	6	$1.20 \times 10^{-7} \pm 6 \times 10^{-9}$	5	6.4
Ocotea sp.	339 ± 7	2	0.085 ± 0.002	2	$4.80 \times 10^{-7} \pm 8 \times 10^{-8}$	16	0.7
Vitamin E	2858 ± 146	ND	ND	ND	0.27 ± 0.05	19	ND

* Average ± standard deviation ** Variance coefficient ND: Not determined.

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