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The first report of chemical and biological study of essential oil from *Begonia reniformis* leaf (Begoniaceae)

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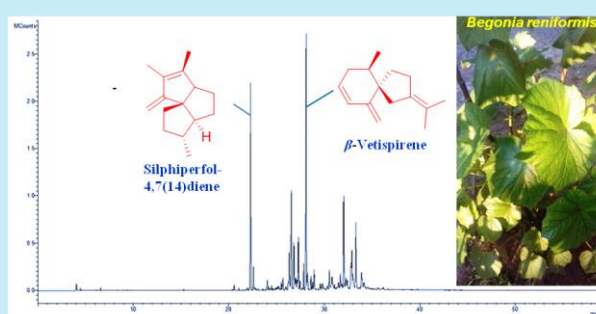
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ABSTRACT: Species of the genus *Begonia* are expensive ornamental plants. There have been few reports of chemical studies with the *Begonia* species. Here we report the first chemical and biological study of essential oil for a species of *Begonia*. The essential oil of the *Begonia reniformis* leaf obtained by hydrodistillation was analyzed via GC-MS and twenty-one compounds were identified. Sesquiterpenes silphiperfol-4,7(14)-diene and β -vetispiene were the major ones with a percentage of 15.7 and 21.0%, respectively. The oil showed weak activity against bacteria *Bacillus subtilis* and *Pseudomonas aeruginosa* with minimum inhibitory concentrations of 625 μ g/mL for each of the two bacteria.



1. Introduction

The family Begoniaceae is characterized by flowering plants that consist of two genera, *Begonia* and *Hillebrandia*, with about 1500 species occurring in all parts of the world^{1,2}. The genus *Hillebrandia* is classified as monotypic because it consists of only one specie *Hillebrandia sandwicensis*, a plant endemic of the Hawaiian Islands³. The species of the genus *Begonia* can be native or horticultural hybrids and are popular as houseplants due to the diversity in their floral form and leaf shape which adds commercial value to their species⁴. In Brazil there are about 213 *Begonia* species, mainly found in the Atlantic Forest, of which 186 are endemic^{5,6}.

The chemistry and biological potential of the *Begonia* species is poorly explored with rare studies available in the literature. In an investigation of the

phytochemistry of leaf extracts of *Begonia malabarica*, the compounds friedelin, *epi*-friedelinol, β -sitosterol, luteolin, quercetin and β -sitosterol-3- β -D-glucopyranoside were isolated⁷. From the *Begonia picta* species, the following flavonoids were isolated: vitexin, iso-vitexin, orientin, iso-orientin and 1,3-dihydroxy-6,7-dimethoxyxanthone. The compounds orientin and iso-orientin were also identified, both of which showed potent antioxidant activity with IC₅₀ values of 54.0 and 53.4 μ mol/L, respectively, when compared with a trolox positive control with an IC₅₀ value of 96.1 μ mol/L⁸. In other phytochemical studies, different classes of anthocyanins were isolated from the extracts of flowers of the *Begonia sp* species, while several steroids and flavonoids were isolated from *B. evansiana* and *B. malabarica* species^{7,9,10}. Despite limited studies of the chemistry of the *Begonia* species,

the few reports available point to flavonoids and steroids as chemotaxonomic metabolites for the genus.

In relation to studies on the chemistry of the essential oil obtained from the *Begonia* species, to our knowledge there are no reports, this work is the first to describe the chemical constituents of the essential oil for a *Begonia* species, the *Begonia reniformis*, a species that is part of the Brazilian flora. To date, we have found no chemical study previously reported for this plant.

2. Experimental

2.1. Material botanic

Begonia reniformis specimens were collected on the Campus of the Rural Federal University of Pernambuco, in the city of Recife, State of Pernambuco in the Northeastern of Brazil, in August 2016. The Instituto Agronômico de Pernambuco – IPA was asked to make the botanical identification and a voucher specimen was deposited at the Dárdano de Andrade Lima Herbarium of the IPA (90664).

2.2. Obtaining of essential oils

The essential oil was obtained from fresh leaf (250 g) via hydrodistillation using a Clevenger-type apparatus. The distillation was carried out for 3 h. The oil obtained was treated with Na₂SO₄.

2.3. Analysis of essential oil

The essential oil was analyzed by GC-MS with a programmed temperature of 60-240°C at 3°C min rate in a Gas Chromatograph (Varian 431-GC) equipped with a fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm) coated with DB-5 and coupled to a Mass Spectrometer (Varian 220-MS). The detailed conditions of GC-MS analysis have been previously reported by our research group^{11,12}. Identification of the chemical constituents was carried out using the Van den dool and Kratz¹³ equation and comparing with published mass spectra¹⁴.

2.4. In vitro assay for antimicrobial activity

The antimicrobial potential of essential oil obtained of *B. reniformis* leaves was evaluated against the

bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (UFPEDA 224) and *Pseudomonas aeruginosa* (ATCC 416) as well as against the fungi *Candida albicans* (ATCC 1007) and *Candida utilis* (ATCC 1009). The microorganisms were maintained in nutrient agar (NA), stored at 4 °C. The antimicrobial activity evaluation was performed by determination of the values of minimum inhibitory concentrations (MIC) as previously reported^{11,12}. The antimicrobials Metronidazol and Fluconazol were used as the positive control.

3. Results and discussion

Essential oil (78 mg) from a mass of the fresh leaves of *B. reniformis* (250 g) was obtained with a yield of 0.03%. The chemical profile of essential oil of *B. reniformis* leaves obtained by GC-MS showed two major peaks at 22.2 and 28.1 minutes with relative concentrations of 15.7 and 21.0%, respectively (Figure 1). Major peaks at 22.2 and 28.1 minutes were identified as sesquiterpenes silphiperfol-4,7(14)-diene and β -vetispiroene, respectively. A total of 21 compounds were identified representing 84.8% of the analyzed oil (Table 1), such as (-)-*cis*-cadine-1(6),4-diene (7.1%), ishwarane (3.9%), Guaiol (8.0%), *cis*-muurola-3,5-diene (3.5%), *trans*-muurola-3,5-diene (3.7%), dauca-5,8-diene (4.3%) and β -atlantol (2.9%). To our knowledge there has been no report of chemical and biological studies of the essential oils of species from Begoniaceae family that has included the genera *Begonia* and *Hillebrandia*. The major compound of essential oil from *B. reniformis* leaves, sesquiterpene β -vetispiroene, has been previously reported in the essential oils from *Vetiveria zizanioides* (Poaceae) roots¹⁵, and its occurrence in other plant species is rare. The second major oil compound has been reported in about fifteen plant species, such as *Remirea maritima* (Cyperaceae), *Piper amalago* (Piperaceae), *Artemisia vulgaris* (Asteraceae), *Clinopodium vulgare* (Labiatae) and *Lomatium rigidum* (Apiaceae)¹⁶⁻²⁰.

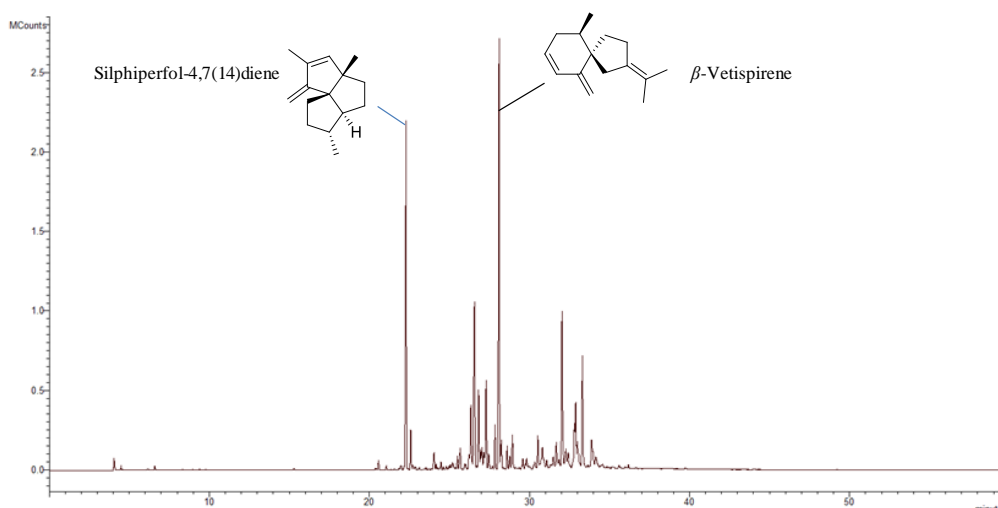


Figure 1. Chromatogram of essential oil of the *B. reniformis* leaf obtained via GC-MS.

Table 1. Chemical constituents identified in the essential oil of the *B. reniformis* leaf.

Compounds ^a	AI ^b	AI ^c	Relative amount (%)
1. Silphiperfol-4,7(14)-diene	1356	1358	15.7
2. Cyclosativene	1363	1369	1.5
3. Isobazzanene	1432	1436	1.0
4. <i>cis</i> -Muurolo-3,5-diene	1448	1448	3.5
5. <i>trans</i> -Muurolo-3,5-diene	1454	1451	3.7
6. (-)- <i>cis</i> -Cadina-1(6),4-diene	1455	1461	7.1
7. Ishwarane	1460	1465	3.9
8. <i>cis</i> -Muurolo-4(14),5-dieno	1465	1465	1.0
9. Dauca-5,8-diene	1471	1471	4.3
10. <i>trans</i> -Cadina-1(6),4-diene	1474	1475	2.0
11. β-Vetispiene	1490	1493	21.0
12. <i>trans</i> -Muurolo-4(14),5-diene	1493	1493	1.2
13. Epizonarene	1502	1501	1.0
14. Vanillin acetate	1518	1524	1.8
15. Raspberry ketone	1549	1545	1.3
16. β-Copaen-4-α-ol	1586	1590	1.1
17. Carotol	1593	1594	1.2
18. Guaiol	1587	1600	8.0
19. β-Atlantol	1608	1608	2.9
20. 1,10-di- <i>epi</i> -Cubenol	1611	1618	1.0
21. <i>cis</i> -Cadin-4-en-7-ol	1634	1635	0.6
Unidentified terpenes ^d			10.5
Unidentified fatty acids ^e			1.1
Unknown compounds			3.6

Total of compounds identified: 84.8 %

^aCompounds are listed in ascending order of their retention times. AI: Arithmetic index. ^bArithmetic index as determined on non-polar DB-5 column. ^cArithmetic index of the literature. ^{d,e}Determined by the fragmentation pattern of their respective mass spectra.

The essential oil of *B. reniformis* leaf was evaluated against six microorganisms including fungi, gram-positive bacteria and gram-negative bacteria to determine the MIC values by the agar dilution method. The samples of natural source as extract and essential oil that show MIC values lower than 100 µg/mL are characterized as strong antimicrobial agent. The samples with MIC values ranging from 100-500, 500-1000 and over 1000 µg/mL are considered to be

moderately weak, or non-active, respectively, against fungi and bacteria according to criteria in the literature²¹. The results in Table 2 show that the oil was biologically active against gram-positive bacteria *B. subtilis* and gram-negative bacteria *P. aeruginosa* with MIC of 625 µg/mL for each of the two bacteria, this is considered to be weak activity.

Table 2. Values of the MIC obtained for essential oil from the *B. reniformis* leaf towards bacteria and fungi

Bacteria	
Gram-positive bacteria	
<i>Staphylococcus aureus</i>	>2500 µg/mL
<i>Bacillus subtilis</i>	625 µg/mL
Gram-negative bacteria	
<i>Escherichia coli</i>	>2500 µg/mL
<i>Pseudomonas aeruginosa</i>	625 µg/mL
Fungi	
<i>Candida albicans</i>	>2500 µg/mL
<i>Candida utilis</i>	>2500 µg/mL

4. Conclusions

The first study on the chemical composition of the essential oil for a species of the Begoniaceae family showed sesquiterpenes as the major constituents in the *B. reniformis* leaf. The sesquiterpenes silphiperfol-4,7(14)-diene and β -vetispirorene were the majority and these are rare in the essential oil of plant. The essential oil showed weak antimicrobial activity against the bacteria *B. subtilis* and *P. aeruginosa*.

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