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FLORAL VOLATILES IN BIOPHYTUM DC. (OXALIDACEAE).

SREESHMA L S* AND BINDU R NAIR

Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram-69558, India

ABSTRACT

The present study is comparative information of the essential oil composition of two commonly available species of *Biophytum*, *B. reinwardtii* and *B. veldkampii*. It was noted that the flowers of *B. reinwardtii* possessed a persistent and pleasant aroma and was intended to detect the phytochemicals responsible for this pleasant aroma. The essential oils of both the species were extracted by hydro-distillation and the components analyzed by GC-MS. The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with the NIST08 library source. The analysis of the floral oil of both species revealed the presence of many compounds [*B. veldkampii* (42) and *B. reinwardtii* (36)], especially fatty acids such as n-Hexadecanoic acid, tetradecanoic acid and 9, 12-Octadecadienoic acid (Z, Z)-. The major component, however in both the species was n-Hexadecanoic acid [*B. reinwardtii* (42.32%) and *B. veldkampii* (51.44%)]. The remaining compounds were present in trace quantities (< 10%) in both species. The essential oil of *B. reinwardtii* contained certain sesquiterpenes such as alpha.-Bisabolol (5.97%), gamma-eudesmol (1.06%), .beta Bisabolene (0.69%), .alpha.-Farnesene (0.57%), Caryophyllene oxide (0.41%), Caryophyllene (0.37%), and cis alpha Bisabolene (0.30%) which are reported to be aromatic and are being widely used in cosmetic and pharmaceutical industries for various purposes. Since these compounds were absent in the essential oil of *B. veldkampii*, it appears that these aromatic sesquiterpenes are responsible for the pleasant aroma of *B. reinwardtii* flower.

KEYWORDS: ***Essential oil, GC-MS, Biophytum reinwardtii, B. veldkampii ,Sesquiterpenes***

SREESHMA L S*

Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram-695581, India.

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INTRODUCTION

The genus *Biophytum* (Oxalidaceae) contains herbaceous annuals or perennials and about 80 species are reported to be distributed in the tropical and subtropical regions of the world.[1]The genus is reported to be represented by 17 species in India [2] and nine species in Kerala.[3.] For the present study, two species of *Biophytum* namely, *Biophytum veldkampii* Shanavas *et al.* and *Biophytum reinwardtii* (Zucc.) Klotzsch are being considered. *Biophytum veldkampii* looks like a miniature palm and is native to India. The plants are usually found in wet lands (mostly plains) of tropical Africa, Asia and India, normally in the shades of trees and shrubs, in grasslands, open thickets, at low and medium altitudes. *Biophytum reinwardtii* (Reinwardt's Tree Plant) is found in India, Ceylon, South China, Malaysia, and also in the Himalayas, from Garhwal to Nepal, at altitudes up to 1000 m. It is an annual herb which also looks like a miniature tree. It was noted that the flowers of *B. reinwardtii* possessed a persistent and pleasant aroma which is not observed in *B. veldkampii*. Therefore, aim of the present study was to conduct the GC-MS analysis of the floral oil of both the species of *Biophytum* and to find out the phytochemicals responsible for the pleasant aroma of flowers of *B. reinwardtii*. GC-MS has proved to be a valuable tool for the analysis of non-polar components and volatile essential oil, fatty acids and lipids in plants.

MATERIALS AND METHODS

The materials for the present study, *B. reinwardtii* and *B. veldkampii* were collected from Peechi (Thrissur) and Kattakada (Thiruvananthapuram) respectively and authenticated by the Curator, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram.

Preparation of oil sample for GC-MS

The flowers of both species were washed gently to remove the fine particles of dust. They were then loaded in the extraction flask. The essential oil was extracted by hydro-distillation for 8 h (50g of sample in 300ml of distilled water) using Clevenger apparatus.[4] The volatile oil obtained was separated from aqueous phase and stored in sealed glass vials protected from the light at 4ºC until analysis. The oil sample was subsequently analysed by GC-MS.

GC-MS Analysis

The analysis of the oil of both species was performed using GC-MS (Model: GC-MS-QP 2010, Shimadzu, Tokyo, Japan) equipped with a VF 5ms fused silica capillary column of 30m length, 0.25mm diameter and 0.25µm film thickness. Conditions
Ionization energy - 70eV
Carrier gas - Helium (99.99%)
Flow rate-1.51ml/min
Injector and mass transfer line temperature- 200°C and 240°C
Oven temperature- 70 to 220°C at 10°C/min
Sample volume- Two microlitres
The relative percentage of the extract was expressed as percentage with peak area normalization.

Identification of components

The identity of the components in the extracts and oil was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with the NIST08 LIB.[5] The relative percentage amount of each component was calculated by comparing its average peak area to the total area.

RESULTS

The phytocomponents present in the oil of *B. veldkampii* and *B. reinwardtii* flowers were identified by GC-MS analysis (total running time being 34 min). The GC-MS chromatograms of oil of both species are presented (Figures. 1 & 2.) and the active compounds, their retention time, area%, molecular formula, weight are provided (Tables 1 &2).

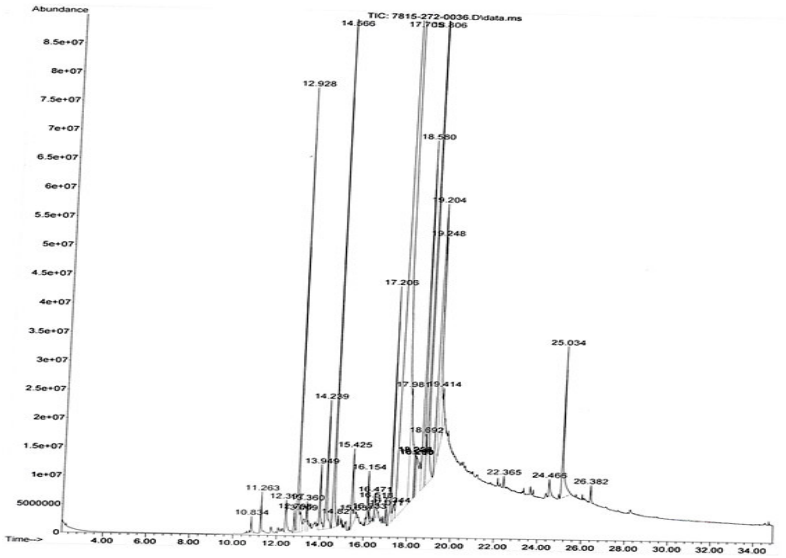

Figure 1
GC-MS Chromatograms of B. reinwardtii flower oil

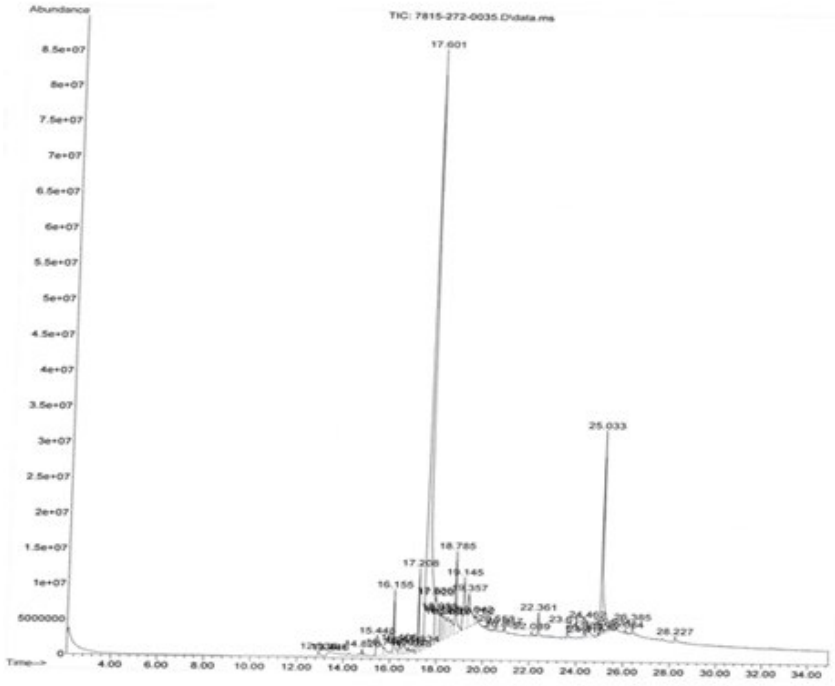

Figure 2
GC-MS Chromatograms of B. veldkampii flower oil

Table 1
Phytoconstituents in the B. reinwardtii flower oil

Peak No	Name of the compound	Retention time	Area %	Molecular formula	Molecular weight
1	Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(methylethenyl)-,(1.alpha., 2.beta., 4.beta)-	10.830	0.21	$C_{15}H_{24}$	204.35
2	Caryophyllene	11.261	0.37	$C_{15}H_{24}$	204.36
3	.beta.-Bisabolene	12.398	0.69	$C_{15}H_{24}$	204.35
4	**cis-.alpha.Bisabolene**	12.799	0.30	$C_{15}H_{24}$	204.35
5	Cyclohexanemethanol,4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-,[1R-(1.alpha.,3.alpha.,4.beta)]-	12.925	4.51	$C_{15}H_{26}O$	222.36
6	.alpha.-Farnesene	13.066	0.57	$C_{15}H_{24}$	204.35
7	Caryophyllene oxide	13.356	0.41	$C_{15}H_{24}O$	220.35
8	.gamma.-eudesmol	13.950	1.06	$C_{15}H_{26}O$	222.36
9	2-Naphthalenemethanol,1,2,3,4,4a,5,6,8a-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-,[2R-(2.alpha.,4a.alpha.,8a.beta)]-	14.240	2.16	$C_{15}H_{26}O$	222.36
10	.alpha.Bisabolol	14.567	5.97	$C_{15}H_{26}O$	222.36
11	2-Benzyl-3-isopropyl-cyclopentanon	14.820	0.08	$C_{15}H_{20}O$	216.31
12	Tetradecanoic acid	15.421	1.57	$C_{14}H_{28}O_2$	228.37
13	Tetradecanoic acid	15.637	0.06	$C_{14}H_{28}O_2$	228.37
14	2-Pentadecanone,6,10,14-trimethyl	16.157	0.62	$C_{18}H_{36}O$	268.47
15	Lanost-9(11)-en-18-oic acid	16.231	0.12	$C_{41}H_{63}NaO_{17}S$	882.98
16	Pentadecanoic acid	16.469	0.30	$C_{15}H_{30}O_2$	242.39
17	Pentadecanoic acid	16.521	0.29	$C_{15}H_{30}O_2$	242.39
18	Hexadecanoic acid, methylester	17.011	0.17	$C_{17}H_{34}O_2$	270.45
19	Isophytol	17.204	2.20	$C_{20}H_{40}O$	296.53
20	Cis-9-Hexadecanoic acid	17.345	0.44	$C_{16}H_{30}O_2$	254.40
21	n- Hexadecanoic acid	17.702	42.32	$C_{16}H_{32}O_2$	256
22	n- Hexadecanoic acid	17.984	4.43	$C_{16}H_{32}O_2$	256
23	n- Hexadecanoic acid	18.207	0.36	$C_{16}H_{32}O_2$	256
24	n- Hexadecanoic acid	18.237	0.36	$C_{16}H_{32}O_2$	256
25	n- Hexadecanoic acid	18.267	0.27	$C_{16}H_{32}O_2$	256
26	n- Hexadecanoic acid	18.289	0.58	$C_{16}H_{32}O_2$	256
27	Phenol, 2-methyl-	18.579	5.77	C_7H_8O	108.13
28	Phenol, 3-Pentadecyl-	18.690	0.64	$C_{21}H_{36}O$	304.50
29	Phytol	18.809	9.30	$C_{20}H_{40}O$	296
30	9,12-Octadecanoicacid(Z,Z)-	19.203	5.80	$C_{18}H_{32}O_2$	280
31	9,12-Octadecanoicacid(Z,Z)-	19.247	4.03	$C_{18}H_{32}O_2$	280
32	9,12-Octadecanoicacid(Z,Z)-	19.411	0.83	$C_{18}H_{32}O_2$	280
33	9,12-Octadecanoicacid(Z,Z)-	22.367	0.11	$C_{18}H_{32}O_2$	280
34	Octadecanoic acid	24.469	0.32	$C_{18}H_{36}O_2$	284.47
35	Tetracosane	25.034	2.59	$C_{24}H_{50}$	338.65
36	Eicosane	26.386	0.20	$C_{20}H_{42}$	282.54

Table 2
Phytoconstituents in the B. veldkampii flower oil

Peak No	Name of the compound	Retention time	Area %	Molecular formula	Molecular weight
1	Cyclohexanemethanol,4-ethenyl-alpha.,..alpha., 4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3alpha.,4.beta.)]	12.933	0.32	$C_{15}H_{26}O$	222.3663
2	Dodecanoic acid	13.378	0.19	$C_{12}H_{24}O_2$	200.32
3	Dodecanoic acid	13.445	0.02	$C_{12}H_{24}O_2$	200.32
4	.beta.-Vatirenene	14.820	0.14	$C_{15}H_{22}$	202.335
5	Tetradecanoic acid	15.451	3.02	$C_{14}H_{28}O_2$	228.37
6	Tetradecanoic acid	15.748	0.69	$C_{14}H_{28}O_2$	228.37
7	2-Pentadecanone,6,10,14-trimethyl	16.157	2.21	$C_{18}H_{36}O$	268.4778
8	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	16.049	0.56	$C_{16}H_{22}O_4$	278.3435
9	Pentadecanoic acid	16.551	0.64	$C_{15}H_{30}O_2$	242.39
10	Pentadecanoic acid	16.617	0.61	$C_{15}H_{30}O_2$	242.39
11	9-Heptadecanone	16.803	0.25	$C_{17}H_{34}O$	254.45
12	Hexadecanoic acid, methyl ester	17.019	0.16	$C_{17}H_{34}O_2$	270.45
13	Isophytol	17.212	2.12	$C_{20}H_{40}O$	296.53
14	Palmitoleic acid	17.338	0.46	$C_{16}H_{30}O_2$	254.40
15	n-Hexadecanoic acid	17.598	51.44	$C_{16}H_{32}O_2$	256
16	n-Hexadecanoic acid	17.888	0.96	$C_{16}H_{32}O_2$	256
17	n-Hexadecanoic acid	17.917	4.49	$C_{16}H_{32}O_2$	256
18	n-Hexadecanoic acid	18.088	0.81	$C_{16}H_{32}O_2$	256
19	n-Hexadecanoic acid	18.133	0.94	$C_{16}H_{32}O_2$	256
20	n-Hexadecanoic acid	18.192	1.08	$C_{16}H_{32}O_2$	256
21	n-Hexadecanoic acid	18.267	0.91	$C_{16}H_{32}O_2$	256
22	n-Hexadecanoic acid	18.460	1.76	$C_{16}H_{32}O_2$	256
23	Phytol	18.787	3.57	$C_{20}H_{40}O$	296
24	Oleic acid	19.143	4.19	$C_{18}H_{34}O_2$	282.46
25	Octadecanoic acid	19.359	2.96	$C_{18}H_{36}O_2$	284.47
26	n-Hexadecanoic acid	19.641	0.36	$C_{16}H_{32}O_2$	256
27	n-Hexadecanoic acid	19.700	0.19	$C_{16}H_{32}O_2$	256
28	n-Hexadecanoic acid	20.562	0.19	$C_{16}H_{32}O_2$	256
29	n-Hexadecanoic acid	20.859	0.11	$C_{16}H_{32}O_2$	256
30	n-Hexadecanoic acid	20.919	0.24	$C_{16}H_{32}O_2$	256
31	n-Hexadecanoic acid	22.092	0.15	$C_{16}H_{32}O_2$	256
32	Bis (2-ethylhexyl) phthalate	22.360	0.77	$C_{24}H_{38}O_4$	390.56
33	9-Eicosene,(E)-	23.615	0.43	C20H40	280.53
34	n-Hexadecanoic acid	24.336	0.31	$C_{16}H_{32}O_2$	256
35	n-Hexadecanoic acid	24.417	0.08	$C_{16}H_{32}O_2$	256
36	Pentadecanoic acid	24.462	1.35	$C_{15}H_{30}O_2$	242.39
37	n-Hexadecanoic acid	24.930	0.07	$C_{16}H_{32}O_2$	256
38	Tetracosane	25.034	10.39	$C_{24}H_{50}$	338.65
39	Quinidine	25.532	0.07	$C_{20}H_{24}N_2O_2$	324.41
40	Octadecanoic acid	26.081	0.12	$C_{18}H_{36}O_2$	284.47
41	Eicosane	26.386	0.41	$C_{20}H_{42}$	282.54
42	Tetrasiloxane, decamethyl-	28.226	0.26	$C_{10}H_{30}O_3Si_4$	310.68

The GC-MS analysis of flower oil of *B. reinwardtii* and *B. veldkampii* revealed the presence of 36 and 42 compounds respectively. The number of compounds was more in *B. veldkampii* compared to *B. reinwardtii*. Apart from very few compounds (mentioned below), the remaining were present only in trace quantities (< 5%). The results revealed that n- Hexadecanoic acid (42.32%) was the major component in both *B. reinwardtii*, and *B. veldkampii*. The next in order in *B. reinwardtii* were Phytol (9.30%), .alpha.Bisabolol (5.97%) and 9,12- Octadecanoic acid (Z,Z)- (5.80%) while in *B. veldkampii*, Hexadecanoic acid was followed by Tetracosane (10.39%). Certain aromatic sesquiterpenes were detected in the floral oil of *B. reinwardtii* (Table 3) but absent in *B. veldkampii*. This probably contributes to the pleasant smell of *B. reinwardtii* flowers.

Table 3
Probable aroma contributing compounds in B. reinwardtii

Compound name	Area%
α-Bisabolol	5.97%
.gamma.-eudesmol	1.06%
beta Bisabolene	0.69%
.alpha.-Farnesene	0.57%
caryophyllene oxide	0.41%
caryophyllene	0.37%
cis-.alpha.Bisabolene	0.30%

DISCUSSION

GC-MS method is a direct and fast analytical tool for the identification of phytoconstituents with the added advantage that only few grams of plant material is required. The gas chromatogram of floral oil of two species of *Biophytum* shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peaks indicate the relative concentrations of the components present in the plant. The mass spectrometer analyses the compounds eluted at different times to identify the nature and structure of the compounds. The larger compounds fragment into small compounds giving rise to various peaks at different m/z ratio. These mass spectra are actually a fingerprint of that compound which can be identified from the data library. In the present study the essential oil from the flowers of two species of *Biophytum* were separated by hydro distillation and analysed by GC-MS. A total of 42 compounds were analysed in *B. veldkampii* and 36 compounds were analysed in *B. reinwardtii.* n-Hexadecanoic acid was the major compound present in both species. *n*-Hexadecanoic or palmitic acid is the most abundant saturated fatty acid in nature, and is most common in animals, plants and microorganisms. It possesses antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, haemolytic, antioxidant, antiandrogenic, flavoring and hemolytic properties.[6] In addition some important sesquiterpenes were present which probably contribute to the unique aroma to the flowers of *B. reinwardtii*. The importance of these compounds is listed below. *Bisabolol or α-bisabolol***,** also known as levomenol, is a natural monocyclic sesquiterpene alcohol. It is a colourless viscous oil that is the primary constituent of the essential oil from *Matricaria recutita* and *Myoporum crassifolium*. It is almost insoluble in water and glycerin, but very soluble in ethanol. Bisabolol has a weak sweet floral aroma and is used in various fragrances. It has also been used for hundreds of years in cosmetics because of its perceived skin healing properties. It is known to have anti-irritant, anti-inflammatory and anti-microbial properties. α- bisabolol has recently been shown to induce apoptosis in models of leukemia.[7] The .gamma.-eudesmol belongs to the eudesmane group, which is probably the largest of the sesquiterpene groups and can be considered to have been derived from farnesyl pyrophosphate cyclization involving a germacrane skeleton as an intermediate. The alcohol beta eudesmol occurs in eucalyptus oil. Beta isomer has a similar but warmer and more herbaceous odour. Eudesmol which finds some use as fixative offers a delicate sweet-woody and warm odour.[8] Bisabolenes are present in the essential oils of a wide variety of plants including cubeb, lemon and oregano.[9] Various derivates also function as pheromones in different insects, such as stink bugs and fruit flies. Bisabolenes are intermediates in the biosynthesis of many other natural chemical compounds, including hernandulcin, a natural sweetener. β-Bisabolene has a balsamic odor and is approved in Europe as a food additive. [10] Alpha - Farnesene*:* The isomeric forms of farnesene possess interesting odours, which are generally not known to perfumers because of commercial non-availability. However, it occurs in many essential oils such as lavender, pepper, ginger oil, hops, ylang-ylang. Beta-farnesene is considered to be one of the most important constituents of lavender oil. The alpha-isomer has not been reported to occur in nature but has been synthesized by dehydration of nerolidol or farnesol. [8] Farnesol occurs in several essential oils such as oils of ambrette seed, neroli, rose and cyclamen jasmine. It has a delicate sweet oily odour developing in to a floral fresh-green note and finds uses in floral, oriental and chypre fragrances. Biogenetically speaking this alcohol occupies a very significant position in sesquiterpene chemistry since it has been established that farnesyl pyrophosphate is the *in vivo* precursor of a variety of sesquiterpenes.[8] Caryophyllene or β-caryophyllene, is a natural bicyclic sesquiterpene that is a constituent of many essential oils, especially the oil from the stems and flowers of *Syzygium aromaticum*, the essential oil of *Cannabis sativa* and *Rosmarinus oficinalis*. It is usually found as a mixture with isocaryophyllene and α-humulene (α-caryophyllene), a ring-opened isomer. Caryophyllene is one of the chemical compounds that contribute to the spiciness of black pepper. Beta-caryophyllene was shown to be selective agonist of cannabinoid receptor type-2 and to exert significant cannabimimetic anti-inflammatory effects in mice. [11] Beta-caryophyllene is a widespread plant natural product and an FDA approved food additive. Caryophyllene oxide, in which the olefin of caryophyllene has become an epoxide, is the component responsible for cannabis identification by drug-sniffing dogs and is also approved for food flavouring. [12,13]Sesquiterpenes offer a great challenge to the Organic Chemist because of their structural complexity. They are of crucial importance to the perfumery industry as they occur widely in essential oils and individual chemicals offer a wide spectrum of interesting odour types.

CONCLUSION

Among the floral volatile constituents detected in the two species of *Biophytum,* the fatty acid n- Hexadecanoic acid, phytol and the alkane hydrocarbon, tetracosane were identified to be the predominant. However, *B.reinwardtii* consisted of some aromatic sesquiterpenes such as alpha.-Bisabolol and gamma-eudesmol, which were absent in *B. veldkampii*. The pleasant aroma characteristic of *B. reinwardtii* flower appears to be due to the presence of these and other minor compounds.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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Reviewers of this article

Dr. Laija .S. Nair . M.Sc., M.Phil ,Ph.D.,

Assistant Professor ,Dept of Botany
University College ,Thiruvananthapuram ,
Kerala.

Prof. Srawan Kumar G.Y

Affiliation
Associate Professor,Nalanda Institute of Pharmaceutical Sciences, Sattenapalli, Guntur, Andrapradesh, India

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