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# Composition and Acaricidal Activity of *Laurus novocanariensis* and *Laurus nobilis* Essential Oils Against *Psoroptes cuniculi*

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**Abstract**

The major components of *Laurus nobilis* and *L. novocanariensis* leaf oils were identified and their acaricidal activity against *Psoroptes cuniculi* evaluated. Monoterpenes were predominant in *L. nobilis* oil (91.8%), while sesquiterpenes were only 1.4%. The main components of this oil were 1,8-cineole (39.2%), α-terpinyl acetate (11.3%), sabinene (10.6%) and linalool (7.4%). The acaricidal activity of *L. nobilis* oil, at a concentration of 10%, led to a mortality rate of 73%; at 5% the average activity was significantly reduced to 51%, while dilutions of 2.5%, 1.25% and 0.625% were ineffective.

*Laurus novocanariensis* oil, compared to *L. nobilis,* was richer in sesquiterpenes; the main constituents were α-pinene (10.4%), 1,8-cineole (9.6%) and β-selinene (7.2%). After 24 h of contact, the oil of *L. novocanariensis* killed all the mites when used at 10% and 5% concentrations. At lower concentrations the mortality significantly decreased; a dilution of 0.625% was ineffective.

**Key Word Index**

*Psoroptes cuniculi*, *Laurus nobilis*, *Laurus novocanariensis*, Lauraceae, acaricide, essential oil composition, α-pinene, sabinene, 1,8 cineole, α-terpinyl acetate.

## Introduction

The biological activity of essential oils against several organisms has been confirmed in many reports, mainly associated with primary components mono- and sesquiterpenoids (1,2).

The acaricidal activity of many essential oils has been previously reported: Charmil gel containing essences of *Cedrus deodara* Loud. (Pinaceae) and *Pangamia glabra* Vent. (Fabaceae) was used against *Sarcoptes scabiei* (L.) (Sarcoptidae) in dogs (3) and pigs (4). A phyto-aromatic gel (Canidor) composed of more than 15 oils from plants was used against the rabbit mite *Psoroptes cuniculi* (Delafond) (Psoroptidae) (5); linalool and the oils from *Lavandula angustifolia* Mill. (Lamiaceae) and *Artemisia verlotorum* Lamotte (Asteraceae) were evaluated against *P. cuniculi* in rabbits (6,7). Acaricidal activity of pine oils and of their main components against *Tyrophagus putrescentiae*, a stored food mite, was also evaluated (8). A moderate activity against house dust mites was shown by laurel leaf oil (9). Different oils, among them *Laurus nobilis* oil and some monoterpenes, were tested against *Dermatophagoides pteronyssinus* (10).

The composition of the leaf oils of *L. azorica* and *L. nobilis* has been reported (11-13). Prior to 2002, *Laurus* ssp.—endemic of the macaronesian archipelagos of Azores, Madeira and Canaries—were represented as a single species, *Laurus azorica*. Recently, laurels from Madeira and the Canaries have been reconsidered as a separated taxon, classified as *Laurus novocanariensis* (14).

The aim of the present study is the identification of the major components of *L. nobilis* and *L. novocanariensis* leaf

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**Table I. Composition (a) of the leaf oils of *L. novocanariensis* and *L. nobilis***

| Constituents | LRI | *L. novocanariensis* % | *L. nobilis* % |
|---|---|---|---|
| (E)-2-hexenal | 854 | - | 0.1 |
| α-thujene | 933 | t | 0.6 |
| α-pinene | 941 | 10.4 | 5.2 |
| camphene | 955 | t | 0.5 |
| sabinene | 978 | 1.0 | 10.6 |
| β-pinene | 981 | 5.7 | 4.7 |
| myrcene | 992 | - | 1.3 |
| α-phellandrene | 1007 | - | 0.5 |
| δ-3-carene | 1013 | 2.7 | 0.1 |
| α-terpinene | 1020 | - | 0.4 |
| o-cymene | 1024 | 1.2 | - |
| p-cymene | 1028 | 1.1 | 0.3 |
| limonene | 1032 | 0.5 | 2.0 |
| 1,8 cineole | 1035 | 9.6 | 39.2 |
| (E)-β-ocimene | 1051 | - | 0.1 |
| γ-terpinene | 1064 | - | 0.7 |
| *cis*-sabinene hydrate | 1070 | - | 0.6 |
| terpinolene | 1089 | - | 0.3 |
| linalool | 1099 | - | 7.4 |
| *trans*-sabinene hydrate | 1100 | - | 0.2 |
| *cis*-p-menth-2-en-1-ol | 1123 | - | 0.1 |
| *trans*-pinocarveol | 1141 | t | - |
| *trans*-p-menth-2-en-1-ol | 1145 | - | 0.1 |
| pinocarvone | 1164 | 0.7 | |
| δ-terpineol | 1166 | - | 0.3 |
| borneol | 1167 | - | 0.2 |
| terpinen 4-ol | 1180 | 0.7 | 2.1 |
| α-terpineol | 1190 | - | 2.5 |
| linalyl acetate | 1258 | - | 0.1 |
| isobornyl acetate | 1286 | - | 0.4 |
| α-terpinyl acetate | 1351 | 2.4 | 11.3 |
| eugenol | 1356 | - | 1.2 |
| α-copaene | 1377 | 5.0 | - |
| β-bourbonene | 1385 | 0.6 | - |
| methyl eugenol | 1404 | 2.3 | 4.5 |
| β-caryophyllene | 1420 | 1.5 | 0.2 |
| (E)-cinnamyl acetate | 1443 | 1.2 | - |
| allo-aromadendrene | 1461 | 3.9 | - |
| γ-muurolene | 1477 | 1.2 | - |
| germacrene D | 1482 | 1.1 | - |
| γ-selinene | 1485 | 0.8 | |
| β-selinene | 1487 | 7.2 | - |
| valencene | 1492 | 4.3 | - |
| bicyclogermacrene | 1495 | - | 0.2 |
| viridiflorene | 1496 | 0.8 | - |
| δ-cadinene | 1524 | 3.8 | 0.1 |
| α-calacorene | 1542 | 0.6 | - |
| elemicin | 1554 | 5.4 | 0.2 |
| spathulenol | 1577 | 1.1 | 0.5 |
| caryophyllene oxide | 1583 | 3.3 | 0.2 |
| allo-aromadendrene epoxide | 1641 | 0.8 | - |
| cubenol | 1643 | 0.6 | |
| β-eudesmol | 1643 | - | 0.2 |
| eudesma-4(15),7-dien-1β-ol | 1651 | 2.2 | |
| **Total identified** | | **83.7** | **99.2** |
| **Yield %** | | **0.8** | **0.6** |

(a) percentages obtained by FID peak-area normalization, all relative response factors being taken as one (HP-5 column); linear retention indices (HP-5 column); t = trace (< 0.1%)

oils and the evaluation of their acaricidal activity against *Psoroptes cuniculi*.

## Experimental

Leaves of *Laurus nobilis* were collected in June 2003 at Alberaccio (Asciano- Pisa, Italy) on wild trees. A voucher was deposited in the Herbarium of Pisa's botanical garden. Leaves of *L. novocanariensis* were collected in May 2001, at Ponta do Pargo on Madeira Island (Portugal) from a single wild tree. A voucher was deposited in the Herbarium of Madeira's botanical gardens.

The leaves were dried at room temperature away from direct sunlight before distillation. For each species, the oil was obtained from 100 g samples of finely cut dry leaves hydrodistillated for 2 h in a Clevenger-type apparatus.

## Chemical Analysis

The oils were analyzed by gas chromatography with FID GC/MS detection. The GC analyses were accomplished with an HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m x 0.25 mm, 0.25 μm film thickness) operated with the following temperature program: 60°C for 10 min, ramp of 5°C/min up to 220°C; injector and detector temperatures 250°C; carrier gas nitrogen (2 mL/min); detector dual FID; split ratio 1:30; injection of 0.5 μL. The identification of the components was performed, for both the columns, by comparison of their retention times with those of pure authentic samples, and by means of their linear retention indices (LRI) relative to the series of n-hydrocarbons. The relative proportions of the oil constituents were expressed as percentages obtained by FID peak-area normalization, all relative response factors being taken as one.

GC/EIMS analyses were performed on a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm, coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220°C and 240°C, respectively; oven temperature programmed from 60°-240°C at 3°C/min; the carrier gas was helium at 1 mL/min; injection of 0.2 μL (10% hexane solution); split ratio 1:30. Identification of the constituents was performed as before by computer matching against commercial (NIST 98 and ADAMS) and homemade libraries of mass spectra built up from pure substances and components of known oils and MS literature data (15-20). Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS using MeOH as CI ionizing gas.

## Biological Analysis

Disks of filter paper were imbibed with 50 μL of each of the oils, diluted to several concentrations. The disks were put in concave glass capsules having the same dimensions of disks. Adult mites, collected from the ears of a massively infested rabbit, were put in groups of 30 over the disks. The capsules with the disks were then covered with parafilm, finely punched to allow the circulation of air without allowing the mites to escape.

**Table II. Mortality of mites (*Psoroptes cunicoli*) at different concentrations of *L. novocanariensis* and *L. nobilis* (differences for columns)**

| E. O. conc. | *L. novocanariensis* mean | *L. nobilis* mean |
|---|---|---|
| 10% | 100 A | 73 A |
| 5% | 100 A | 51 B |
| 2.5% | 22 B | 9 C |
| 1.25% | 8 BC | 0 C |
| 0.65% | 0 C | 0 C |
| Control | 0 C | 0 C |

A, B: P<0.01

The mobility control of mites was performed after 24 h of contact with the substances. To verify mortality, immobile mites were collected one by one and placed on disks inside capsules without tested substances. Control was performed after another 24 h; the persistence of the immobility of the mites after their stimulation with a needle indicated their death.

The oils of *L. nobilis* and *L. novocanariensis* were used at 10%, 5%, 2.5%, 1.25% and 0.625%. Dilutions were obtained with paraffin oil as the solvent. Paraffin was also used as control to ensure its innocuousness for mites.

## Statistical Analysis

Data were subjected to ANOVA in which oil type (*Laurus novus* and *Laurus novocanariensis*) and different concentration levels (10, 5, 2.5, 1.25, 0.65, control) were considered as fixed effects and percentage mortality as the independent variable. Statistical analysis was undertaken using the statistical package JMP (SAS Institute, 2002).

## Results and Discussion

The yield of the leaf oil was 0.8% for *L. novocanariensis* and 0.6% for *L. nobilis*. In *L. nobilis* oil the monoterpenes were predominant (91.8%), while sesquiterpenes represented only 1.4%. Aromatic compounds were present at 5.9%; 71.4% of all constituents were oxygenated. The main components were 1,8-cineole (39.2%), α-terpinyl acetate (11.3%), sabinene (10.6%), linalool (7.4%), α-pinene (5.2%), β-pinene (4.7%) and methyl eugenol (4.5%). *Laurus novocanariensis* leaf oil components proved more difficult to identify. Unambiguous identification was only achieved for about 80% of components, of which monoterpenes accounted for 36% and identified sesquiterpenes 38.8%. The aromatic compounds were 8.9% of the oil, while the oxygenated components were 30.3%. The main constituents were α-pinene (10.4%), 1,8-cineole (9.6%), β-selinene (7.2%), β-pinene (5.7%), elemicin (5.4%) and α-copaene (5%). *Laurus novocanariensis* leaf oil, compared to *L. nobilis*, was richer in sesquiterpenes, especially considering that many of the unidentified components had an m/z of 204 and an LRI that indicated they were probably sesquiterpene compounds as well. The most remarkable differences were observed with the greater amounts of α- and β-pinene, δ-cadinene, elemicin, caryophyllene oxide and δ-3-carene in *L. novocanariensis*, which also contained other components such as α-copaene, allo-aromadendrene, β-selinene or valencene. On the contrary, *L. nobilis* had higher contents of 1,8-cineole, sabinene, α-terpenyl acetate and methyl eugenol; it also contained linalool and α-terpineol, which were absent in *L. novocanariensis*.

The oil of *L. novocanariensis*, at concentrations of 10% and 5% after 24 h of contact with mites, killed all the mites, showing an acaricidal activity of 100%; at 2.5%, the mortality of the mites significantly decreased to 22%; at 1.25% and at 0.65% concentrations, the mortality was further reduced, respectively, to 8% and 0%—largely ineffective. From these data, it can be established that the minimum concentration leading to death of the mite is 5%.

The acaricidal activity of *L. nobilis* oil, at a concentration of 10%, led to a mortality of 73% (average of three independent assays), while at 5% the average activity was significantly reduced to 51%. Dilutions of 2.5%, 1.25% and 0.625% were found to be significantly ineffective.

Compared to *L. nobilis*, *L. novocanariensis* oil showed a more intense and significant activity at 5% (P<0.05) and 10% (P<0.01).

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