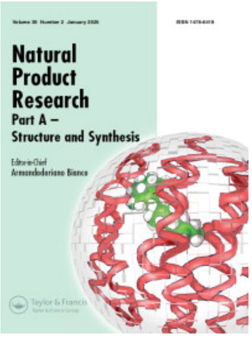

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RAPID COMMUNICATION

First comprehensive report on the chemical composition of the floral perfume of *Notylia* (Orchidaceae)

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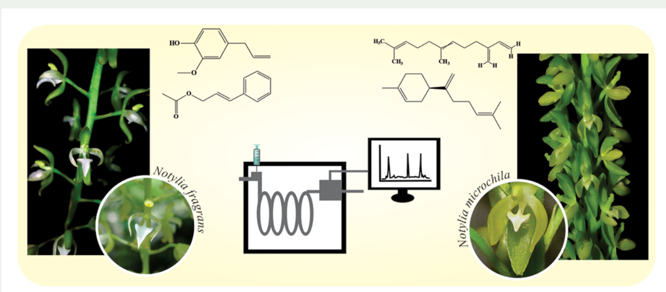
ABSTRACT

This study presents the first complete analysis of the chemical composition of the flowers of the species *N. fragrans* and *N. microchila*. The compounds were extracted *via* distillation and simultaneous extraction and analysed using gas chromatography coupled to mass spectrometry. A total of 82 compounds were identified and the results reveal significant differences in the abundance of compounds between the species. While *N. fragrans* has eugenol as its majority compound, *N. microchila* exhibits high concentrations of (*E*)-*β*-farnesene. In addition, remarkable concentrations of secondary compounds such as methyl-eugenol, *β*-bisabolene, germacrene D, 1-octadecene, hexadecyl acetate and ipsdienol were observed and are demonstrably related to the processes of attraction of pollinators, such as euglossine bees, by Orchidaceae. The findings of the study offer valuable insights into the ecology and reproductive biology of the species studied, and also present taxonomic potential for the phytochemical data.



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1. Introduction

Notylia Lindl. is a Neotropical genus that belongs to the family Orchidaceae and includes around 60 species (Chase et al. 2005; Chase 2009). It is inserted in the subtribe Oncidiinae, which is widely recognised for its variations of sizes, shapes and colours of its flowers, in addition to offering floral resources such as oils, nectar, resins and perfumes to attract pollinators (Neubig et al. 2012; Castro and Singer 2019). In Brazil, 26 species of the genus are known, 16 of which are endemic, and these are distributed in all the regions of the country (Flora e Funga do Brasil 2024).

Although *Notylia* is widely distributed and has an interesting pollination ecology, recent studies of this genus have been restricted to floristic studies (Pessoa et al. 2022; Silva et al. 2022). Therefore, although it is known that *Notylia* species have notably perfumed flowers that attract males Euglossini bees (Karremans 2023), little is known about the chemical composition of the perfumes of the flowers of this species. Whitten et al. (1986, 1988) indicated the presence of ipsdienol and (*E*)-nerol as the main constituents of the floral perfume of *N. latilabia* Ames & C.Schweinf., while Gerlach and Schill (1991) identified *p*-dimthoxybenzene and (*E*)-*β*-ocimene as the major compounds in *N. bunchtienii* Schltr., *N. venezuelana* Schltr. and *N. barkeri* Lindl. These compounds are linked to the system pollination used by Euglossini; however, there is no complete record of the volatile compounds present in the perfume of any of the species of the genus, so a more thorough phytochemical study is necessary.

In this sense, the objective of this study was to create the first detailed report of the chemical composition of the floral volatile compounds for two species of the genus, namely *N. fragrans* Wullschl. ex H. Focke and *N. microchila* Cogn. Thus, we seek to contribute to the expansion of knowledge about the chemical composition of the floral volatile compounds found in the Orchidaceae family.

2. Results and discussion

We identified 82 volatile compounds extracted from the four specimens analysed (Table S2). Analysis of the data revealed marked differences between the chemical composition of *N. fragrans* and *N. microchila*, indicating a taxonomic potential for perfumes. In *N. fragrans*, the predominant compound in the sampled specimens is eugenol, which ranges from 73.46% in SAM 1 to 35.99% in SAM 2. For *N. microchila*, (*E*)-*β*-farnesene is the most abundant compound, ranging from 32.97% in SAM 3 to 46.61% in SAM 4.

The chemical composition of the floral aroma of *N. fragrans* in the samples SAM 1 and SAM 2 differed in their concentrations and the amount of compounds (Table S2; Figure S2 and S3). In sample SAM 1, eugenol (73.46%), (*E*)-cinnamon acetate (17.02%) and methyl-eugenol (7.28%) are the majority compounds, while sample SAM 2 presented eugenol (35.99%), hexadecyl acetate (14.66%) and 1-octadecene (10.49%). However, eugenol was the majority compound found in both samples (SAM 1 and SAM 2) of *N. fragrans* and also in a low concentration in SAM 4 of *N. microchila*.

Eugenol (the aroma of cloves) is a phenylpropanoid present in the flowers of Orchidaceae pollinated by Euglossini bees, as observed in the chemical analyses carried out for the genus *Aspasia* Lindl., *Catasetum* Rich. ex Kunth, *Cypripedium* L., *Dressleria* Dodson, *Gymnadenia* R. Br., *Gongora* Ruiz & Pav., and *Stanhopea* Frost ex Hook. (Williams 1974; Hills 2012; Gupta et al. 2014; Casique et al. 2018; Braunschmid et al. 2020; D'Auria et al. 2020; Engels et al. 2020; Vasconcelos et al. 2023); and this is its first report for the genus *Notylia*. The composition of floral perfumes is important for attracting different species that act in pollination systems involving male Euglossini bees (Dressler 1982; Sazima et al. 1993). Different components may mediate such interactions, whereby males collect these perfumes as part of the ritual for attracting females, and certain species may have preferences for specific chemical compounds, without this being a potential proxy for reproductive isolation in closely related orchid species (Dressler 1982; Nunes et al. 2016; Brandt et al. 2020; Opedal et al. 2020).

The compounds methyl-eugenol, (*E*)-cinnamyl acetate, hexadecyl acetate and 1-octadecene (Table S2) have already been reported in Orchidaceae for the genus *Bulbophyllum, Dendrobium, Epipogium, Stanhopea* and *Zygopetalum* and are known for being attractive to euglossine bees (Williams and Whitten 1983; Tatsuka 1988; Tan et al. 2006; Tan and Nishida 2012; Jakubska Busse et al. 2014; Casique et al. 2018). However, they are cited here for the first time for the genus *Notylia*.

The composition of samples SAM 3 and SAM 4 of *N. microchila* also differed in their concentrations and the amount of compounds (Table S2; Figure S4 and S5). SAM 3 consists mainly of the compounds (*E*)-*β*-farnesene (32.97%), *β*-bisabolene (14.96%) and germacrene D, while SAM 4 is composed of (*E*)-*β*-farnesene (46.61%), *β*-bisabolene (12.09%) and ipsdienol (7.48%). (*E*)-*β*-farnesene is a natural volatile compound commonly found in essential oils of citrus plants and in the skin of apples, and is responsible for their characteristic odour (Sacchetti et al. 2005; Bozin et al. 2006; Hazekamp et al. 2016).

(*E*)-*β*-farnesene is a sesquiterpene found in large concentrations in SAM 3 and SAM 4 (Table S1), which has also been identified in the chemical analysis of *Notylia bunchtienii* (Gerlach and Schill 1991) and other genus of the Orchidaceae family such as *Catasetum*, *Gongora* and *Stanhopea* (Gerlach 2013; Milet Pinheiro and Gerlach 2017; Casique et al. 2018; Vasconcelos et al. 2022, 2023), and is related to the process of attracting pollinators. Therefore, the data found in this study add to the evidence that species of *Notylia* are pollinated by male *Euglossini* and *Eulaema* bees (Warford 1992; Singer and Koehler 2003).

The compounds *β*-bisabolene, ipsdienol and germacrene D were present in significant amounts in SAM 3 and SAM 4 (Table S2). Ipsidienol is a terpene already described in the genus *Notylia,* and was identified in the analysis of *N. latilabia* (Whitten et al. 1986, 1988). It was also reported as a compound present in flowers pollinated by *Euglossa* (Eltz et al. 2005). The compounds *β*-bisabolene and germacrene D are sesquiterpenes until recently only known for other genus of Orchidaceae such as *Catasetum* and *Stanhopea* (Gerlach 2013; Milet Pinheiro and Gerlach 2017; Casique et al. 2018) and are reported here for the first time for the genus *Notylia*.

Furthermore, a chemometric investigation of the samples was conducted employing multivariate statistical techniques (Figure S6) to ascertain the compounds with the greatest influence on the formation of the groups. As illustrated in Figure S6, the principal component analysis (PCA) revealed that PC1 accounted for 50.4% of the total variance, while PC2 explained 29.7%. Moreover, an analysis of the compounds with the highest weights for group formation is feasible. As illustrated in Figure S7, the hierarchical cluster analysis (HCA) reveals the formation of three distinct groups. However, there is minimal overlap between the two sets of data. For instance, Group I, constituted by samples 1 and 2, exhibits a similarity of 27.84%, whereas samples 3 and 4 demonstrate a similarity of only 18.83%, which is insufficient to form a group. The observed similarities between the specimens may be attributed to genetic factors, while the noted differences may be attributed to the collection sites. Previous studies in the literature have reported that these factors can exert both qualitative and quantitative influences on the chemical composition of volatile compounds (Tsusaka et al. 2019; Walia et al. 2020).

3. Experimental

See Supplementary Material.

4. Conclusion

This study presents a comprehensive analysis of the chemical composition of the floral perfumes of two species of *Notylia*, with identified compounds demonstrating a clear link to the pollination process. These findings also indicate notable variations in the composition of the aromas observed in the species under analysis and in other previously studied species, thus providing potential for taxonomic classification based on phytochemical data. The taxonomy of *Notylia* is complex due to the similarity of its flowers and the absence of a comprehensive revision. Consequently, phytochemical studies of this nature have the potential to serve as a tool for species delimitation, in addition to assisting in the comprehension of evolutionary processes associated with reproductive isolation, which are generated by variation in the chemical composition of aromas.

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No potential conflicts of interest are reported by the authors.

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References

Bozin B, Mimica-Dukic N, Simin N, Anackov G. 2006. Characterization of the volatile composition of essential oils of some lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. J Agric Food Chem. 54(5):1822–1828. doi:10.1021/jf051922u.

Brandt K, Machado IC, Navarro D, Dötterl S, Ayasse M, Milet Pinheiro P. 2020. Sexual dimorphism in floral scents of the neotropical orchid *Catasetum arietinum* and its possible ecological and evolutionary significance. AoB Plants. 12(4):1–12. doi:10.1093/aobpla/plaa030.

Braunschmid H, Guilhot R, Dötterl S. 2020. Floral scent and pollinators of *Cypripedium calceolus* L. at different latitudes. Diversity. 13(1):5. doi:10.3390/d13010005.

Casique JV, Silva EF, Andrade EHA, Mastroberti AA, Aguiar Dias ACA. 2018. Anatomical analyses of floral and extrafloral secreting structures indicate the presence of nectaries and colleters in *Stanhopea grandiflora* Lindl. Braz J Bot. 41(3):725–738. doi:10.1007/s40415-018-0469-5.

Castro JB, Singer RB. 2019. A literature review of the pollination strategies and breeding systems in Oncidiinae orchids. Acta Bot Bras. 33(4):618–643. doi:10.1590/0102-33062019abb0111.

Chase MW. 2009. Subtribe oncidiinae. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN. Genera orchidacearum: epidendroideae. Oxford: University Press. p. 211–391.

Chase MW, Hanson L, Albert VA, Whitten WM, Williams NH. 2005. Life history evolution and genome size in subtribe oncidiinae (orchidaceae). Ann Bot. 95(1):191–199. doi:10.1093/aob/mci012.

D'Auria M, Lorenz R, Mecca M, Racioppi R, Romano VA, Viggiani L. 2020. Fragrance components of *Gymnadenia conopsea* and *Gymnadenia odoratissima* collected at several sites in Italy and Germany. Nat Prod Res. 36(13):1–5. doi:10.1080/14786419.2020.1851227.

Dressler RL. 1982. Biology of the orchid bees (Euglossini). Annu Rev Ecol Syst. 13(1):373–394. doi:10.1146/annurev.es.13.110182.002105.

Eltz T, Roubik DW, Lunau K. 2005. Experience-dependent choices ensure species-specific fragrance accumulation in male orchid bees. Behav Ecol Sociobiol. 59(1):149–156. doi:10.1007/s00265-005-0021-z.

Engels ME, Rocha LCF, Koch AK, Gerlach G. 2020. The genus *Gongora* (Orchidaceae, Stanhopeinae) from state of Mato Grosso. Brazil Rodriguésia. 71:1–9. doi:10.1590/2175-7860202071068.

Flora e Funga do Brasil. 2024. Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br/.

Gerlach G. 2013. Stanhopeinae Mesoamericanae, V. El aroma floral de las *Stanhopeas* de Mexico. Lankesteriana. 9(3):431–442. doi:10.15517/lank.v0i0.12105.

Gerlach G, Schill R. 1991. Composition of orchid scents attracting euglossine bees. Botanica Acta. 104(5):379–384. doi:10.1111/j.1438-8677.1991.tb00245.x.

Gupta AK, Schauvinhold I, Pichersky E, Schiestl FP. 2014. Eugenol synthase genes in floral scent variation in *Gymnadenia* species. Funct Integr Genomics. 14(4):779–788. doi:10.1007/s10142-014-0397-9.

Hazekamp A, Tejkalová K, Papadimitriou S. 2016. Cannabis: from cultivar to chemovar II – A metabolomics approch to cannabis classification. Cannabis Cannabinoid Res. 1(1):202–215. doi:10.1089/can.2016.0017.

Hills HG. 2012. Taxonomic revision of Dressleria (Orchidaceae, Catasetinae). Phytoneuron. 48:1–28.

Jakubska Busse A, Jasicka-Misiak I, Poliwoda A, Święczkowska E, Kafarski P. 2014. The chemical composition of the floral extract of *Epipogium aphyllum* Sw. (Orchidaceae): a clue for their pollination biology. Arch Biol Sci. 66(3):989–998. doi:10.2298/ABS1403989B.

Karremans AP. 2023. Desmistifying orchid pollination. Richmond: Kew Publishing, Royal Botanic Gardens, Kew. p. 440.

Milet Pinheiro P, Gerlach G. 2017. Biology of the Neotropical orchid genus *Catasetum*: a historical review on floral scent chemistry and pollinators. Perspect Plant Ecol Evol Syst. 27:23–34. doi:10.1016/j.ppees.2017.05.004.

Neubig KM, Whitten WM, Williams NH, Blanco MA, Endara L, Burleigh JG, Silvera K, Cushman JC, Chase MW. 2012. Generic recircum scriptions of Oncidiinae (Orchidaceae: cymbidieae) based on maximum likelihood analysis of combined DNA datasets. Bot J Linn Soc. 168(2):117–146. doi:10.1111/j.1095-8339.2011.01194.x.

Nunes CE, Peñaflor MFG, Bento JMS, Salvador MJ, Sazima M. 2016. The dilemma of being a fragrant flower: the major floral volatile attracts pollinators and florivores in the euglossine-pollinated orchid *Dichaea pendula*. Oecologia. 182(4):933–946. doi:10.1007/s00442-016-3703-5.

Opedal ØH, Martins AA, Marjakangas EL. 2020. A database and synthesis of euglossine bee assemblages collected at fragrance baits. Apidologie. 51(4):519–530. doi:10.1007/s13592-020-00739-4.

Pessoa E, Silva MJC, Oliveira MS, Silva-Junior WR, Ferreira AWC. 2022. An updated checklist reveals strong incongruences with previous studies: insights after revisiting a regional orchid list. Acta Bot Bras. 36:1–14. doi:10.1590/0102-33062020abb0487.

Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R. 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem. 91(4):621–632. doi:10.1016/j.foodchem.2004.06.031.

Sazima M, Vogel S, Cocucci A, Hausner G. 1993. The perfume flowers of Cyphomandra (Solanaceae): pollination by euglossine bees, bellows mechanism, osmophores, and volatiles. Pl Syst Evol. 187(1-4):51–88. doi:10.1007/BF00994091.

Silva MJC, Pansarin ER, Pessoa E, Silva EO, Albuquerque PMC, Oliveira MS, Silva-Junior WR, Santos KN, Lima JF, Ferreira AWC. 2022. Synopsis of orchidaceae from Fazenda Sete Irmãos: a fragment of Amazon Forest in northwestern Maranhão, Brazil. Rodriguésia. 73:1–19. doi:10.1590/2175-7860202273044.

Singer RB, Koehler S. 2003. Notes on the pollination biology of *Notylia nemorosa* (Orchidaceae): do pollinators necessarily promote cross pollination? J Plant Res. 116(1):19–25. doi:10.1007/s10265-002-0064-4.

Tan KH, Nishida R. 2012. Methyl eugenol: its occurrence, distribution, and role in nature, especially in relation to insect behavior and pollination. J Insect Sci. 12(56):56–60. doi:10.1673/031.012.5601.

Tan KH, Tan LT, Nishida R. 2006. Floral phenylpropanoid cocktail and architecture of *Bulbophyllum vinaceum* orchid in attracting fruit flies for pollination. J Chem Ecol. 32(11):2429–2441. doi:10.1007/s10886-006-9154-4.

Tatsuka K, Kohama M, Suekane S. 1988. Floral fragrance components of *Zygopetalum mackayi* (Orchidaceae). Agric Biol Chem. 52(6):1599–1600. doi:10.1080/00021369.1988.10868877.

Tsusaka T, Makino B, Ohsawa R, Ezura H. 2019. Genetic and environmental factors influencing the contents of essential oil compounds in *Atractylodes lancea*. PLoS One. 14(5):e0217522. doi:10.1371/journal.pone.0217522.

Vasconcelos FM, Andrade EHA, Teixeira LOA, Figueiredo PLB, Maia JGS. 2023. Volatile constituents from *Catasetum* (Orchidaceae) species with occurrence in the Brazilian Amazon. Plants. 12(4):703. doi:10.3390/plants12040703.

Vasconcelos FM, Andrade EHA, Teixeira LOA, Maia JGS. 2022. Volatile constituents of floral scents from *Encyclia cordigera* (Kunth) Dressler and *E. randii* (Barb. Rodr.) Porto & Brade (Orchidaceae). J Braz Chem Soc. 33(1):96–101. doi:10.21577/0103-5053.20210127.

Walia S, Mukhia S, Bhatt V, Kumar R, Kumar R. 2020. Variability in chemical composition and antimicrobial activity of *Tagetes minuta* L. essential oil collected from different locations of Himalaya. Indus Crops Product. 150:112449. doi:10.1016/j.indcrop.2020.112449.

Warford N. 1992. Pollination biology the reciprocal agreement between *Notylia* and *Euglossa viridissima*. Am Orchid Soc Bull. 61(4):885–889.
Whitten WM, Hills HG, Williams NH. 1988. Occurrence of ipsdienol in floral fragrances. Phytochemistry. 27(9):2759–2760. doi:10.1016/0031-9422(88)80657-5.
Whitten WM, Williams NH, Armbruster WS, Battiste MA, Strekowski L, Lindquist N. 1986. Carvone oxide: an example of convergent evolution in euglossine pollinated plants. Syst. Botany. 11(1):222–228. doi:10.2307/2418960.
Williams NH. 1974. Taxonomy of the genus *Aspasia* Lindley (Orchidaceae: oncidieae). Brittonia. 26(4):333–346. doi:10.2307/2805817.
Williams NH, Whitten WM. 1983. Orchid floral fragrances and male euglossine bees: methods and advances in the sesquidecade. Biol Bull. 164(3):355–395. doi:10.2307/1541248.