BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
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Revisión / Review

Chemical composition, antioxidant, antimicrobial activity, toxicity, genetic analysis and popular use of *Eugenia luschnathiana* (O. Berg) Klotzsch ex B. D. Jacks: a literature review

[Composición química, actividad antioxidante, antimicrobiana, toxicidad, análisis genético y uso popular de *Eugenia luschnathiana* (O. Berg) Klotzsch ex B. D Jack: una revisión de la literatura]

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Section Review

Received: 20 July 2020
Accepted: 6 October 2020
Accepted corrected: 11 October 2020
Published: 30 May 2021

Citation:
Henriques MQS, Barbosa DHX, Alves DN, Melo AKV, Castro RD. Chemical composition, antioxidant, antimicrobial activity, toxicity, genetic analysis and popular use of *Eugenia luschnathiana* (O. Berg) Klotzsch ex B. D. Jacks: a literature review
Bol Latinoam Caribe Plant Med Aromat
20 (3): 215 - 225 (2021).
https://doi.org/10.37360/blacpma.21.20.3.17

Abstract: This review describes the geographical distribution, botanical data, popular use, chemical composition, pharmacological activities and genetic aspects related to *Eugenia luschnathiana*, a native Brazilian plant popularly known as "bay pitomba". *E. luschnathiana* leaves are characterized morphologically by the presence of a petiole, an attenuated base, acuminated apex, elliptical shape, and parallel venation. The major chemical compounds found in E. luschnathiana are sesquiterpenes. Literature reports showed that *E. luschnathiana* extracts have antioxidant properties and antimicrobial activity against Gram-negative and Gram-positive bacteria. The extracts from the leaf, fruit and stem, and a concentrated residual solution of its essential oil, displayed negligible toxicity. Lastly, a cytogenetic analysis indicated that some markers can be used for the study of genetic diversity, population structure, and genetic improvements. The information available on *E. luschnathiana* supports the hypothesis that this plant may be a source of compounds with promising pharmacological activity.

Keywords: *Eugenia luschnathiana*; Pharmacological effects; Plant extracts; Antimicrobial agents.

Resumen: Esta revisión describe la distribución geográfica, datos botánicos, uso popular, composición química, actividad farmacológica y el análisis genético de *Eugenia luschnathiana*, una planta originaria del Brasil conocida popularmente como "pitomba da baía". Las hojas de *E. luschnathiana* se caracterizan por la presencia de pecíolo, base atenuada, ápice acuminado, forma elíptica y venación paralela. Su composición química presenta mayormente sesquiterpenos. Los informes en la literatura muestran que los extractos de *E. luschnathiana* presentan propiedades antioxidantes y actividad antimicrobiana contra las bacterias Gram-negativas y Gram-positivas. Los extractos de la hoja, fruto y tallo, y una solución residual concentrada del aceite esencial, presentaron baja toxicidad. Por último, un análisis citogenético indicó que algunos marcadores pueden utilizarse para estudios de diversidad genética, estructura poblacional y mejoramiento genético. Las informaciones disponibles acerca de *E. luschnathiana* proponen la hipótesis de que esta planta puede ser una fuente de compuestos con actividad farmacológica prometedora.

Palabras clave: *Eugenia luschnathiana*; Efectos farmacológicos; Extractos vegetales; Agentes antiinfecciosos

INTRODUCTION

Due to its various pharmacological activities and phytochemical profile, the Myrtaceae family, found especially in subtropical and tropical regions, representing about 140 genera and 5800 species is widely described and studied (Stefanello *et al*., 2011; Sobeh *et al*., 2019). Myrtaceae belongs to the division Magnoliophyta, the class Magnoliopsida, and the order Myrtales (Morais *et al*., 2014). Plants of this family are widely found in Australia and Asia, and in the Atlantic rainforests of the Americas (Morais *et al*., 2014). They are widely investigated in natural product research, and their use as a functional food with biological activity has been well documented (Freitas *et al*., 2016; de Souza *et al*., 2018;). Previous studies reported that *Eugenia calycina* leaf and bark extracts have antimicrobial activity (antifungal and antibacterial) and low cytotoxicity (Ferreira *et al*., 2014; Sousa *et al*., 2015).

The genus Eugenia is composed of about 1115 species which correspond to one third of the family Myrtaceae (Morais *et al*., 2014), among which is *Eugenia luschnathiana* (O. Berg) Klotzsch ex BD. Jacks, which is known in popular community as "bay pitomba". The genus is chiefly represented by *E. axillaris*, *E. beaurepaireana*, *E. brasiliensis*, *E. dysenterica*, *E. umbeliflora*, and *E. uniflora* L., which are recognized for their pharmacological antioxidant, antibacterial, antidiarrheal, antifungal, cytotoxic, anti-inflammatory, and antipyretic activities (Magina *et al*., 2009; Galheigo *et al*., 2016; Dos Santos *et al*., 2018; Hegazi *et al*., 2019).

Eugenia luschnathiana appears in several states in northeastern Brazil. It usually reaches about 2 to 5 meters in height, presenting white buds, and white or green flowers with yellowish globose-shaped fruits (de Lucena *et al*., 2014; Do Brasil, 2020)

Extracts obtained from different species of the genus Eugenia were evaluated for their phytochemical properties, antioxidant activity, cytotoxicity, and antimicrobial activity. Information concerning biological properties is also found in ethnopharmacological studies (Lemos & Araujo, 2015; Guerra *et al*., 2016). However, studies on the species *E. luschnathiana* are scarce, and therefore the aim of the present study was to develop a review of the literature regarding its geographic distribution, botanical properties, popular use, chemical composition, antioxidant activity, antimicrobial activity, cytotoxicity, and a genetic analysis.

The review was performed between April and June 2020, with published works on *Eugenia luschnathiana* from consultation in several academic databases, including the National Library of Medicine - PubMed (**https://pubmed.ncbi.nlm.nih.gov**), Scopus (**https://www.scopus.com**), Web of Science (**www.webofknowledge.com**), Virtual Health Library – Bireme **https://bvsalud.org**), Chemical Abstracts Service (CAS) - SciFinder (**https://scifinder.cas.org**), and the Scientific Electronic Library Online - Scielo (**https://scielo.org**) using the descriptor "*Eugenia luschnathiana*". The bibliographic searches were conducted independently by four researchers, with no restrictions on language or year of publication. A total of 13 articles were found that will be described in detail.

BOTANICAL ASPECTS, GEOGRAPHICAL DISTRIBUTION, AND POPULAR USE

Botanical aspects

Eugenia luschnathiana presents ellipticall leaves, with an attenuated base, acuminated apices, leathery texture, and revolute margins; it presents indeterminate inflorescence, with persistent bracts and bract flowers (Do Brasil, 2020). The plant contains unicellular trichomes, a uniseriate epidermis, a concave-convex shape in the cross-section of the medial region of the vein, and secretory cavities located close to the collenchyma and druses (Donato & Morretes, 2009; Esposito-Polesi *et al*., 2011; Lemos *et al*., 2018).

E. luschnathiana species are native to Brazil and commonly occur as trees or shrubs. Its leaves have characteristics that distinguish it from other species of the Eugenia genus, such as the distribution pattern of primary ribs; midvein sulcate on adaxial lamina surface; raceme ending in a vegetative stem; a pyloric disc; hairy stylus; and the presence of globose and subglobose fruits (Amorim & Alves, 2012). The leaves have a great capacity for ecological adaptation, which can be verified through the presence of stomata, in addition to the great thickness of the cuticle on the adaxial face and several trichomes on the abaxial face (Esposito-Polesi *et al*., 2011). Another relevant characteristic refers to the great thickness of the leaf blade and palisade parenchyma, in addition to a spongy parenchyma with the presence of druses (Lemos *et al*., 2018).

It is an undemanding species in terms of water consumption, which can be evidenced by the presence of a thick cuticle and hypostomatic leaf, composed of several trichomes on the abaxial surface and fibers in the region of the vascular bundles, in addition to epidermal secretion cells arranged in rays (Lemos *et al*., 2018). It may also present paracytic stomatal cells (Donato & Morretes, 2009) or anomocytic cells (Defaveri *et al*., 2011). The leaf edge is quite versatile, ranging from straight (in the sun), to bent towards the abaxial face (in the shade) (Donato & Morretes, 2009; Lemos *et al*., 2018).

Figure No. 1
***E. luschnathiana* leaves present petioles, attenuated base, acuminated apex, elliptical shape, and parallel lineal venation.**

Geographic distribution of E. luschnathiana

Present in the Atlantic Forest and the Caatinga, *E. luschnathiana* is an endemic Brazilian species, yet usually located in the coastal plains. Its geographical distribution (Northeastern Brazil) occurs in the states of Bahia, Ceará, Rio Grande do Norte, Paraíba, and Pernambuco. In addition, occurrences are expected in the states of Espírito Santo, Minas Gerais, São Paulo and Paraná (Silva, 2009; Amorim & Alves, 2012; Leitão *et al*., 2014; Lemos *et al*., 2018; Do Brasil, 2020).

Popular usage of E. luschnathiana

The popular use of *E. luschnathiana* was reported by (Guerra *et al*., 2016) in a study conducted with 250 inhabitants of the rural community settlement *Reforma Agrária Sítio Novo*, located in the State of Bahia, Brazil. Tea from the leaves was singularly used with an anti-thermal therapeutic indication, and presenting a Relative Importance (RI) value of 0.29. The Relative Importance Index is obtained from the number of indications and the number of diseases that a plant can treat, and used to verify the usefulness of medicinal plants The maximum value obtainable is RI = 2 (Bennett & Prance, 2000). The greater the number of indications for the plant, the greater its RI, suggesting that the low *E. luschnathiana* RI value presented in the cited Guerra study was related to its low consumption as a medicinal plant, and to the absence of studies regarding its chemical composition and pharmacological effects. Studies with species from the Myrtaceae family have shown higher RI values, such as *Psidium guajava* L and *Eucalyptus globulus* Labill (Lemos & Araujo, 2015; Guerra *et al*., 2016) reflecting their greater consumption as medicinal plants as compared to *E. luschnathiana*.

The Importance Value Index (IVI) is used to characterize the relevance of a species in a community, being the sum of the abundance, frequency, and relative dominance of the species (Curtis & McIntosh, 1951). (Castro *et al*., 2012) performed a floristic survey and phytosociological studies of 382 plant species (including *E. luschnathiana*) collected in a district of the municipality of *São Gonçalo do Amarante*, located in the coastal region of Ceará, Brazil. In the phytosociological study, the authors observed a low IVI value of 0.61 for *E. luschnathiana*, which may be a result of the species' scarcity in the area at the time the collection was made. Yet the ecological value of *E. luschnathiana* continues due to its ability to attract pollinators, seed dispersers, and herbivores (Lemos *et al*., 2018).

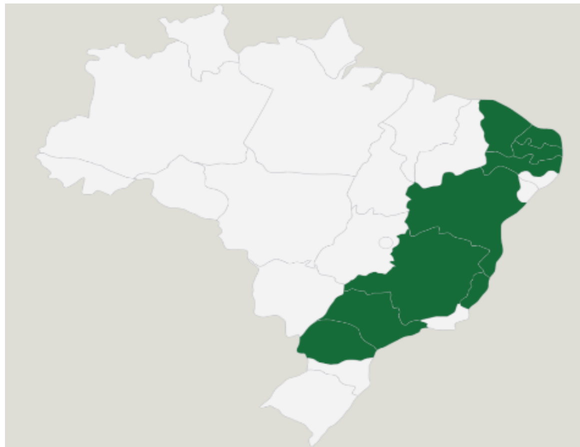


Figure No. 2
The geographic distribution of *E. luschnathiana* in the Brazilian territory

PHYTOCHEMISTRY

Chemical composition of essential oil and extracts of E. luschnathiana

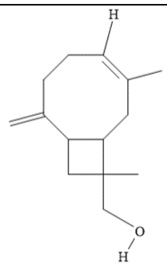
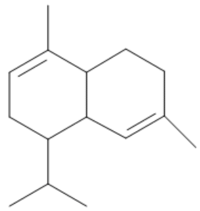
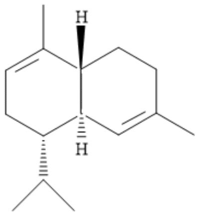
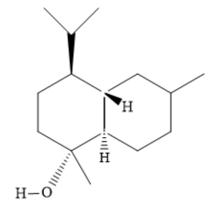
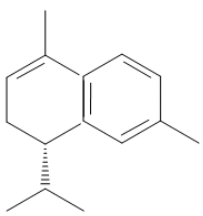
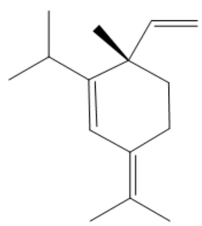
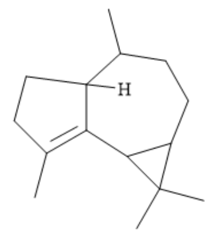
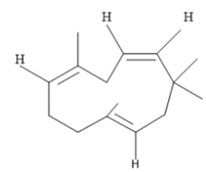
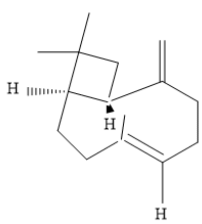
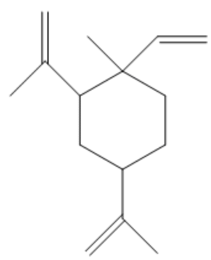
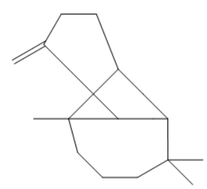
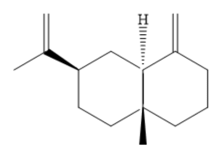
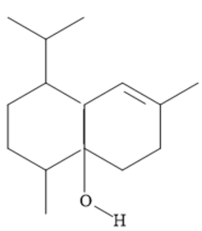
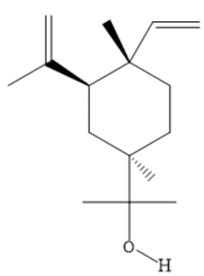
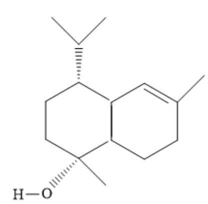
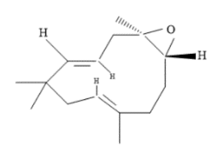
Essential oils obtained from Eugenia species present sesquiterpenes such as β-Karyophylene in their composition (de Souza *et al*., 2018). As shown in Tables No. 1 and No. 2, there is a similarity between the phytochemical profiles of essential oil obtained from *E. luschnathiana* leaves and those extracted from other species belonging to the same family. Studies analyzing the chemical composition of *E. luschnathiana* leaf essential oil confirm the presence of the majority of sesquiterpenes, as well as esters, alcohols, and other terpenes (Monteiro *et al*., 2016; Araújo, 2018).

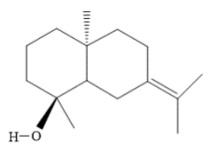
Chemical similarity between species suggests similar pharmacological activity. Certain species present chemical similarity to *E. luschnathiana* as well as presenting known pharmacological activities: Anti-inflammatory (*E. beaurepaireana, E. brasiliensis,* and *E. dysenterica*); Antidiarrheal (*E. axillaris, E. beaurepaireana*, and *E. dysenterica*); Antimicrobial (*E. axillaris, E. bacopari, E. beaurepaireana, E. brasiliensis, E. calycina, E. chlorophyla, E. dysenterica,* and *E. uruguayensis*) (de Souza *et al*., 2018).

The chemical composition of *E. luschnathiana* leaf hydroethanolic extract reveals a phytochemical profile similar to those of other species of the Eugenia genus, such as: *Eugenia involucrata, Eugenia brasiliensis, Eugenia pyriformis, Eugenia stipata,* and *Eugenia victoriana*. The similarity between chemical components, such as: quinic acid, malic acid, gallic acid, ellagic acid, myricetim-O-rhamnoside, and arjunolic acid, can be verified by UPLC-qTOF / MS-MS. These constituents may thus be considered as possible chemical markers for the Eugenia genus (Santos *et al*., 2020).

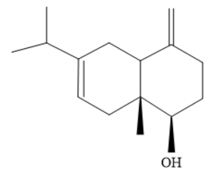
The *E. luschnathiana* leaf extract was also submitted to saponification, and it was possible to identify through Gas Chromatography coupled to mass spectrometry (GC-MS), certain unsaponifiable compounds in decreasing percentages: Estra-1,3,5 (10)-trien-17α-ol, Espatulenol, Clovan-2,9-diol, Hexadecanoic acid, 4,8,12,16-tetramethylheptadecan-4-olide, Tetracycle [6.3.2.0 (2.5).0(1, 8)] tridecan-9-ol4,4, -dimethyl, Calacorene epoxide, Naphthalene, 1,6-dimethyl-4 (1-methyl-ethyl), Cariophylene oxide, aromadendrene oxide, and Cubenol. The large presence of sesquiterpene compounds, such as Spathulenol, Clovane-2,9- diol, Caryophyllene oxide, and Aromatic oil oxide is again emphasized, an important characteristic frequently seen in the phytochemical profiles of other species of the Eugenia genus (Araújo, 2018).

Table No. 1
Chemical constituents found in common for *E. luschnathiana* leaf essential oils from 2 studies (Monteiro, et al., 2016; Araújo, 2018).

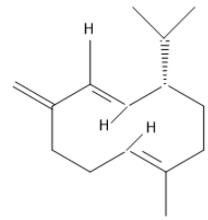
			
14-hydroxy-9-epi-(E)-cariophylene	α-Amorphene	δ-Cadinene	α-Cadinol
			
α-Calacorene	δ-Elemene	α- Gurjunene	α-Humulene
			
β- Karyophyllene	δ-Elemene	β-Longipinene	β-Selinene
			
Cubenol	Elemol	Epi-α-Muurolol	Humulene Epoxide II



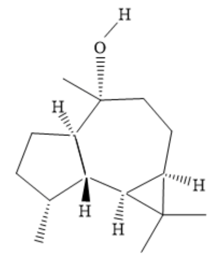
Eudesm-7(11)-en-4-ol



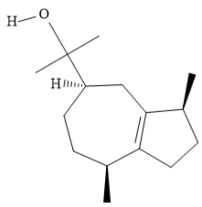
Eudesma-4(15),7-dien1β-ol



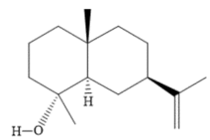
Germacrene D



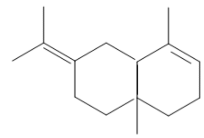
Globulol



Guaiol



Selin-11-en-4-α-ol



Selina-3,7(11)-diene

Table No. 2
Principal chemical constituents encountered in essential oils of E. luschnathiana and other species of the same family (Souza *et al*., 2018)

Species:	Major chemical constituents found in the leaf:
E. arenosa; E. klappenbachiana; E. xiririicana	Globulol
E. argentea	β-Karyophylene
E. austin-smithii; E. cartagensis; E. pitanga	Germacrene D
E. biflora; E. calycina; E. cúprea; E. involucrata; E. patrisii; E. riedeliana;Eugenia sp; E. sulcata; E. uruguayensis	β-Cariofilene
E. candolleana	β –Elemene
E. catharinensis; E. dimorpha;E. supraaxilaris	α-Humulene
E. bacopari	δ-Cadinene
E. axillaris	Germacrene D
E. zuchowskiae; E. repanda	α-Humulene
E. beaurepaireana; E. neonitida; E. stitipata	Germacrene D
E. moraviana; E. ramboi	δ-Elemeno; β-Karyophyllene
E. joensonii; E. platysema	β-Selinene
E. brasiliensis	Cubenol
E. chlorophyla	Globulol; α-Cadinol
E. dysenterica	β-Karyophyllene; δ-Cadinene
E. foetida	α-Cadinol
E. hiemalis	β-Karyophyllene; Germacrene D
E. multicostata	Globulol

E. piauhiensis	β-Karyophyllene; β-Elemene
E. protenta	Selin-11-en-4-α-ol; δ-Elemene; Germacrene D
E. punicifolia	β-Karyophyllene; α-Cadinol
E. rhombea	α-Cadinol; Cubenol
E. rocana	14-hydroxy-9-epi- (E)-cariophylene

Another study using tandem mass spectrometry, with electrospray ionization by HPLC/UV and HPLC/UV/electrospray (HPLC/UV/ESI-MS/MS), investigated the presence of bilirubin in 10 plant species (including *E. luschnathiana*) to verify its distribution in the plant kingdom and its contributions to plant color. Bilirubin was found in the fruit of *E. luschnathiana*, yet there was no association with color, unlike *P. guyanense*, in which high concentrations of bilirubin in its arils were associated with color. Thus, further study involving samples collected under varying conditions and at different times are needed to capture the role of bilirubin in *E. luschnathiana* (Pirone *et al*., 2010).

PHARMACOLOGICAL STUDY

E. luschnathiana antioxidant, antimicrobial activity, and toxicity

The antioxidant activity of *E. luschnathiana*, collected in the *Rio Grande do Norte*, a state in the Brazilian northeast, has been demonstrated by investigations involving ethanol extracts from the stems and leaves, their fractions, fruit extract, essential leaf oil, and a concentrated hydro-distillated solution residual of the essential oil using the 2,2-diphenyl-picrilhidrazil (DPPH) radical capture test. The Inhibitory Concentration (IC) values obtained for the stem, leaf, and fruit extracts were respectively IC_{50} - 65.84 µg/mL, 147.41 µg/mL and 204.65 µg/mL. For the fractions, the more nonpolar fractions (such as hexane) presented higher IC_{50} values, due to the higher fat presence in their compositions, and thus the fewer number of insaturations or electrons available for free radical stabilization. Polar fractions such as ethyl acetate and hydroalcoholic fractions presented lower IC_{50} values, and, consequently, better antioxidant activities. The concentrated hydro-distillated essential oil solution residual presented even better activity than the leaf extract, indicating that temperature and the use of different solvents in the extraction process may result in different metabolite compositions. The essential oil revealed little reducing potential against DPPH (3714.41 IC_{50}), this, due to an absence of phenolic compounds, and an abundance of sesquiterpenes in its composition (Araújo, 2018).

The antioxidant activity of *E. luschnathiana*, looking for compounds such as anthocyanins and flavonoids in the fruit extract. The extract was submitted to the DPPH capture test and presented low free radical activity (IC_{50} = 38.0 µg/mL), which might be related to the presence of flavonoids in its composition. The total anthocyanin content (TAC) remained undetected, and the total phenolic content (TPC) was 22.0 mgC3G/g dry weight. The authors concluded that because of synergism, it would not be possible to correlate the individually quantified phenolics with antioxidant activity, since it could involve not yet identified compounds (Reynertson *et al*., 2008).

Low IC_{50} values for extracts of species of the genus Eugenia, such as *Eugenia copacabanenesis* Kiaersk and *Eugenia uniflora* have been reported in the literature. Such plants are considered natural sources of antioxidants due to the high content of phenolic substances present among their components (Carvalho Junior *et al*., 2014; Sobral-Souza *et al*., 2014).

The antimicrobial activity of *E. luschnathiana* was evaluated by (Araújo, 2018). Three samples of *E. luschnathiana* essential oil (collected at different times) were used. The samples (1, 2, and 3) presented low Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) values against Gram-positive *Staphylococcus aureus* and *Staphylococcus epidermidis* and against Gram-negative *Escherichia coli.* Yet, for Gram-negative *Pseudomonas aeruginosa*, sample 2 presented no antimicrobial activity. Of the three, sample 1 presented the best activity, which may well be related to the constituent spatulenol present in its composition, whose antimicrobial activity has already been reported in the literature (do Nascimento *et al*., 2018). Species such as *E. calycina, E. pyriformis, E. umbelliflora, E. uniflora*, and *E. uruguayensis* have also demonstrated satisfactory inhibitory potential against Gram-negative and Gram-positive bacteria, fungi, and yeasts. The antimicrobial activity of these species is attributed to bioactive compounds present in their constitutions (de Souza *et al*., 2018).

The toxicity of *E. luschnathiana* against the microcrustacean *Artemia salina* Leach was investigated. Extracts from the leaves, fruits, and stems, and concentrated residual solution from essential oil hydro-distillation were found to be non-toxic. However, three *E. luschnathiana* essential oil samples (collected at different times of the year) proved to be highly toxic to *A. salina*. Such results are preliminary and need further investigation (Araújo, 2018).

GENETICS

Cytogenetic analysis of E. luschnathiana and transferability of microsatellite markers (SRR)

(Pedrosa *et al*., 1999) performed cytogenetic analysis of *E. luschnathiana* MYR-411 collected in the State of Pernambuco, Brazil. In his study, nuclei were observed varying between semi-reticulated and arreticulate. The chromosome number of *E. luschnathiana* was primarily described presenting a value of 2n = 22, according to the basic chromosome number n = 11, proposed for genera of the family Myrtaceae (Costa, 2004), and can be considered a strong synapomorphy of the Myrteae tribe of which the genus Eugenia is part of. however, (Costa, 2004) verified polyploidy in 20% of Myrteae tribe species, which may be related to adaptive and evolutionary mechanisms in these plants.

(Bernardes *et al*., 2018) analyzed the transferability of molecular microsatellites (SRR) from *P. guajava* to *E. luschnathiana* using polymerase chain reaction (PCR) techniques. A low rate of 33% for transferability of microsatellites was verified, yet the transferability rate was higher for other species of the genus Eugenia. This result is in line with (Nogueira *et al*., 2016), in which of 152 SRRs of *P. guajava* tested, 49 were transferred to *E. luschnathiana*. The transferability of microsatellites found between species of the same family indicates genomic conservation between them through high degrees of polymorphism, codominance, and reproducibility (Ellegren, 2004; Parida *et al*., 2009; Kalia *et al*., 2011). With species of different genera, low transferability rates are expected (Barbara et al., 2007), since there are more taxonomic differences between species of different genera as compared to species of the same genus. However, as a result of high microsatellite reproducibility and the high cost of PCR testing to produce specific microsatellites for each species, these studies are important for future conservation and genetic improvement programs, in addition to precise investigations into genetic diversity and population structure (Ferreira-Ramos *et al*., 2014).

Table No. 3
List of published studies on *E. luschnathiana* included in the research

SUBJECT MATTER		AUTHOR AND YEAR OF PUBLICATION
GEOGRAPHICAL AND BOTANICAL DISTRIBUTION		Amorim & Alves, 2012.
		Araújo, 2018.
		Leitão *et al.*, 2014.
		Lemos *et al.*, 2018.
		Monteiro *et al.*, 2016.
		Pirone *et al.*, 2010.
		Santos *et al.*, 2020.
PHARMACOLOGICAL STUDY	Antioxidant activity	Araújo, 2018.
		Reynertson *et al.*, 2008.
	Antimicrobial activity	Araújo, 2018.

	Toxicity	Araújo, 2018.
GENETICS	Cytogenetics	Pedrosa *et al.*, 1999.
	Microsatellite Transferability (SRR)	Bernardes *et al.*, 2018.
		Nogueira *et al.*, 2016.
POPULAR MEDICINE	Importance Value to the Population	Castro *et al.*, 2012.
	Popular use	Guerra *et al.*, 2016.

CONCLUSIONS

In view of the above it, and due to its wide availability and potential for use in the food industry, *Eugenia luschnathiana*, an endemic Brazilian plant, presents great economic potential. Extracts obtained from the species present antioxidant and antimicrobial activities with evidence of variable toxicity. The leaves present a high percentage of sesquiterpenes and are easily recognized through their botanical characteristics. Further studies with *Eugenia luschnathiana* are needed to increase our knowledge concerning its pharmacological and toxicological properties.

ACKNOWLEDGMENTS

This study was financed in party by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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