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Botanic Aspects Of The Leaves And Chemical Constituents In The Essential Oil From Two Varieties Of Basil (Ocimum spp) Grown In Brazil.

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ABSTRACT

The screening of the biological activity aromatic plants used in folk medicine have been investigated for their possible therapeutic benefits, especially their essential oils and active compounds, being promising new sources of medicinal drugs. The leaves of two varieties of live specimens of *Ocimum* L., *O. basilicum var. basilicum* L. and *var. thyrsiflora* were botanical analyzed, essential oil extract by hydrodistillation, and constituents determined by GC-MS. The results of the GC-MS analysis for the *O. basilicum var. Basilicum* showed a predominance of linalool, α-muurolol, eugenol, 4-terpineol, and eucalyptol, as major constituents, whereas for the variety *var. thyrsiflora*, linalool, eugenol, α-muurolol, 4-terpinene, and germacrene D were found as majority compound.

Keywords: Basil, *Ocimum basilicum* L., Essential Oil, Natural Products

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INTRODUCTION

The Lamiaceae (Labiatae) family is one of the most important families, with innumerable plant species. Basil (*Ocimum basilicum* L.) is an aromatic plant belonging to this family, and widely used in cooking tradition in the mediterranean área and also in tradicional medicine due for its beneficial health properties [1-3]. The genus *Ocimum* L. consists of 70 species and subspecies, found and cultivated in Mediterranean areas and semitropical and tropical regions of America, Asia, and Africa [4, 5].

Essential oils usually have a complex and wide variation in their chemical composition, which include terpenes (monoterpenes and sesquiterpenes), terpenoids (isoprenoids), and aliphatic and aromatic compounds such as aldehydes and phenols [1, 6]. This chemical variation is due to many factors, such as geographical origins, nutritional status, genetic factors, weather conditions, cultivation process, harvest time and extraction method, plant species, and parts used [1, 6].

The classification of plants of the genus of basil is complex, due to natural and artificial crossings, which resulted in a great genetic variation, morphological aspects and chemical composition of the plant and its essential oils [1, 6]. The essential oils from the plants of the genus *Ocimum* ssp have several biologically active constituents, and the chemical composition of the oil can vary greatly, influencing on the pharmacological activities, e.g., antioxidant, antiviral, antimicrobial, anti-inflammatory, analgesic, and diuretic, according to its major constituents [1, 7-9]. The Basil leaves contain essential oil at a percentage of 0.2- 1%, exhibiting a wide variety of chemical compounds, rich in monoterpenoids, sesquiterpenoids, and flavonoids, and the main components being linalool, and other compounds are also frequent such as 1,8-cineole, estragole (methyl chavicol), o-cymene, citral, alpha-pinene, camphene, beta-pinene, geraniol, geranial, eugenol and methyl eugenol is linalool [1, 7, 9].

MATERIAL AND METHODS

Procedures in bothanical analysis

Two varieties of live specimens of *Ocimum* L., *O. basilicum var. basilicum* L. and *var. thyrsiflora* were cultivated at Maringá State University (UEM). The vouchers were deposited in the Herbarium of the Maringá State University (HUEM).

Third node leaf plants were collected for each treatment, fixed in FAA 70 (50ml formaldehyde 37%, 50 mL ethanol 70% and 900 mL glacial acetic acid) and stored in 70% ethanol (Johansen 1940). For anatomical studies, the leaves were embedded in Leica™ historesin following the manufacturer's instructions, sectioned in serial cross sections using a 6 µm-thick rotating microtome, and stained with Toluidine blue in acetate buffer (pH 4.7). Slides were mounted in Entellan® mounting medium.

Histochemical test were performed with Sudan IV to identify lipid content in freehand cross-sections of fresh leaves.

Histological analyses were conducted with a Leica ICC50 microscope coupled with a digital camera and processed using the Leica Application Suite software, version 1.8. Boards were assembled in the Corel DRAW X8 using photomicrographs with the respective scales (obtained with a micrometer blade in the same optical conditions employed for each case).

Extraction and CG-MS analyses of the Essential Oils

The fresh leaves of two varieties of *O. basilicum var. basilicum* L. and *var. thyrsiflora* were used for essential oil extraction by hydrodistillation using a Clevenger-type apparatus. Approximately 556 g of the leaves were subjected to steam distillation for 2 h. The oil was dried over sodium sulfate and stored in an amber flask at 4 °C.

The essential oils were analyzed by GC-MS using a gas chromatography Shimadzu QP2010 couple to mass spectrometer with electron ionization (EI) source, which was applied an ionization energy of 70 eV. The chromatographic column was a Rtx-5MS (30 m x 0.25 mm, 0.25 mm in thickness) using He as carrier gas (pressure 79.7 kPa and column flow rate 1.30 mL/min). The split ratio was 1:40 and the temperature programming was 60 to 220˚C increasing by 3˚C/min. WILEY 7, NIST 11 and FFNSC data

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banks were used to identify the constituents and their retention index (calculated by the injection of a series of alkanes C9 to C22) were compared with the reported by Adams [10].

RESULTS AND DISCUSSION

The basil varieties studied showed some different morphological traits and essential oil components. In both species the leaves are dorsiventral and amphistomatic (Fig. 1 and 2). In the leaf mesophyll, the palisade parenchyma is made up of a single cell layer and the spongy parenchyma ranging from 4-5 cells layers in *O. Basilicum var. basilicum* (Fig. 1A-B, DE, GH) and 3-4 in *O. basilicum var. thyrsiflora* (Fig. 2A-B, E, GH). The height of the palisade parenchyma cells in *O. Basilicum var. basilicum* (Fig. 1A-B, D-E, G-H) is remarkably greater than in *O. basilicum var. thyrsiflora* (Fig. 2A-B, E, G-H). In this species, the consistency of the leaf is more delicate and the blade is thinner. The vascular tissue of the midrib consists of an arc-shaped collateral bundle (Fig. 1A, D, G, 2A, G).

In the epidermis, uniseriate, non-branched, multicellular non-glandular trichomes with a wider basal cell and tapered ends are observed (Fig. 2G). Major and minor peltate glandular trichomes, with a short unicellular peduncle, are embedded in depressions in the epidermis on the adaxial and abaxial sides. These trichomes secrete lipophilic material (Fig. 1B, E, I, 2B-C, E-F, I). Minor peltate secretory trichomes were observed in *O. Basilicum var. basilicum,* only (Fig. 1I). Secretory trichomes elevated above the level of the epidermis or occurring in minor depressions of the epidermis are also seen. They have a short peduncle and a unicellular globoid head, both of which are unicellular (Fig. 1F-H, 2G).

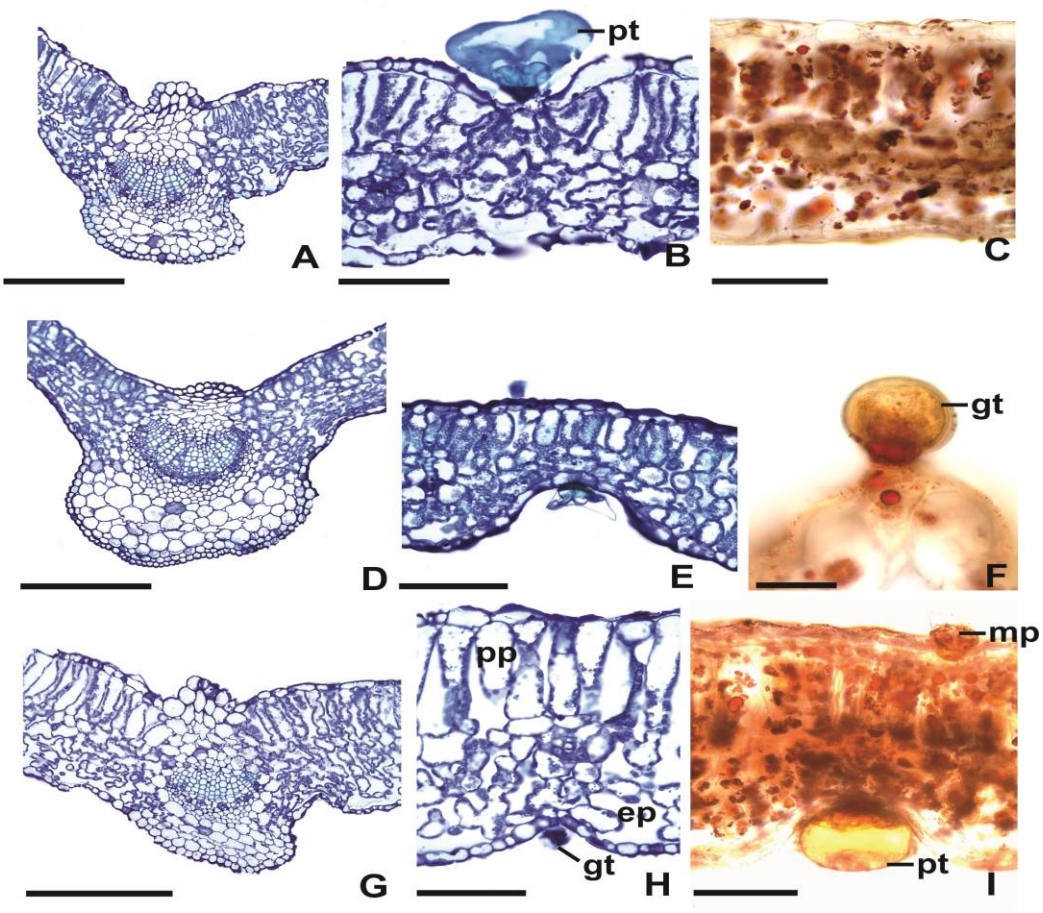

Figure 1 – Cross sections of *O. Basilicum var. basilicum* leaves cultivated under different water conditions. A-C, F. Stress from lack of water (50% less water). D-E. In field capacity (100% field capacity). G-H- Excess water stress (50% more water). Note in C, F and I drops of lipophilic material on the Sudan IV stained sections. (ep – spongy parenchyma, gt – globoid glandular trichome, mp – peltate minor glandular trichome, pp – palisade parenchyma, pt – peltate major glandular trichome). Scales: A, D and G = 300 µm, B-C, E, H-I = 100 µm, F = 50 µm.

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Qualitative analysis of the leaves of both species reveals that the anatomical changes are more visible in *O. basilicum var. basilicum*, a species in which an increase in the height of the palisade parenchyma cells is observed both in the treatment with 50% less water in the soil and in the treatment with 50% percent more watering compared to control with soil in field capacity (Fig. 1A-B, DE, GH). In the spongy parenchyma there is a clear change in the shape of the cells, which are isodiametric in 50 and 100% of water (Fig. 1B, E) and more lobed and with wider intercellular spaces in 150% (Fig. 1H).

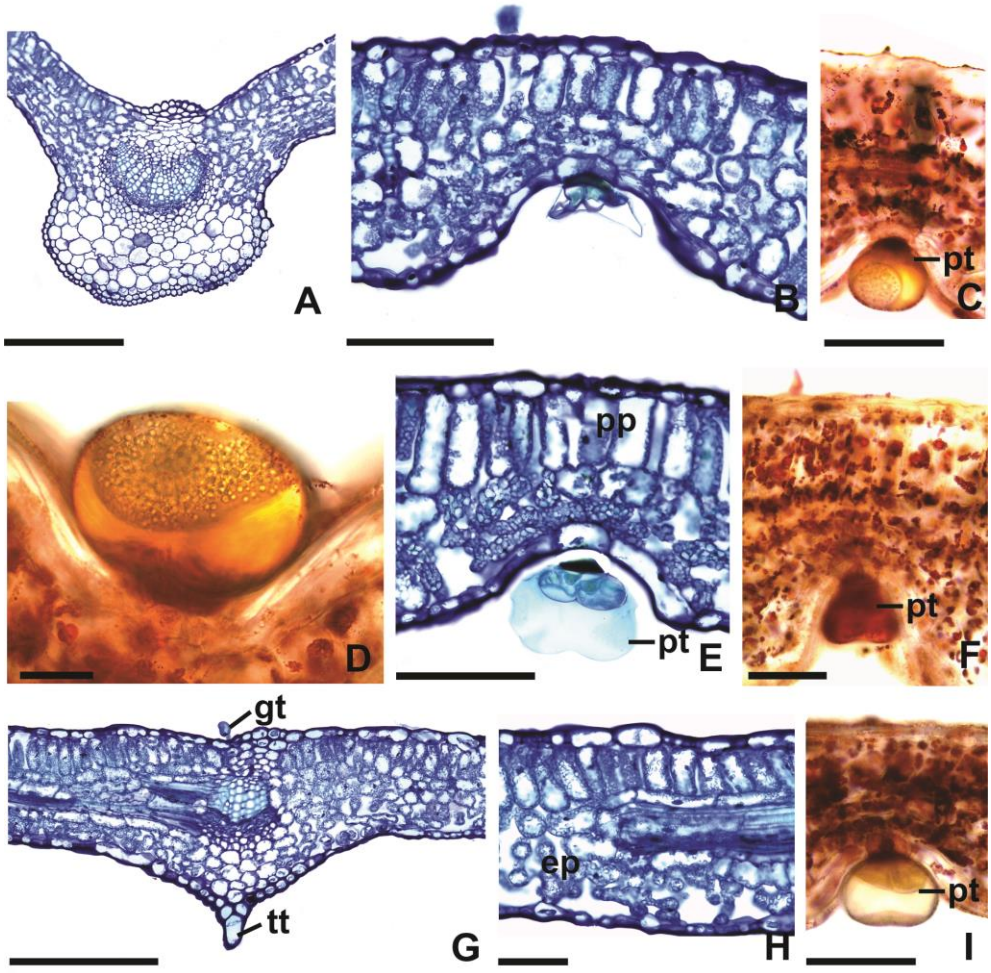

Figure 2 – Cross sections of *O. basilicum var. thyrsiflora* leaves cultivated under different water conditions. A-D. Stress from lack of water (50% less water). E-F. In field capacity (100% field capacity). G-I- Excess water stress (50% more water). Note in C, F and I drops of lipophilic material on the Sudan IV stained sections and in C-D between the distended cuticle and cell wall of secretory cells. (ep – spongy parenchyma, gt – globoid glandular trichome, mp – peltate minor glandular trichome, pp – palisade parenchyma, pt – peltate major glandular trichome, tt – non-glandular trichome). Scales: A, G = 300 µm, B-C, E-F, H-I = 100 µm, D = 50 µm.

The composition of the essential oils depends on various factors, including environmental conditions, the soil composition and cultivation method, the season and time of the day when the plant was picked, the storage and processing conditions, the oil extraction method, and analysis of the chemical components [1, 7, 9]. In this work, the obtained pale yellow essential oils obtained from *O. basilicum var. basilicum* L. (OBB) and *var. thyrsiflora* (OBT) were dried over sodium sulfate and stored at 4 °C in dark vials until tested. The yield of OBB and OBT were similar 0.08 % v/w for both essential oils. The chemical composition of two oils were investigated by gas chromatography-mass spectrometry (GC-MS). The results of the GC-MS analysis for the *O. basilicum var. Basilicum* showed a predominance of linalool (55.87 %), α-muurolol (9.74 %), eugenol (8.89 %), 4-terpineol (4.60%), and eucalyptol (2.83 %), whereas for the variety *var. thyrsiflora*, linalool (51.10 %), eugenol (16.48 %), α-muurolol (11.67 %), 4-terpinene (5.88 %), and germacrene D (2,73) were found as majority compound.

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Table 1: Compounds identified from essential oils by GC-MS.

Compound	RT (min)	RI	Area (%)	
			O. Basilicum var. basilicum	*O. basilicum var. thyrsiflora*
Eucalyptol	7.960	1033	2.83	2,02
***trans*- β-Ocimene**	8.430	1047	1.14	1,91
γ-Terpinene	8.810	1058	0.32	0,97
Linalool	10.340	1103	55.87	36,83
Camphor	12.120	1147	0.67	0,86
4-Terpineol	13.405	1180	4.60	5,88
α-Terpineol	13.970	1194	0.65	0,72
Geraniol	16.658	1258	0.23	0,94
Bornyl acetate	17.890	1288	1.94	2,06
Eugenol	21.030	1363	8.89	11,65
Neryl acetate	22.000	1387	0.37	1,07
β-Cubebene	22.180	1391	1.98	0,14
β-Elemene	22.235	1392	-	2,81
***cis*-α-Bergamotene**	24.080	1438	1.56	1,44
α-Guaiene	24.180	1440	0.18	0,66
α-Humulene	24.750	1454	0.32	0,79
(*E*)-β-Famesene	24.920	1458	0.07	0,35
***cis*-Muurola-4(15),5-diene**	25.130	1464	0.20	0,72
Germacrene D	25.820	1481	1.64	2,73
Bicyclogermacrene	26.560	1499	0.61	1,58
α-Bulnesene	26.870	1507	0.97	1,66
γ-Cadinene	27.220	1516	1.80	2,46
1-*epi*-Cubenol	31.070	1617	0.98	1,52
α-Muurolol	32.090	1645	9.74	7,58
Others			2.44	10.65

RT: retention time; RI: retention index on Rtx-5MS.

CONCLUSION

The screening of the biological activity aromatic plants used in folk medicine have been investigated for their possible therapeutic benefits, especially their essential oils and active compounds, being promising new sources of medicinal drugs. The results related to the chemical composition of both basil species showed a predominance of linalool as the major constituent. The profile of different essential oil and their constituents is important in determining the effectiveness of basil varieties of their use for food, aromatherapy, biological activities, and therapeutic purposes. Furthermore, the conditions for plant cultivation processes are responsible for the essential oil profile for their utilization.

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