

ANTIFUNGAL ACTIVITY AND CHEMICAL CONSTITUENTS OF THE ESSENTIAL OIL FROM LEAVES OF *Nectandra longifolia* (Ruiz & Pav.) Nees

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ACTIVIDAD ANTIFÚNGICA Y CONSTITUYENTES QUÍMICOS DEL ACEITE ESENCIAL DE LAS HOJAS DE *Nectandra longifolia* (Ruiz & Pav.) Nees

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ABSTRACT

"*Yellow moena*" (*Nectandra longifolia*: Lauraceae) is a fragrant leaves-possessing plant commonly used for timber purposes. Nevertheless, so far, there is not available information about its chemical composition. Thus, in the present work is described the components of the essential oil from *N. longifolia* leaves. The oil was extracted by steam distillation and analyzed by GC/FID and GC/MS. Twenty eight compounds were therefore identified. The main components were found to be terpinen-4-ol (53.1%), α -terpinene (6.8%), α -phellandrene (4.5%), and β -pinene (3.8%). The chemical composition of essential oil from *N. longifolia* leaves was consequently in agreement to that of several neotropical Lauraceae plants. In addition, the oil exhibited good antifungal activity against *F. oxysporum* through dose-dependent mycelial growth inhibition (using amended media and fumigant assays).

Keywords: Lauraceae, *Nectandra longifolia*, essential oil, monoterpenes.

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RESUMEN

La "moena amarilla" (*Nectandra longifolia*, Lauraceae) es una planta con hojas fragantes que comúnmente se utiliza para propósitos maderables. Sin embargo, a la fecha, no hay información disponible sobre su composición química. Por consiguiente, en el presente trabajo se describen los componentes del aceite esencial obtenido a partir de las hojas de *N. longifolia*. El aceite se extrajo por destilación por arrastre con vapor y se analizó por CG/FID y CG/EM. De esta manera se identificaron veintiocho compuestos como componentes del aceite obtenido, cuyos principales fueron terpinen-4-ol (53.1%), α -terpineno (6.8%), α -felandreno (4.5%), and β -pineno (3.8%). La composición química del aceite esencial de las hojas de *N. longifolia* fue consecuente con aquellas registradas en aceites de otras plantas neotropicales de la familia Lauraceae. Adicionalmente, el aceite exhibió actividad antifúngica frente a *F. oxysporum* a través de inhibición del crecimiento micelial dependiente de la dosis (usando ensayos de medio suplementado y acción fumigante).

Palabras Clave: Lauraceae, *Nectandra longifolia*, aceite esencial, sesquiterpenos.

INTRODUCTION

Lauraceae is a family of plants mainly distributed in tropical and subtropical regions and composed by 52 genera and nearly 3000 species (Chanderbali et al., 2001). The Lauraceae family is well-known for its role in traditional medicine and common uses (van der Werff and Richter, 1996) and for those phytochemical studies performed on lauraceous plants finding several metabolites, including alkaloids, lignans, neolignans, and flavonoids (Pagotto et al, 1998) with important biological activities (Oliveira-Filho et al., 2015). Plants of this family are also recognized to produce fragrant materials, because of that a plethora of studies had been conducted in order to determine the composition of essential oils of several Lauraceae specimens (Takaku et al., 2007).

Nectandra genus (Lauraceae) is considered endemic in neotropical regions (Macías-Villamizar et al., 2015) with occurrence from Florida to Argentina

but predominantly found in South America (Ciccio et al., 2009). The plant list (www.theplantlist.org; accessed on 26 march 2016) includes 357 scientific plant names at species level for the genus *Nectandra*, but 117 are considered as accepted names and the other 184 as synonyms (Macías-Villamizar et al., 2015). In Colombia, thirty four species are known, distributed especially in zones below 1800 m. *N. longifolia*, known in Colombia as "yellow moena", can be found in Amazonia and Orinoquia regions. It is found in areas with high and constant rainfall, but also in areas with a marked dry season. Its wood is considered as a good quality material, soft, lightweight, fairly durable; because of that It is prized in carpentry, cabinet making and construction. Despite its fragrant leaves, this plant has no background in essential oil composition studies; therefore the exposed information herein is reported for the

first time as well as the antifungal activity against *Fusarium oxysporum*, a commercially-important phytopathogen. Thus, in this paper is presented the chemical and biological analysis of the *N. longifolia*-derived essential oil as prospecting parameter for further studies with this plant.

MATERIALS AND METHODS

Plant Material

Leaves of *N. longifolia* were collected in Casanare, Colombia (coordinates: +5.183, -72.532) in March, 2012. A voucher specimen, numbered COL528624, has been deposited at the Herbario Nacional Colombiano of the Instituto de Ciencias Naturales – Universidad Nacional de Colombia.

Essential oil extraction

Dry leaves (122 g) were finely chopped and steam distilled for 2 h to obtain the essential oil, which was dried over anhydrous sodium sulfate affording the crude oil (0.045 g).

Chemical Analyses

GC/FID. This analysis was carried out on a Shimadzu GC-17A gas chromatograph equipped with a FID and operated in split mode (1:15, injected volume 1 μ L), using a fused silica capillary column HP-5, 30 m x 0.25 mm, 0.5 μ m coating thickness. The operational conditions used were as follows: temperature program from 50°C (4 min) to 300°C (20 min) at 4°C/min, split/splitless injector (300°C), carrier gas was helium at 1.0 mL/min, and makeup gas was nitrogen at 30 mL/min. Quantitation was made by using the Class 5000 software. Relative percentages were calculated by electronic integration of FID, whose peak areas were used without response factor correction. Retention indices (RI) were calculated in order to support the identification using linear hydrocarbons (C₈-C₂₄) (certified standard, Supelco®).

GC/MS. This analysis was carried out on a Shimadzu GC-17A gas chromatograph coupled to a Shimadzu GCMS-QP5050A mass spectrometer (70 eV) using a fused silica capillary column HP-5ms, 30 m x 0.25 mm, 0.5 μ m coating thickness, using identical program temperature as GC/FID. Interface temperature 300°C. Detector voltage: 1.20 kV. Acquisition mass range: 42-800u. Acquisition mode: full scan; scan interval: 0.35 s. Solvent delay: 3 min. The components of the oil were identified by detailed/extensive analyses of their mass spectra, by comparison with those of the compound library (NIST02) and by comparison of their experimental retention indices (RI) with those reported (Adams, 2007).

Antifungal Assays

The phytopathogen *Fusarium oxysporum* was obtained from a commercial culture of Cape gooseberry (*Physalis peruviana*) in the department of Cundinamarca, Colombia. The fungus was cryopreserved in nutrient broth:glycerol 70:30 at -20 °C and reactivated in nutrient agar plates at 25 °C for 48 h for usage. The *in-vitro* antifungal activity against *F. oxysporum* was evaluated through the inhibition of mycelial growth by two protocols (i.e., amended-medium and fumigant assays)

Amended-medium assay. Potato Dextrose Agar (PDA) culture medium was supplemented with the *N. longifolia* essential oil. The final doses (100-0.01 μ g/mL) were reached by a direct mixture between the medium (PDA before solidifying) and the oil, adding the required amount of medium and oil for succeeding the required dose to be evaluated. This mixture was vigorously stirred until achieving a homogenous dispersion before solidifying. The amended medium was quickly added into 6-cm Petri dishes and then a 4-mm agar-mycelial plug from actively growing cultures of *F. oxysporum* was inoculated into the center of the Petri dish (Ochoa-Fuentes *et al.*, 2012). Each trial consisted of a randomized

design with three replicates compared to a plate control (untreated PDA). Prochloraz® was used as positive control. Petri dishes were sealed with parafilm to prevent the leaking of test oil. After inoculation and sealing, the plates were incubated at room temperature (average, 17 °C). Assay was completed once the colony on the untreated dish wholly covered the Petri dish (average, 3 days). Mean colony

area (mm²) was therefore measured for treated and untreated plates and the percent mycelial growth inhibition (MGI) was then calculated for each replicate. %MGI = (mycelial growth area in untreated dish - mycelial growth area in treated dish / mycelial growth area in untreated dish) * 100.

Fumigant assay. PDA culture medium was placed into 6-cm Petri dishes and then a 4-mm agar-mycelial

Table 1. Essential Oil Composition of *N. longifolia* leaves.

No	RI ^b	Compounds ^a	%	No	RI ^b	Compounds ^a	%
1	935	α -pinene	1.4	15	1100	β -linalol	0.4
2	944	camphene	0.6	16	1178	terpinen-4-ol	53.1
3	972	β -pinene	3.8	17	1192	α -terpineol	0.3
4	989	β -myrcene	2.5	18	1349	α -cubebene	1.7
5	1003	α -phellandrene	4.5	19	1377	α -copaene	0.7
6	1018	α -terpinene	6.8	20	1436	β -caryophyllene	1.1
7	1021	ρ -cymene	0.6	21	1498	bicyclogermacrene	2.1
8	1039	limonene	2.3	22	1507	β -bisabolene	0.6
9	1028	1,8-cineole	1.9	23	1512	α -muurolene	0.7
10	1040	β -E-ocimene	0.9	24	1529	δ -cadinene	1.5
11	1046	β -Z-ocimene	1.5	25	1550	α -cadinene	1.2
12	1164	borneol	2.4	26	1605	guaiol	0.8
13	1069	γ -terpinene	0.7	27	1654	α -muurolol	1.1
14	1084	α -terpinolene	2.4	28	1660	α -cadinol	0.9
Monoterpene hydrocarbons (%)							12 (74.3%)
Oxygenated monoterpenes (%)							5 (11.4%)
Sesquiterpene hydrocarbons (%)							8 (9.6%)
Oxygenated sesquiterpenes (%)							3 (4.3%)
Total							28 (98.5%)

^aCompounds are listed in order of RI values; ^bRI = Retention Indices (experimental) as calculated from retention times recorded on GC using a HP-5 column and a homologous series of *n*-alkanes C₈-C₂₄, under identical GC conditions.

plug from actively growing cultures of *F. oxysporum* was inoculated into the center of the Petri dish. Lids were placed upside down on the Petri dishes. Essential oil was introduced onto an 8-mm paper disc (Whatman N° 4), which was placed on the agar-free lid of the Petri dish (Kim and Park, 2012). Each 8-mm paper disc was prepared adding oil aliquots (1-50 μ L) in order to attain the corresponding doses within the plates (8-390 μ L/L air). Each trial also consisted of a randomized design with three replicates compared to a plate control (untreated PDA). Petri dishes were then sealed with parafilm. After inoculation and sealing, the plates were incubated at room temperature (17 °C average). Assay was completed once the colony on the untreated plate wholly covered the Petri dish (average, 3 days). Mean colony area (mm²) was therefore measured for treated and untreated plates and the percent mycelial growth inhibition (MGI) was then calculated for each replicate. %MGI = (mycelial growth area in untreated dish - mycelial growth area in treated dish / mycelial growth area in untreated dish) * 100.

Statistics

Percent MGI values were used to calculate the half-maximal effective doses (ED₅₀) through non-linear regressions using the software GraphPad prism 5.00 (GraphPad software, San Diego, CA, USA).

RESULTS AND DISCUSSION

The gas chromatogram of the essential oil obtained by steam distillation from leaves of *N. longifolia* showed the presence of thirty three signals, but twenty eight compounds were found to be the main components (98.5%). The cumulative relative abundance of remaining signals was below 0.2%. Main compounds were identified by GC/MS and CG/FID (table 1). The main constituents of the essential oil were terpinen-4-ol (53.1%), α -terpinene (6.8%), α

-phellandrene (4.5%), and β -pinene (3.8%) (Figure 1). The oil was found to be rich in monoterpene hydrocarbons (74.3%) and oxygenated monoterpenes (11.4%), being the menthane-containing monocyclic monoterpenes the most abundant moiety in *N. longifolia* leaves-derived essential oil.

So far, no oil from *N. longifolia* has been described. In addition, despite the several studies performed on oils obtained from plants of other Lauraceae genera (Palazzo *et al.*, 2009; Unlu *et al.*, 2010; Fidelis *et al.*, 2013; Takaku *et al.*, 2007; Chaverri and Cició, 2005; Bruni *et al.*, 2004), the *Nectandra* plants suffer of a lack of chemical studies performed on their essential oils, since only seven oils from *Nectandra* plants had been reported (Macias-Villamizar *et al.*, 2015). However, all of these studies coincide that the composition could fluctuate even inter and intra genera, including those plants producing phenylpropanoid-abundant essential oils (Unlu *et al.*, 2010). Additionally, the nature of Lauraceous oils might be divided into monoterpene chemotypes, such as *N. angustifolia* (Torres *et al.*, 2005), and sesquiterpene chemotypes, such as *N. antillana* (Pino *et al.*, 2014) and *N. leucantha* (Grecco *et al.*, 2015), including common chemical components such as α - and β -pinene, β -caryophyllene, bicyclogermacrene, germacrene-D, menthane derivatives, among others (Amaral *et al.*, 2015). Thus, as expected, *N. longifolia* essential oil shares some components to that of other neotropical Lauraceae plants involving a monoterpene chemotype.

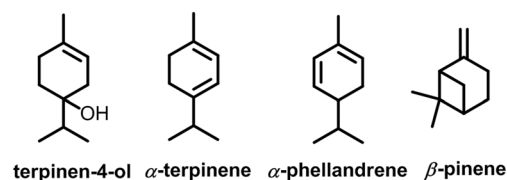


Figure 1. Main components of the essential oil from *N. longifolia* leaves.

Some works have described the good antifungal activity of monoterpene-enriched essential oils (Lucini *et al.*, 2006; Parveeen *et al.*, 2013; Tyagi and Malik, 2010; Terzi *et al.*, 2007). Therefore, the *in vitro* antifungal activity of *N. longifolia*-derived essential oil was evaluated against *F. oxysporum*, a commercially-important phytopathogen. The activity was evaluated using two protocols (i.e., amended-medium and fumigant assays) in order to assess the capability of the oil for inhibiting the mycelial growth of the phytopathogen. The essential oil exhibited a dose-dependent behavior on both protocols as

0.12 $\mu\text{g/mL}$ (Morcia *et al.*, 2012). Additionally, there is important evidences that monoterpenes increase the concentration of lipid peroxides producing fungal cell damage (Lucini *et al.*, 2006) or they bind to ergosterol generating a subsequent destabilization of fungal cell membranes (Miron *et al.*, 2014), which explain the inhibition of fungal development showed by monoterpenes as well as by the essential oil from *N. longifolia* leaves.

In conclusion, the composition of essential oil from *N. longifolia* leaves no significantly differs from reported data of other Lauraceae plants,

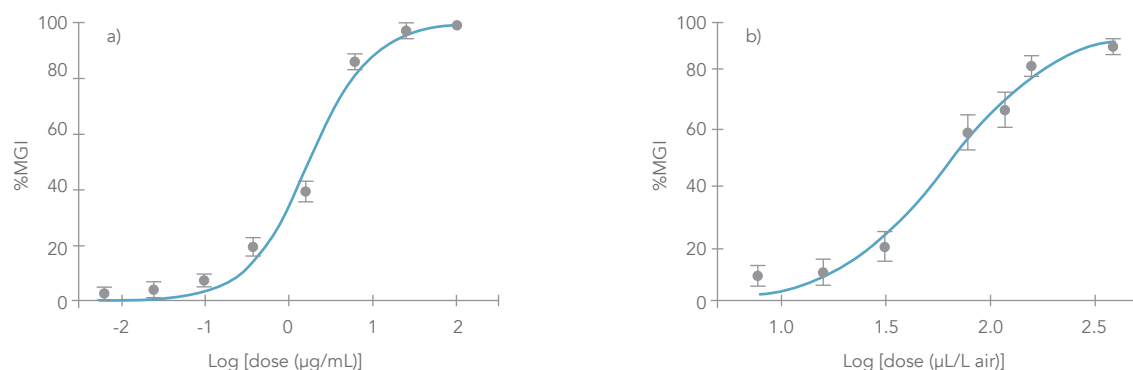


Figure 2. Dose-response curves constructed from the mycelial growth inhibition exhibited by essential oil from *N. longifolia* leaves through **a)** amended-medium assay and **b)** fumigant assay.

observed in Figure 2. ED_{50} values could be thus calculated by non-linear regression. The ED_{50} value for the oil - determined by amended-medium assay - was found to be comparable to the positive control, prochoraz® (1.80 ± 0.29 vs 1.68 ± 0.37 $\mu\text{g/mL}$, respectively), whereas by fumigant assay the ED_{50} was found to be 61.7 ± 8.8 $\mu\text{L/L}$ air. The antifungal activity exhibited by *N. longifolia* essential oil can be rationalized by the abundant presence of terpinen-4-ol (51.3 %), whose activity was individually assessed against other fungi, affording an $ED_{50} = 0.49 \pm$

constituting this specific oil as a source of monoterpene-type compounds. However, seasonal/circadian/ontogenetic variations should be evaluated, since intrinsic and external factors that affect the oil's content and composition, suggesting an important requirement for further studies involving *N. longifolia*. In addition, the oil exhibited good antifungal activity against *F. oxysporum* with $ED_{50} = 1.80 \pm 0.29$ $\mu\text{g/mL}$ and 61.7 ± 8.8 $\mu\text{L/L}$ air obtained after evaluation through amended-medium and fumigant assays, respectively. The antifungal results

can be explained by the abundant presence of terpinen-4-ol (53.1%) as the main constituent of the oil.

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