Revista Brasileira de Farmacognosia xxx (2017) xxx–xxx

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original article

Anatomy and volatile oil chemistry of *Eucalyptus saligna* cultivated in South Brazil

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ARTICLE INFO

Article history:
Received 26 January 2018
Accepted 6 March 2018
Available online xxx

Keywords:
Anatomy
Histochemistry
Essential oil
Eucalipto
Microscopy

ABSTRACT

Eucalyptus saligna Sm., Myrtaceae, commonly known as Sydney blue gum, is often confused with several other species in the genus. The leaf volatile oils of the species have been reported to have antimicrobial, insecticidal, nematicidal, repellent and cytotoxicity properties. The present work provides anatomy as well as volatile oil chemistry of the species collected from South Brazil. The anatomy and histochemistry of the leaves and stems were investigated by light and scanning electron microscopy, and the leaf and stem volatile oils were analyzed by GC–MS. Amphistomatic leaves, anomocytic stomata, presence of papillae and epicuticular waxes, slightly biconvex midrib with a bicollateral vascular bundle in open arc and two dorsal traces, secretory cavities, calcium oxalate druses and prismatic crystals, rounded petiole with a bicollateral vascular bundle in open arc with invaginated ends and rounded stem with sclerenchyma abutting the internal and external phloem are observed in this species. The main components of the volatile oil were *p*-cymene (28.90%) and cryptone (17.92%). These characteristics can help in the identification and quality control of *E. saligna*.

Introduction

Eucalyptus L'Hér. is one of the principal genera of Myrtaceae. The genus comprises more than 800 species, most are native to Australia (Flores et al., 2016) and many of them are cultivated elsewhere. Species of *Eucalyptus* are used in the production of paper, timber, honey, and volatile oils, which have extensive use in pharmaceutical and perfumery industries (Brooker and Kleinig, 2006; Flores et al., 2016; Barbosa et al., 2016).

Eucalyptus saligna Sm., Myrtaceae, commonly known as "Sydney blue gum" (Ritter, 2014), is a large tree, with a rough and persistent bark. The leaves are petiolate, about 9–19 cm long and 1.8–3.5 cm wide. They are lanceolate or falcate in shape, with acuminate apex, acute to attenuate base (commonly asymmetric), entire margin and prominent reticulate veins (Flores et al., 2016). Several species of *Eucalyptus* are morphologically similar and called by the same common name "eucalipto" in Brazil, causing confusions in the identification. For example, *E. saligna* is often confused with *E. deanei* Maiden, *E. dunnii* Maiden, *E. grandis* W.Hill or *E. botryoides* Sm. (Flores et al., 2016).

Several bioactivities of the volatile oils have been reported for *E. saligna*, such as antimicrobial (Sartorelli et al., 2007; Barbosa et al., 2016), insecticidal (Brooker and Kleinig, 2006; Barbosa et al., 2016), nematicidal (Salgado et al., 2003), repellent (Tapondjou et al., 2005; Ceferino et al., 2006) and cytotoxicity activities (Bhuyan et al., 2017). The biological activities of the species are mainly due to chemical compounds present in the volatile oil (Barbosa et al., 2016). Various studies have shown qualitative and quantitative differences in the volatile oil compositions in *E. saligna* collected from different geographical regions (Barbosa et al., 2016).

Considering the morphological similarities between different species of *Eucalyptus*, and the fact that *E. saligna* shows differences in the chemical composition of volatile oils sourced from different locations, the aims of this study were to illustrate the anatomical

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https://doi.org/10.1016/j.bjp.2018.03.001

features of the leaves and stems that can facilitate correct identification of the species and to characterize the volatile oil composition of *E. saligna* collected in Paraná, South Brazil.

Materials and methods

Plant material

Samples of leaves and stems of *E. saligna* Sm., Myrtaceae, were collected from plants growing in the campus of the State University of Ponta Grossa, Ponta Grossa, Paraná, Brazil (latitude 25°09′36″ S and longitude 50°10′18″ W) in March 2016. At least six samples of mature leaves (cut from median, intercostal and margin regions) were obtained from the sixth node and below, as well as stem fragments 5–15 cm from the shoot were collected. The plant material was identified using floras (Chippendale, 1988; Boland et al., 2006; Flores et al., 2016) and a voucher specimen was stored the under the number 21836 HUPG in the Herbarium of the State University of Ponta Grossa.

Sample preparation for light microscopy

Leaf and stem samples of *E. saligna* were cut into fragments and fixed in FAA solution consisting of a mixture of 70% ethanol (90%), formaldehyde (5%) and acetic acid (5%) (Johansen, 1940) and stored in 70% ethanol/water solution (Berlyn and Miksche, 1976). Semi-permanent mounts were prepared by free-hand sectioning from the third inferior portion of the midrib of the plant material using razor blades and mounting them on glass slides in a drop of glycerin. The sections were stained using astra blue/basic fuchsine (Roeser, 1962) or toluidine blue (O'Brien et al., 1964). Kraus and Arduin (1997) methods were used to analyze epidermal features. Epicuticular wax classification was based on Barthlott et al. (1998). The preparations were analyzed and photographed using an Olympus CX31 light microscope equipped with a C-7070 digital camera.

Sample preparation for field emission scanning electron microscopy

Fixed samples of leaves and stems were gradually dehydrated by passing through a series of ethanol/water solutions with increasing concentrations of ethanol (80%, 90% and 100%), then dried in a critical point dryer. The dried samples were mounted on aluminum stubs using glued carbon tabs and then sputter coated with gold using a Quorum SC7620 (Quorum Technologies, Laughton, UK) sputter coater. The samples were analyzed and photographed using a Mira3 (Tescan, Brno-Kohoutovice, Czech Republic) field emission scanning electron microscope (FESEM). Qualitative X-ray microanalyses were performed on crystals and in cells without crystals (control) using an EDS (*Energy-dispersive X-ray spectroscopy*) detector attached to the Mira3 SEM. This procedure as well as FESEM and light microscope studies were conducted at the multi-user laboratory (C-Labmu) of the State University of Ponta Grossa, Paraná, Brazil.

Histochemical analyses

Histochemical analyses were carried out using cross-sections of fresh leaves and stems obtained by the same method used in the anatomical assay. The following standard solutions were employed for histochemical tests: Sudan III for lipophilic substances (Foster, 1949), ferric chloride 2% (Johansen, 1940) and potassium dichromate 10% (Gabe, 1968) for phenolic components, phloroglucinol/HCl to test lignin (Sass, 1951) and iodine-iodide for starch (Berlyn and Miksche, 1976). Controls were made in parallel with the tests, and semi-permanent slides were prepared as described above.

Extraction of volatile oil and GC–MS analysis

Dried plant material (300 g) was subjected to hydrodistillation for 4 h, in triplicate, using a modified Clevenger-type apparatus for the extraction of volatile oils. The resultant oils were dried using anhydrous Na_2SO_4 and stored in glass vials with Teflon-sealed caps at 4 ± 0.5 °C with no light.

Volatile oils of *E. saligna* were analyzed on a Shimadzu 2010 Plus gas chromatograph coupled with a Shimadzu TQ8040 mass selective detector and equipped with a Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 μm), operated under programmed temperature from 60 to 250 °C at 3 °C/min and an injector temperature of 250 °C, with an injection volume of 1 μl of the sample (1% (w/v) in hexane), in split mode (ratio 1:40). The interface ion source was at 300 °C, mass range of *m/z* 40–400, using helium as a carrier gas, with a flow of 1.0 ml/min, with the ionization mode: electron impact 70 eV. Quantitative analysis was performed using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector under the same conditions previously described. The relative areas were the average of triplicate analysis.

Experimental retention indices (RI) were calculated by injection of *n*-alkane series standard from nine to twenty carbon atoms and volatile oil samples under the same conditions. The identification of the components was based on the comparison of the RI, and mass spectra of each substance with spectra from the NIST02 library and with literature data (Adams, 2007). The identification of the main compound of volatile oil was confirmed using the standard of *p*-cymene. This analysis was carried out at the Federal University of Paraná. The results are shown in Table 1.

Results and discussion

Anatomical studies

In *E. saligna*, leaf (Fig. 1a) the adaxial and abaxial epidermises show straight anticlinal walls (Fig. 1b and c) and are covered externally by smooth cuticle (Fig. 1g). Anomocytic stomata are present on both adaxial (Fig. 1b) and abaxial (Fig. 1c) epidermises, characterizing the leaf as amphistomatic. The average size of stomata

Table 1
Chemical compounds in the volatile oil of *Eucalyptus saligna*.

Compound	RI cal.	RI lit.	Peak area (%)	Identification
α-Thujene	927	924	0.94	RI, MS
α-Pinene	933	932	1.04	RI, MS
α-Phellandrene	1006	1002	1.04	RI, MS
α-Terpinene	1017	1014	0.57	RI, MS
p-Cymene	1025	1020	28.90	RI, MS
Sylvestrene	1029	1025	4.89	RI, MS
1,8-Cineole	1032	1026	2.15	RI, MS
p-Cymenene	1091	1089	0.80	RI, MS
Sabina ketone	1152	1154	2.25	RI, MS
Terpinen-4-ol	1179	1174	5.33	RI, MS
Cryptone	1188	1183	17.22	RI, MS
α-Terpineol	1193	1186	0.73	RI, MS
Cuminaldehyde	1242	1238	5.25	RI, MS
trans-Ascaridol glycol	1277	1266	7.32	RI, MS
Thymol	1305	1289	0.89	RI, MS
Spathulenol	1582	1577	4.84	RI, MS
Compounds identified			84.16	RI, MS
Monoterpene hydrocarbons			38.18	RI, MS
Oxigenated monoterpenes			41.14	RI, MS
Sesquiterpenes			4.84	RI, MS

RI lit., retention index literature from Adams (2007); RI calc, calculated retention index; MS, mass spectra from NIST02 library.

Please cite this article in press as: Saulle, C.C., et al. Anatomy and volatile oil chemistry of *Eucalyptus saligna* cultivated in South Brazil. Revista Brasileira de Farmacognosia (2017), https://doi.org/10.1016/j.bjp.2018.03.001