

## CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF *ARAUCARIA EXCELSA* R. Br. CULTIVATED IN EGYPT

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### ABSTRACT:

The essential oil of leaves, stem bark and wood of *Araucaria excelsa* were prepared by hydrodistillation. Analysis of the oils by GC and GC-MS resulted in the identification of 78 compounds. The diterpene; phyllocladene (37.86%) is the major compound of leaves oil, while  $\alpha$ -pinene (16.46%) and caryophyllene oxide (15.71%) are the major components of bark oil. The diterpenoids; kaurene, isopimarol and phyllocladanol are the major constituents of wood oil in relative percentage of 18.59%, 12.09% and 10.73%, respectively. The major component of the leaves oil; phyllocladene was isolated and its structure was established by spectral data (UV, IR, MS,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR). All the prepared oils and phyllocladene exhibited marked antimicrobial and antifungal activities.

### INTRODUCTION

Araucariaceae (Araucaria family, Monkey-Puzzle tree family) is characterized by ever-green resinous trees represented by only two genera *Araucaria* and *Agathis* comprising about 38 species<sup>(1,2)</sup>. *Araucaria excelsa* R. Br. (Norfolk - Island - Pine) is a pyramidal tree of height about 200 ft which introduced to Egypt and cultivated as an ornamental plant<sup>(1,3)</sup>.

Chemical investigation of the oil obtained from the terminal leafy branchlets of *Araucaria excelsa* growing in New Zealand resulted in the isolation and identification of three compounds,  $\alpha$ -pinene, phyllocladene and isophyllocladene<sup>(4,5)</sup>. Also, a number of known diterpenes were isolated from oleoresin of *Araucaria excelsa*<sup>(6,7)</sup>.

Nothing is available in literature concerning the chemical constituents and the biological activity of the Egyptian species. Thus, it was deemed of interest to study the chemical constituents and the antimicrobial activity of *Araucaria excelsa* cultivated in Egypt.

In the present work, the volatile oils prepared from leaves, stem bark and wood were analyzed using GC and GC-MS. The main component of leaves oil was isolated and its structure was elucidated using different spectral analysis (UV, IR, MS,  $^1\text{H}$ -,  $^{13}\text{C}$ -NMR, APT, DEPT, HSQC and HMBC). The qualitative and quantitative variations of the different three oils have been recorded. Also, the antimicrobial activities of the oils were evaluated.

### EXPERIMENTAL

#### Material and methods:

**Plant material:** The branches of *Araucaria excelsa* R. Br. (Araucariaceae) were collected in September 1999 from plants cultivated in Al-Montazah garden, Alexandria, Egypt. The plant was kindly identified by Dr. Nabil El-Hadidi, Professor of Plant Taxonomy, Faculty of Science, Cairo University. A voucher specimen is kept at the Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Egypt.

Melting point was determined on a digital melting point apparatus, electrothermal LTD, England and was

uncorrected; UV spectrum was recorded on Shimadzu UV-260 (Japan) and IR on Beckham (IR) 4220 double beam spectrophotometer; EIMS was recorded on a Finnigan MAT 4500 spectrometer operating at 70 eV;  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were obtained on a Varian MAT at 300 and 75 MHz, respectively; silica gel 60 (Merck, 0.015 - 0.040 mm) was used for column chromatography; precoated TLC sheets (silica gel 60 GF<sub>254</sub>, Merck) were used.

#### Methods:

##### Preparation of oils:

The volatile oils were prepared from fresh leaves, stem bark and wood by steam distillation using E.P. method<sup>(8)</sup> and the percentage yield (v/w) was found to be 1.40%, 0.20% and 0.05%, respectively. The oils were dried with anhydrous sodium sulphate and kept at 4°C in sealed vials for analysis.

##### Analysis of oil:

GC analysis was carried out on a Varian 3400 Gas Chromatograph equipped with a fused silica column (DB5, 30 m  $\times$  0.25 mm I.D., 0.25  $\mu\text{m}$  film thickness). J&W P/N: 122 - 5032 under the following conditions: carrier gas: He with flow rate 2 ml/min<sup>-1</sup>; detector: FID; temp. 300°C; inj. temp.: 250°C; split ratio: 1 : 10; oven temp. program: initial temp.: 50°C for 4 min, 50 - 90°C at 4°C/min<sup>-1</sup>, 90 - 300°C at 10°C/min<sup>-1</sup> then hold for 10 min. Kovats retention indices (RI) were calculated using co-chromatographed standard n-alkane mixture (C-8 to C-24)<sup>(9)</sup>. Quantitation of the components was performed on the bases of their GC peak areas. For GC-MS analysis: GC conditions were mentioned above, and the capillary column was directly coupled with a quadrupole mass spectrometer (Finnigan MAT SSQ 7000). EIMS were recorded at 70 eV.

Qualitative analysis of the oil constituents was performed by comparing their retention indices and mass fragmentation patterns with the published data<sup>(10-14)</sup> and also by the aid of the computer library search. The results of GC and GC-MS analysis are recorded in Table 1.

### Isolation of the major compound:

The fresh leaves of *Araucaria excelsa* (1.5 Kg) was subjected to hydrodistillation to give 20 ml of yellowish-white fragrant oil. On cooling this oil, white precipitate was separated. TLC investigation of the precipitate (light petroleum - EtOAc, 9.5 : 0.5) revealed one major spot ( $R_f$  0.43). About 1.1 g of the precipitate was packed on silica gel column (80 g, 2.5 × 50 cm) eluted with n-hexane and the polarity was increased in gradient manner with light petroleum then with benzene. White needle-shaped crystals (730 mg) were successfully isolated from the fractions eluted with light petroleum/benzene (9:1). The isolated compound showed melting point 96-97°C;

UV:  $\lambda_{max}$  MeOH 227 nm; IR:  $\nu_{max}^{KBr}$  3070, 2900, 2850, 1650, 1440, 1380, 1360 and 870  $cm^{-1}$ ; EIMS m/z (rel. abund %): 272 ( $M^+$ , 100), 257 (59), 230 (36), 229 (87), 228 (16), 187 (24), 175 (18), 148 (23), 147 (24), 137 (19), 133 (63), 131 (18), 123 (39), 121 (18), 119 (33), 109 (31), 108 (26), 107 (27), 106 (26), 105 (46), 95 (35), 93 (37), 91 (71), 81 (45), 79 (47), 77 (28), 69 (51), 67 (45), 55 (69) and 53 (24).  $^1H$ - and  $^{13}C$ -NMR data are recorded in Table 2.

### Antimicrobial Activity

The antimicrobial activity of the oils of different plant parts as well as the isolated compound phyllocladene was carried out, adopting the agar diffusion method<sup>(15)</sup>. Each cup was accurately filled with 70  $\mu$ l of 10% of each oil and the isolated compound in dimethylformamide (DMF). The potency was tested against *Staphylococcus aureus*, *Sarcina lutea* and *Bacillus subtilis* (Gram +ve bacteria), *Escherichia coli* (Gram -ve bacteria), *Candida albicans* and *Aspergillus* spp (fungi). The microorganisms used were obtained from stock cultures of the Department of Microbiology, Faculty of Pharmacy, Zagazig University. The plates were incubated overnight at 37°C in case of bacteria and at 30°C in case of fungi. The diameters of inhibition zones were measured (mm) against Penicillin (150  $\mu$ g/ml) and Nystatin (250  $\mu$ g/ml) as reference antibiotic and antifungal, respectively. The results are recorded in Table 3.

## RESULTS AND DISCUSSION

Results of the qualitative and quantitative analysis of the essential oils of leaves, stem bark and wood of *Araucaria excelsa* are recorded in Table 1.

The previous work on the New Zealand species *Araucaria excelsa* established identification of  $\alpha$ -pinene, phyllocladene and isophyllocladene<sup>(4,5)</sup>. In the present work, we have identified altogether 78 compounds from the oils of the studied organs (leaves, stem bark and wood). Diterpenoidal compounds compose the main constituents of leaves oil (53.99%) and wood oil (49.69%), while sesquiterpenoids constitute the major constituents of stem bark oil (41.36%).

From the volatile oil of leaves (pale yellow, aromatic odour) 38 compounds were identified

constituting 97.0%. Phyllocladene (37.86%) was isolated and its structure was elucidated by different spectral methods. In addition,  $\gamma$ -cadinene (9.14%),  $\alpha$ -himachalene (5.03%) (sesquiterpene hydrocarbons), limonene (6.43%) and *p*-mentha-1(7),8-diene (5.66%) (monoterpene hydrocarbons) were the submajor components.

In the stem bark oil (yellow, aromatic odour), oxygen containing sesquiterpenes constitute (31.13%), the major compound is caryophyllene oxide (15.71%), while 14-hydroxy-9-epi-(*E*)-caryophyllene constitute (8.26%) and khusinol (6.94%). From monoterpene hydrocarbon (24.22%)  $\alpha$ -pinene was found to be 16.46%. Oxygenated monoterpenes constitute only 2.14%. Abietadiene (8.49%), isokaurene (4.44%), kaurene (2.59%) and a mixture of abietatriene and manool (10.07%) were the major diterpenoid components. Abital was identified in leaves and bark oils (2.86% and 3.56%, respectively).

Oil of the wood (pale yellow, aromatic odour) was richer in the delayed compounds than the earlier eluted ones; in particular, oil is rich with diterpenes; kaurene (18.59%), isopimarol (12.09%) and phyllocladanol (10.73%).  $\alpha$ -Copaene (8.57%),  $\gamma$ -muurolene (4.31%) and  $\gamma$ -cadinene (2.37%) are the major sesquiterpene hydrocarbons, while caryophyllene oxide (6.90%) and 1-epi-cubenol (3.06) are the major components of oxygen - containing sesquiterpenes group. Monoterpene hydrocarbons constitute 4.87%; the major component is tricyclene (3.75), while oxygen containing monoterpenes constitute 4.30% and borneol (1.71%) is the major one.

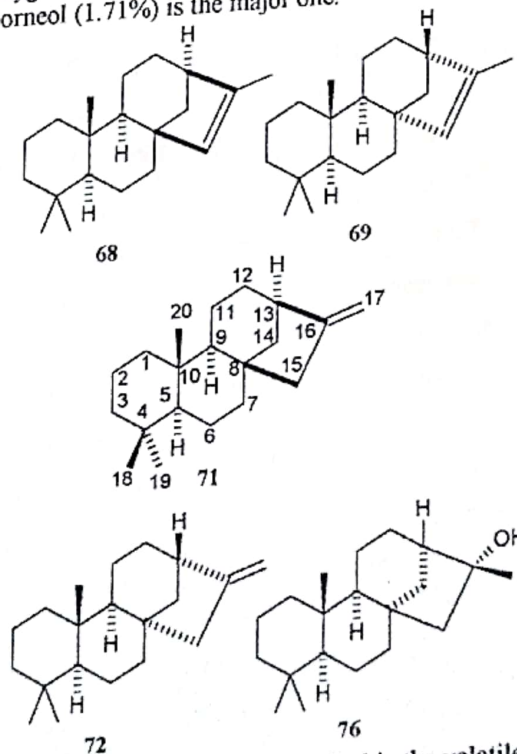


Fig. 1: Kaurene diterpenes identified in the volatile oils of *Araucaria excelsa* R. Br.

The above results obviously show qualitative and quantitative differences in the composition of the essential oils obtained from the different plant parts of

*Araucaria excelsa*.

The major component of leaves oil was identified as phyllocladene; previously identified in the oil of the New Zealand species; by comparing its retention index (2009) and mass fragmentation pattern with the published data<sup>(10)</sup>. Phyllocladene (Fig. 1) was isolated and its structure was confirmed through different spectral data. EIMS showed M<sup>+</sup> at m/z 272 for molecular formula C<sub>20</sub>H<sub>32</sub>. IR spectrum displayed absorption bands at 3070 cm<sup>-1</sup> (=CH<sub>2</sub>), 1650 cm<sup>-1</sup> (C=C) and 870 cm<sup>-1</sup> (=CH<sub>2</sub> bending)<sup>(16)</sup>. <sup>13</sup>C-NMR data (Table 2) showed the presence of 20 carbons; three methyls, ten methylene, three methines and four quaternary carbons through APT and DEPT experiments. <sup>1</sup>H-NMR spectrum (Table 2) displayed the characteristic exocyclic methylene protons (H-17) at 4.70 ppm (1H, d, J = 1.2 Hz) and 4.74 ppm (1H, d, J = 1.2 Hz) and three methyl singlets at 0.86, 0.81 and 0.92 ppm for H-18, 19 and 20, respectively.

In the HMBC spectrum (Fig. 2, Table 2) H-13 resonance at 2.52 ppm gave long range correlation with carbon signals at δ 34.01 (C-14), 50.35 (C-15), 157.63 (C-16) and 102.32 (C-17) supporting kaurene structure. The exact chemical shift of (C-3) and (C-1) was confirmed through long range correlation between (H<sub>2</sub>-1) and carbon signals at δ 56.93 (C-9) and δ 15.11 (C-20).

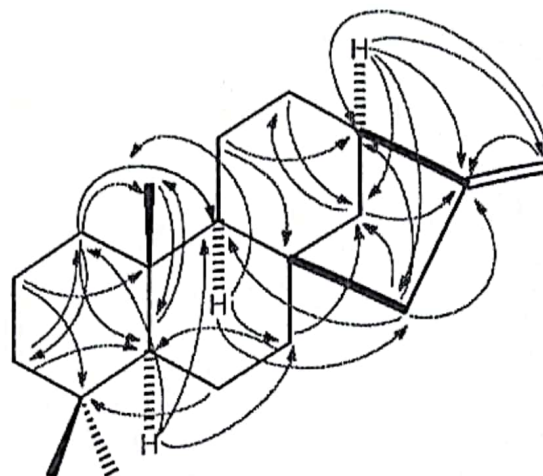


Fig. 2: HMBC of phyllocladene

The obtained results as well as the available literature of the related compounds<sup>(17-22)</sup> confirmed the structure of phyllocladene (8β, 13β-kaur-16-ene). To the available knowledge, <sup>1</sup>H- and <sup>13</sup>C-NMR assignments of phyllocladene are addressed herein for the first time.

Concerning the antimicrobial activity (Table 3), the volatile oils of stem bark and wood showed strong antimicrobial activity against the tested strains for both bacteria and fungi. The leaves oil and its major component (phyllocladene) exhibited significant activity against the tested strains for both bacteria and fungi except *sarcina lutea* (Gram +ve cocci).

Table 1: GC-MS Analysis of the essential oils of leaves, stem bark and wood of *Araucaria excelsa* R. Br.

| No. | Compound              | RI*  | Parent ion (M <sup>+</sup> ) | Base peak (B.p) | Major peaks               | Percentage |       |      |
|-----|-----------------------|------|------------------------------|-----------------|---------------------------|------------|-------|------|
|     |                       |      |                              |                 |                           | Leaves     | Bark  | Wood |
| 1   | Tricyclene            | 933  | 136                          | 93              | 91,77,41,121,105,67       | 0.24       | 1.61  | 3.75 |
| 2   | α-Thujene             | 938  | 136                          | 93              | 91,77,41,121,105,51,65    | 0.20       | 1.69  | --   |
| 3   | α-Pinene              | 945  | 136                          | 93              | 91,92,79,77,41,121,105    | 0.13       | 16.46 | --   |
| 4   | Verbenene             | 972  | 134                          | 91              | 41,119,77,65,51,105       | --         | 0.67  | --   |
| 5   | Sabinene              | 982  | 136                          | 93              | 41,91,77,79,51,65,121,107 | --         | 1.00  | --   |
| 6   | β-Pinene              | 985  | 136                          | 93              | 79,69,53,121,105,107      | --         | 0.45  | --   |
| 7   | Myrcene               | 994  | 136                          | 41              | 93,69,79,53,81,107        | --         | 0.16  | --   |
| 8   | p-Mentha-1(7),8-diene | 1001 | 136                          | 93              | 41,91,77,79,53,121,105,67 | 5.66       | --    | --   |
| 9   | α-Phellandrene        | 1006 | 136                          | 93              | 91,77,41,79,69,53,65,121  | 3.47       | --    | --   |
|     | Unidentified          | 1008 | 136                          | 93              | 41,69,79,91,77,53,121,107 | 1.55       | --    | --   |
| 10  | Isosylvestrene        | 1011 | 136                          | 93              | 121,79,67,41,107,53       | 0.19       | --    | --   |
| 11  | p-Cymene              | 1030 | 134                          | 119             | 91,41,67,77,53,65,105     | --         | 1.45  | 1.12 |
| 12  | Limonene              | 1036 | 136                          | 68              | 93,67,79,53,41,107,121    | 6.43       | 0.61  | --   |
| 13  | γ-Terpinene           | 1067 | 136                          | 93              | 91,77,121,41,105,65       | 1.01       | --    | --   |
| 14  | Terpinolene           | 1085 | 136                          | 93              | 121,91,79,41,77,105,53,67 | 1.27       | --    | --   |

Table 1: continued

|    |                                  |      |      |     |   |          |      |      |
|----|----------------------------------|------|------|-----|---|----------|------|------|
| 15 | $\alpha$ -Pinene oxide           | 1093 | 152  | 41  | 43,67,53,55,109,93,83                   | --       | 0.36 | --   |
| 16 | <i>cis</i> -Thujone              | 1096 | 152  | 41  | 67,53,55,109,81,95                      | --       | 0.46 | --   |
| 17 | <i>trans</i> -Thujone            | 1096 | 152  | 41  | 67,55,82,81,109,95                      | 0.25     | --   | --   |
| 18 | $\alpha$ -Campholenol            | 1126 | 152  | 108 | 93,67,41,81,55,119                      | 1.01     | 0.12 | --   |
| 19 | <i>trans</i> -Pinocarveol        | 1139 | 152  | 41  | 55,91,79,109,70,119,134                 | 0.26     | 0.15 | --   |
| 20 | <i>cis</i> -Verbenol             | 1141 | 152  | 41  | 94,109,79,67,59,119,137                 | 0.30     | 0.37 | --   |
| 21 | Camphor                          | 1143 | 152  | 95  | 41,81,55,108,109,67,137                 | --       | --   | 0.58 |
| 22 | <i>trans</i> -Verbenol           | 1144 | 152  | 41  | 55,91,81,67,109,119,137                 | 1.75     | --   | --   |
| 23 | <i>p</i> -Menth-8-en-1-ol**      | 1148 | 154  | 43  | 93,71,79,55,121,107,139                 | 1.85**   | --   | --   |
| 24 | <i>p</i> -Menthan-8-ol**         | 1148 | n.d* | 59  | 43,81,123,67,155                        | --       | --   | --   |
| 25 | Borneol                          | 1163 | 154  | 95  | 41,43,110,55,67,136,121,111             | --       | --   | 1.71 |
| 26 | 3-Thujanol**                     | 1165 | n.d. | 43  | 95,55,121,67,81,107,136                 | } 0.32** | --   | --   |
| 27 | $\alpha$ -Phelandrene-8-ol**     | 1165 | n.d. | 59  | 79,91,94,43,109,119                     |          | --   | --   |
| 28 | <i>p</i> -Menth-1-en-8-ol        | 1191 | n.d. | 59  | 43,136,121,93,81,67,107                 | 0.72     | --   | 0.86 |
| 29 | Myrtenol                         | 1210 | 152  | 79  | 91,41,108,107,67,119,121, 136           | 0.11     | --   | --   |
| 30 | Verbenone                        | 1215 | 150  | 107 | 41,91,135,79,55,67,122                  | 0.26     | --   | --   |
| 31 | <i>trans</i> -Carveol            | 1223 | 152  | 109 | 41,84,55,91,69,119,137,123              | 0.25     | --   | --   |
| 32 | <i>trans</i> -Ocimenone          | 1245 | 150  | 41  | 91,135,107,79,55,67,95,83               | --       | 0.53 | --   |
| 33 | 2-Ethylmenthone                  | 1276 | 182  | 55  | 41,69,83,97,111,125,140,153,167         | 0.25     | --   | 1.15 |
| 34 | Methyl nerolate                  | 1284 | 182  | 41  | 69,83,91,109,95,123,139,151             | --       | 0.15 | --   |
| 35 | $\alpha$ -Cubebenc               | 1355 | 204  | 41  | 105,119,161,81,91,55,65,133             | --       | --   | 0.26 |
| 36 | Cyclosativene                    | 1373 | 204  | 41  | 105,119,161,91,79,55,133, 189,147       | --       | 4.88 | --   |
| 37 | $\alpha$ -Copaene                | 1379 | 204  | 161 | 119,105,41,91,81,77,55,67, 133,189,147  | 1.59     | --   | 8.57 |
| 38 | $\beta$ -Bourbonene              | 1388 | 204  | 81  | 80,123,41,161,105,91,53,133             | --       | --   | 1.13 |
|    | Unidentified                     | 1412 | 202  | 43  | 41,55,91,118,131,105,81,77,159,1 61,175 | --       | --   | 0.17 |
| 39 | $\beta$ -Caryophyllene           | 1420 | 204  | 41  | 91,79,53,105,133,67,119,161,189         | --       | 2.33 | --   |
| 40 | $\beta$ -Cedrene                 | 1420 | 204  | 161 | 41,120,91,105,79,133,69,147             | --       | --   | 0.29 |
| 41 | $\beta$ -Gurjurene               | 1433 | 204  | 161 | 41,91,105,119,77,133,147, 189           | --       | --   | 0.52 |
| 42 | $\alpha$ -Himachalene            | 1446 | 204  | 41  | 93,79,91,133,105,119,161, 189           | 5.03     | --   | --   |
| 43 | Seychellene                      | 1456 | 204  | 41  | 91,79,133,105,161,119,147, 189,175      | 0.39     | 2.13 | --   |
| 44 | $\gamma$ -Muuroleene             | 1478 | 204  | 161 | 41,105,119,91,79,133,189, 149,147       | --       | --   | 4.31 |
| 45 | $\alpha$ -Muuroleene             | 1500 | 204  | 105 | 161,41,93,79,119,133,189, 147           | --       | --   | 1.81 |
| 46 | <i>trans</i> - $\beta$ -Guaizene | 1510 | 204  | 41  | 105,91,161,119,133,189,147,175          | --       | 0.37 | --   |
| 47 | $\gamma$ -Cadinene               | 1519 | 204  | 161 | 91,41,105,79,119,133,147, 189,175       | 9.14     | 0.52 | 2.37 |

Table I: continued

|    |  |      |      |     |   |      |       |        |
|----|--|------|------|-----|---|------|-------|--------|
| 48 | <i>cis</i> -Calamenene                             | 1525 | 202  | 159 | 41,131,91,105,115,144,172                                 | --   | --    | 0.26   |
| 49 | $\Delta$ -Cadinene                                 | 1528 | 204  | 161 | 119,105,41,91,134,81,189, 145                             | 0.30 | --    | --     |
| 50 | $\alpha$ -Calacorene                               | 1547 | 200  | 157 | 142,41,115,91,55,105,77,129                               | --   | --    | 0.26   |
| 51 | Caryophyllene oxide                                | 1588 | 220  | 41  | 79,91,67,55,107,121,135,149,161, 177,187                  | 0.63 | 15.71 | 6.90   |
| 52 | Humulene-1,2-epoxide                               | 1608 | 220  | 43  | 138,67,109,96,81,123,161, 149                             | --   | --    | 1.29   |
| 53 | $\beta$ -Oplopanone                                | 1610 | 220  | 43  | 177,91,79,107,121,159,135, 205,187                        | --   | --    | 0.81   |
| 54 | 1- <i>epi</i> -Cubanol                             | 1630 | 222  | 41  | 161,119,105,95,55,81,133, 149                             | --   | --    | 3.06   |
| 55 | Cedr-8(15)-en-9- $\alpha$ -ol                      | 1646 | 220  | 43  | 121,79,55,159,69,135,177, 187,205                         | --   | --    | 1.97   |
| 56 | 14-Hydroxy-9- <i>epi</i> ( $\beta$ )-caryophyllene | 1665 | 220  | 41  | 91,55,79,105,119,133,149, 159,177,189                     | --   | 8.26  | 1.81   |
| 57 | Khusinol   | 1674 | 220  | 41  | 91,55,79,105,159,121,133, 177,187,202                     | --   | 6.94  | 1.44   |
| 58 | Cedr-8(15)-en-9- $\alpha$ -ol acetate              | 1740 | 262  | 43  | 220,91,105,55,131,177,81, 69,187,202                      | --   | --    | 1.10   |
| 59 | $\beta$ -Acoradienol                               | 1760 | 220  | 41  | 55,91,79,67,105,135,119,147,159, 177,189                  | --   | 0.22  | --     |
| 60 | ( <i>Z</i> )-Lanceol                               | 1766 | 220  | 43  | 55,93,67,159,119,105,79,134,148, 173,202                  | --   | --    | 0.35   |
| 61 | <i>trans</i> -Dihydro-occidentalol acetate**       | 1785 | 264  | 43  | 81,109,67,55,163,191,149, 135,177,206                     | --   | --    | 0.23** |
| 62 | Hinesol acetate**                                  | 1785 | 264  | 43  | 161,119,105,91,147,79,55,189,20 4,133                     | --   | --    |        |
| 63 | Laurenene  | 1877 | 272  | 41  | 91,55,79,105,76,257,133,148,159, 187,173,201,216          | 3.35 | --    | --     |
| 64 | Oplopanonyl acetate                                | 1887 | 280  | 43  | 81,91,55,107,121,135,150,67,177, 220,205                  | --   | --    | 0.29   |
| 65 | <i>epi</i> -Laurenene                              | 1895 | 272  | 41  | 91,55,105,79,67,119,257,133,161, 201,216                  | 0.38 | --    | --     |
| 66 | Isopimar-9(11),15-diene                            | 1904 | 272  | 41  | 55,91,79,105,272,161,119, 133,175,230                     | 0.31 | --    | 0.35   |
|    | Unidentified                                       | 1915 | 272  | 43  | 41,55,256,91,121,69,145,159,257, 185,175                  | --   | --    | 0.35   |
|    | Unidentified                                       | 1923 | 272  | 41  | 91,69,55,95,105,123,137,229,257, 133,148,187,159,175, 201 | 4.04 | --    | --     |
|    | Unidentified                                       | 1925 | n.d. | 43  | 41,121,55,95,256,80,107,135,159, 175                      | --   | --    | 0.49   |
| 67 | Sclarene   | 1965 | 272  | 41  | 91,55,79,67,105,119,133,257,187, 175,161,201              | 3.08 | --    | --     |
| 68 | Isophyllocladene                                   | 1969 | 272  | 120 | 41,106,105,91,67,79,257,133,161, 187,230                  | --   | --    | 0.90   |

Table 1: *continued*

|    |                                  |      |        |     |   |       |           |       |
|----|----------------------------------|------|--------|-----|---|-------|-----------|-------|
| 69 | Isokaurene                       | 1990 | 272    | 41  | 91,55,79,67,105,119,147,131,229,<br>187,175,163                   | 0.80  | 4.44      | 0.38  |
| 70 | epi-13-Manoyl oxide              | 2007 | n.d.   | 43  | 55,81,95,257,123,109,161,<br>177,275,137,191                      | --    | --        | 3.40  |
| 71 | Phyllocladene                    | 2009 | 272    | 41  | 55,91,79,67,105,123,119,133,145,<br>229,257,175,187,201           | 37.86 | 0.48      | --    |
| 72 | Kaurene                          | 2031 | 272    | 41  | 55,91,79,69,105,123,119,133,147,<br>229,257,175                   | 5.12  | 2.59      | 18.59 |
| 73 | Abietatriene**                   | 2053 | 270    | 255 | 41,173,159,55,69,185,213,<br>143,199                              | --    | } 10.07** | 0.40  |
| 74 | Manool**                         | 2058 | 272    | 43  | 55,81,95,71,137,257,123,109,161,<br>177,229                       | --    |           | 3.20  |
|    | Unidentified                     | 2062 | 286    | 81  | 41,55,93,107,135,120,147,<br>187,257,161,177,230,243, 201,<br>271 | --    |           | 0.41  |
| 75 | Abietadiene                      | 2086 | 272    | 41  | 55,81,91,105,133,229,257,<br>145,187,173,201                      | --    | 8.49      | --    |
|    | Unidentified                     | 2141 | **n.d. | 43  | 257,55,95,161,81,105,133,<br>286,171,213,237                      | --    | --        | 0.41  |
| 76 | Phyllocladanol                   | 2215 | 290    | 43  | 41,123,95,232,109,134,81,69,55,1<br>47,272,257,191                | 0.23  | -         | 10.73 |
| 77 | Isopimarol                       | 2314 | 288    | 257 | 41,105,81,67,91,55,119,133,147,1<br>61,175,243,201,274            | --    | --        | 12.09 |
| 78 | Abital                           | 2315 | 286    | 187 | 131,43,105,51,55,79,145,243,255,<br>117,159,215,197               | 2.86  | 3.56      | --    |
|    | Unidentified                     | 2390 | 286    | 43  | 55,91,81,105,159,123,185,<br>251,201,241,271                      | --    | 2.46      | --    |
|    | Total                            |      |        |     |   | 97.00 | 97.23     | 97.89 |
|    | Monoterpene hydrocarbon          |      |        |     |   | 18.60 | 24.10     | 4.87  |
|    | Oxygen containing monoterpenes   |      |        |     |   | 7.33  | 2.14      | 4.30  |
|    | Sesquiterpene hydrocarbons       |      |        |     |   | 16.45 | 10.23     | 19.78 |
|    | Oxygen containing sesquiterpenes |      |        |     |   | 0.63  | 31.13     | 19.25 |
|    | Diterpenes                       |      |        |     |   | 53.99 | 29.63     | 49.69 |

RI\* = retention index, data were measured relative to n-alkanes on DB5 column under conditions listed in the experimental section.

\*\* = co-eluted components

n.d\* = not detected

Table 2:  $^1\text{H}$ -,  $^{13}\text{C}$ -NMR and HMBC data of phyllocladene ( $\text{CDCl}_3$ , TMS as internal stander).

| No. | $\delta\text{H}$   | $\delta\text{C}$ | DEPT          | HMBC               |
|-----|--|------------------|---------------|--------------------|
| 1   | 0.77, 1H, dd, $J = 14, 4$ Hz<br>1.63, 1H, dd, $J = 14, 9$ Hz     | 41.06            | $\text{CH}_2$ | 3, 5, 9, 20        |
| 2   | 1.38, 2H, dm, $J = 13$ Hz  | 19.00            | $\text{CH}_2$ | 1, 3, 4, 10        |
| 3   | 1.79, 1H, dd, $J = 17, 4.8$ Hz<br>2.77, 1H, dd, $J = 17, 1.8$ Hz | 42.03            | $\text{CH}_2$ | 1, 5               |
| 4   | --   | 41.47            | Cq            | --                 |
| 5   | 0.84, 1H, m  | 56.54            | CH            | 1, 3, 7, 9, 18, 20 |
| 6   | 1.28, 1H, m<br>1.54, 1H  | 20.31            | $\text{CH}_2$ | 4, 5, 7            |
| 7   | 1.52, 2H   | 37.85            | $\text{CH}_2$ | 5, 9, 14           |
| 8   | --   | 43.64            | Cq            | --                 |
| 9   | 1.10, dd, $J = 7.5, 4$ Hz  | 56.93            | CH            | 1, 7, 15, 20       |
| 10  | --   | 39.49            | Cq            | --                 |
| 11  | 1.54, 2H   | 18.48            | $\text{CH}_2$ | 8, 13              |
| 12  | 0.88, 1H, m<br>1.44, 1H, m                                       | 33.19            | $\text{CH}_2$ | 14                 |
| 13  | 2.52 (brs)   | 42.70            | CH            | 14, 15, 16, 17     |
| 14  | 1.54, 1H<br>1.52, 1H   | 34.01            | $\text{CH}_2$ | 12, 16             |
| 15  | 1.15, 1H, dd, $J = 11, 2.4$ Hz<br>1.53, 1H                       | 50.35            | $\text{CH}_2$ | 9, 13, 14, 16      |
| 16  | --   | 157.63           | Cq            | --                 |
| 17  | 4.70, 1H, d, $J = 1.2$ Hz<br>4.74, 1H, d, $J = 1.2$ Hz           | 102.32           | $\text{CH}_2$ | 13, 16             |
| 18  | 0.86, 3H, s  | 33.67            | $\text{CH}_3$ | 3, 5, 19           |
| 19  | 0.81, 3H, s  | 21.88            | $\text{CH}_3$ | 3, 5, 18           |
| 20  | 0.92, 3H, s  | 15.11            | $\text{CH}_3$ | 5, 9, 10           |

Assignments were made by 2D( $^1\text{H}$ - $^1\text{H}$  COSY,  $^1\text{H}$ - $^{13}\text{C}$  HSQC and HMBC) spectra, APT and DEPT experiments and comparison with related compounds<sup>(17-22)</sup>.

\* Overlapping signals.

Table 3: Antimicrobial screening of volatile oils from leaves, stem bark, wood and phyllocladene of *Araucaria excelsa* R. Br.

| Material               | Diameter of inhibition zone in mm |                      |                    |                   |                    |                   |
|------------------------|-----------------------------------|----------------------|--------------------|-------------------|--------------------|-------------------|
|                        | Gram +ve bacteria                 |                      |                    | Gram -ve bacteria | Fungi              |                   |
|                        | <i>S. aureus</i>                  | <i>Sarcina lutea</i> | <i>B. subtilis</i> | <i>E. coli</i>    | <i>C. albicans</i> | <i>Asper. sp.</i> |
| Volatile oil of leaves | 15                                | -                    | 24                 | 16                | 10                 | 13                |
| Volatile oil of bark   | 17                                | 25                   | 30                 | 26                | 16                 | 17                |
| Volatile oil of wood   | 18                                | -                    | 28                 | 24                | 19                 | 14                |
| Phyllocladene          | 10                                | 15                   | 25                 | 25                | -                  | -                 |
| Penicillin             | -                                 | -                    | -                  | -                 | 20                 | 22                |
| Nystatin               | -                                 | -                    | -                  | -                 | -                  | -                 |

- = No zone of inhibition.

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## التركيب الكيميائي والفاعلية المضادة للميكروبات للزيت الطيار لنبات

### أروكاريا إكسلسا آ. ب. المنزرع في مصر

عفاف السيد عبد الغنى

قسم العقاقير - كلية الصيدلة - جامعة الزقازيق - الزقازيق - مصر

تم فى هذا البحث فصل الزيت الطيار من الأجزاء المختلفة (الأوراق وقلف وخشب الساق) لنبات أروكاريا إكسلسا آر. ب.ر (شجرة عيد الميلاد) من الفصيلة الأروكارية وتحديد مكونات الزيت المستخلص من كل جزء باستخدام كروماتوجرافيا الغاز الشعرية المتصلة بمطياف الكتلة وقد أمكن التعرف على ثمانية وسبعون مركب من مكونات الزيوت ووجد اختلافاً كبيراً وكيمياً بين محتويات كل جزء ، وتبين أن مركب الفيلوكلادين هو المركب الرئيسى فى زيت الأوراق (37,86%) وقد تم فصله والتعرف عليه بالطرق الطيفية المختلفة.

كما تم اختبار فاعلية الزيوت ضد بعض الميكروبات وثبت أن لها تأثيراً قوياً وشاملاً لكل من البكتريا والفطريات المختارة.